EFFECTS OF EPINEPHRINE ON LIPID METABOLISM IN RESTING SKELETAL MUSCLE

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The effects of epinephrine on lipid metabolism in resting skeletal muscle. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E300–E309, 1998.—The effects of physiological (0, 0.1, 2.5, and 10 nM) and pharmacological (200 nM) epinephrine concentrations on resting skeletal muscle lipid metabolism were investigated with the use of incubated rat epitrochlearis (EPT), flexor digitorum brevis (FDB), and soleus (SOL) muscles. Muscles were chosen to reflect a range of oxidative capacities: SOL > EPT > FDB. The muscles were pulsed with [1-14C]palmitate and chased with [9,10-3H]palmitate. Incorporation and loss of the labeled palmitate from the triacylglycerol pool (as well as mono- and diacylglycerol, phospholipid, and fatty acid pools) permitted the simultaneous estimation of lipid hydrolysis and synthesis. Endogenous and exogenous fat oxidation was quantified by 14CO2 and 3H2O production, respectively. Triacylglycerol hydrolysis was elevated above control at all epinephrine concentrations in the oxidative SOL muscle, at 2.5 and 200 nM (at 10 nM, P = 0.066) in the FDB, and only at 200 nM epinephrine in the EPT. Epinephrine stimulated glycogen breakdown in the EPT at all concentrations but only at 10 and 200 nM in the FDB and had no effect in the SOL. We further characterized muscle lipid hydrolysis potential and measured total hormone-sensitive lipase content by Western blotting (SOL > FDB > EPT). This study demonstrated that physiological levels of epinephrine cause measurable increases in triacylglycerol hydrolysis at rest in oxidative but not in glycolytic muscle, with no change in the rate of lipid synthesis or oxidation. Furthermore, epinephrine caused differential stimulation of carbohydrate and fat metabolism in glycolytic vs. oxidative muscle. Epinephrine preferentially stimulated glycogen breakdown over triacylglycerol hydrolysis in the glycolytic EPT muscle. Conversely, in the oxidative SOL muscle, epinephrine caused an increase in endogenous lipid hydrolysis over glycogen breakdown.

Although synthesis of TGm is primarily regulated by the availability of glycerol 3-phosphate and free fatty acids (FFAs) (1, 29), synthesis of TG in cardiac muscle also appears to be partially governed by adrenergic mechanisms through cAMP-dependent downregulation of glycerol-3-phosphate acyltransferase (18). Whether epinephrine affects TG synthesis in skeletal muscle has not been investigated. Therefore, the purpose of this study was to investigate the effect of physiological levels of epinephrine on both the hydrolysis and synthesis of TGm, its intermediates [di- and monoacylglycerol (DG and MG, respectively)], and other intramuscular lipids [phospholipids (PLs)] and FFAs in resting skeletal muscle incubated in vitro. An epinephrine concentration ~20-fold greater than the highest physiological concentration was also included. The soleus (SOL), epitrochlearis (EPT), and flexor digitorum brevis (FDB) muscles were chosen because they were well suited to incubation and represented a range of oxidative and glycolytic capacities (12). We further characterized these muscles by comparing the HSL protein concentrations with Western blotting.

Because the SOL is primarily oxidative in nature and has previously shown the greatest reliance on lipid metabolism (12), we hypothesized that epinephrine would increase TGm hydrolysis in the SOL (but not in
the EPT or FDB) and would downregulate TG synthesis in the SOL, with no effect in EPT or FDB muscles.

METHODS

Animals

Female Sprague-Dawley rats (n = 43) weighing on average 184 ± 4 g were used in the experiments. The animals were housed in a controlled environment with a 12:12-h light-dark cycle and fed Purina rat chow ad libitum. This study was approved by the University of Guelph Animal Care Committee.

Incubation Media

Krebs-Ringer bicarbonate (KRB) buffer was prepared weekly from refrigerated stock solutions. Final concentrations were 125 mM sodium chloride, 5 mM potassium chloride, 2.7 mM calcium chloride, 1.25 mM potassium phosphate (monobasic), 1.35 mM magnesium sulfate, and 21 mM sodium bicarbonate. Unless otherwise mentioned, all chemicals were obtained from Sigma Chemical (St. Louis, MO). Glucose (5 mM) and 4% (wt/vol) fat-free BSA (Boehringer Mannheim, Montreal, PQ) were dissolved at 45°C. Palmitic acid was dissolved in ethanol (3.2%, wt/vol), and a small volume (0.8%, vol/vol) was added to the KRB-albumin at 45°C (minimum 20 min) to achieve a final concentration of 1.0 mM. The FFA concentration was confirmed by analysis (FFA kit, WAKO Chemical, Richmond, VA). The buffer was divided into three parts for each of the pulse, wash, and chase phases. An appropriate volume of [1-14C]palmitate in ethanol (Amersham, Oakville, ON) was added to the pulse buffer to bring the final specific activity to 2 µCi/ml. Similarly, [9,10-3H]palmitate was added to the chase buffer to the same final specific activity. No radioactive label was added to the wash buffer. Buffer (1.5 ml) was pipetted into 20-ml scintillation vials (Fisher Scientific, Ottawa, ON), gassed with 95% O2-5% CO2 for 10–15 min, sealed, and warmed to 30°C in a shaking water bath. After the palmitate was dissolved for 20 min at 45°C and gassed for 15 min, the ethanol concentration was negligible. Ethanol concentration was measured as previously described (5). After 20 min at 45°C, the ethanol concentration was 0.11% (n = 2) and was reduced further after being gassed for 15 min to 0.006% (n = 3).

Incubations

Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (6 mg/100 g body wt), and the muscles were excised as quickly as possible with care to dissect tendon to tendon. SOL strips were cut with the use of a 27-gauge needle by sliding carefully from the medial to the distal tendon in a smooth motion. The EPT muscle averaged 43.5 ± 1.4 mg, the FDB averaged 30.4 ± 0.9 mg, and the SOL strips averaged 28.1 ± 1.0 mg wet wt.

Each muscle was placed into a vial containing 1.5 ml of pregassed pulse buffer at 30°C. The vial was resealed and placed in a shaking water bath (Isotemp, Fisher Scientific) for 1 h (Fig. 1). This allowed prelabeling of the endogenous pools with [14C]palmitate. Previous studies demonstrated that [14C]palmitate incorporation into the TG pool was linear over 3 h, and little further increase in incorporation was gained at palmitate concentrations higher than 1.0 mM (12). At the end of the pulse phase, the muscle was removed, blotted, and placed in a vial containing wash buffer for an additional hour. This phase allowed movement of the [14C]palmitate from the interstitial spaces into the cell and caused little decrease in the labeling of the endogenous pools as previously shown (12). After the pulse/wash phases, one-half of the muscles were blotted, weighed, and extracted for total lipids. The remaining paired muscles were blotted and transferred to vials containing 1.5 ml of the chase buffer ([3H]palmitate).

Fig. 1. Experimental design. Pulse phase preloads endogenous lipid pools with [14C]palmitate. Wash phase allows interstitial [14C]palmitate to be taken up by cell. Chase phase is experimental phase during which effect of epinephrine was studied (control = 0 nM). [3H]palmitate labels exogenous fatty acid incorporation or oxidation, and changes in 14C monitor endogenous pools. All buffers were gassed with 95% O2-5% CO2 and maintained at 30°C.

<table>
<thead>
<tr>
<th>1 HOUR</th>
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<td>PULSE</td>
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<tr>
<td></td>
<td>CHASE</td>
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14C- palmitate
1.0 mM palmitate
5.0 mM glucose

3H-palmitate
1.0 mM palmitate
5.0 mM glucose

Control (0 nM) or
0.1, 2.5, 10, or 200 nM

Epinephrine

... muscles transferred to wash buffer.

... pre-chase muscles sampled for lipid extraction and paired muscles transferred to chase buffer.

... post-chase muscles sampled for lipid extraction, samples of buffer taken for 14CO2 and 3H2O content.
Incubation media gases were checked in a separate experiment and were measured at the end of 3 h (PO$_2$ 304 ± 33 mmHg, PCO$_2$ 27 ± 3 mmHg, pH 7.42 ± 0.03 n = 6). Viability of the muscles over the 3-h period has previously been established by measuring the content of high-energy phosphates (12).

Epinephrine

All epinephrine stock dilutions were prepared in KRB buffer with 2 mg/ml ascorbic acid to prevent oxidation of the hormone. A 1.0 mM stock solution of epinephrine bitartrate was prepared daily. Just before the chase phase, the stock solution was diluted such that 50 µl could be added to each 10 ml of the final chase buffer to achieve concentrations of 0.1, 2.5, 10, and 200 nM. Final ascorbic acid concentration in the chase buffer was 10 µg/ml.

Epinephrine concentrations were measured before and at the end of the chase phase of the incubations to determine stability of the hormone due to oxidation. Analysis was performed by HPLC (Waters, Mississauga, ON) as previously described (42). When the desired concentration was 0.1 nM, the actual measured concentration was 0.30 ± 0.07 nM at the start of the chase phase, falling to 0.23 ± 0.04 nM at the end of the 1-h chase. A concentration of 2.5 nM was 2.4 ± 0.3 nM, falling to 2.2 ± 0.2 nM; 10 nM was 9.5 ± 1.1 nM, falling to 7.9 ± 0.5 nM; and 200 nM was 178 ± 4 nM, falling to 151 ± 2 nM at the end of the experiment. This change in epinephrine concentration has also been observed by others (28).

Extraction for Total Lipids

Muscles removed from the incubation medium were placed into 2.0 ml of 1:1 chloroform-methanol (vol/vol) and homogenized using a polytron (Brinkman Instruments, Mississauga, ON), and the connective tissue was weighed. This was subtracted from the total weight to give a net muscle weight.

Samples were centrifuged at 3,800 g (4°C) for 10 min. The supernatant was removed with glass Pasteur pipettes and transferred to a clean centrifuge tube. The muscle residue was rinsed with 2.0 ml of 1:1 chloroform-methanol, vortexed, and centrifuged as before. The resultant supernatant was added to that from the previous step. Deionized water (2.0 ml) was added to separate the aqueous compounds and methanol from the lipophilic molecules and the chloroform. The mixture was shaken, tubes were centrifuged as before, and the water/methanol phase was aspirated and discarded. The chloroform phase (containing the total lipids extracted from muscle) was transferred to a glass tube and evaporated with an incubation was confirmed (94.4%).

H$_2$O produced through exogenous FFA oxidation was separated from the labeled [3H]palmitate by shaking 0.5 ml of the buffer with 2.5 ml of 2:1 chloroform-methanol. A quantity of 1.0 ml of 2 M HCl-KCl was added to further separate the phases, and the mixture was shaken well and centrifuged at 3,800 g for 5 min. The aqueous phase (1.0 ml) was removed and treated again as described. After the final centrifugation, 0.5-ml aliquots were quantified by liquid scintillation counting.

Glycogen

Because of the small size of the muscles analyzed for lipids, it was necessary to incubate muscles separately for glycogen analysis. Incubations were performed under identical conditions at 30°C. At the end of the experiment, muscles were rapidly frozen in liquid nitrogen, lyophilized, and dissected free of connective tissue.

For glycogen determination, free glucose was first destroyed by digestion with 0.1 M NaOH for 10 min at 80°C. Muscle extracts were neutralized (equi-volume 0.1 M HCl in 0.2 M citric acid-sodium phosphate buffer, pH 5.0), and the glycogen was converted to glucose enzymatically with amyloglucosidase (Boehringer Mannheim). Glucose was measured spectrophotometrically using NADH-linked enzyme assays as previously described (5).

Western Blotting for HSL

Muscles were homogenized in 210 mM sucrose, 2 mM EGTA, 40 mM sodium chloride, 30 mM HEPES, 5 mM EDTA, and 2 mM phenylmethylsulfonyl fluoride, pH 7.4. Proteins were precipitated with 0.5 M potassium chloride and 25 mM tetrasodium pyrophosphate and spun down in an ultracentrifuge (Beckman XL-90) at 175,000 g for 75 min at 4°C. Protein pellets were rehomogenized in 10 mM Tris-1.0 mM EDTA, pH 7.4, and SDS (Bio-Rad, Mississauga, ON) was added to bring the final concentration to 5%; centrifugation followed at 1,000 g for 10 min at room temperature. Adipose tissue homogenized in PBS (pH 7.4) with 1% (vol/vol) Triton was used as a control. Protein concentration was measured using the BCA protein kit (Pierce, Rockford, IL).

One hundred micrograms of muscle protein (50 µg of adipose protein) were separated with the use of standard SDS-PAGE (4% stacking gel, 10% running gel) and transferred to membranes (Immobilon polyvinylidene difluoride, Bio-Rad) by electrophoretic transfer at 110 V for 1 h in 25 mM Tris, 192 mM glycine, and 20% (vol/vol) methanol, pH 8.4. Membranes
were blocked overnight with 10% (wt/vol) skim milk powder in Tris-buffered saline, pH 7.5, with 0.1% (vol/vol) Tween 20 (Bio-Rad) and then incubated with polyclonal rabbit anti-HSL (1:5,000; kindly donated by Dr. F. B. Kraemer, Stanford Univ. Medical Center). Secondary incubation with donkey anti-rabbit IgG-horseradish peroxidase conjugate (Amersham Life Science, Oakville, ON) allowed detection with the use of an enhanced chemiluminescence kit (Amersham Life Science). Films were scanned for density and quantitated using Abaton Scan 300 (Fremont, CA).

Calculations and Statistics

Incorporation of labeled palmitate into the lipid fractions was calculated by use of the specific activity of the labeled palmitate in the incubation buffer (dpm/nmol) and by normalizing for the wet muscle weight to give measurements of nanomoles per gram palmitate incorporated or oxidized. During the chase phase, all the 14C-labeled data were related to the specific activity of [14C]palmitate in the pulse buffer as previously described (12).

An additional twofold correction was applied to the 14CO2 production to account for 14C-labeled oxidative intermediates, which are aqueous and not released as CO2 as previously reported (12, 36).

The [14C]palmitate lost in the lipid fraction during the chase phase was calculated between the paired muscles, which were sampled at the end of either the pulse or wash phase. The pre- and postchase differences were analyzed for each muscle by one-way ANOVA (epinephrine concentration). Differences among the three muscles (e.g., HSL protein) were analyzed by one-way ANOVA (muscle). A Fisher's protected least significant difference post hoc test was used to distinguish differences revealed by the ANOVA. Significance was accepted as P < 0.05.

RESULTS

Relative Amounts of HSL Protein in the Three Muscles

HSL was detected in all three types of muscles examined. However, the SOL muscle contained almost twofold more HSL protein than EPT and FDB (Figs. 2 and 3). One hundred micrograms of protein from the FDB and EPT muscles tested produced single bands that were much less intense than those from 50 µg adipose tissue protein, which lead to a double band in the 84- to 89-kDa region (Fig. 2). With 100 µg of SOL muscle protein, a double band was visible, but the prominent band was at the higher molecular mass (Fig. 2). When 10 µg of adipose tissue protein was loaded, only the higher molecular mass band was detectable (data not shown).

Preexperimental Labeling of Lipid Pools in Muscle

Labeling of the TGm pool at the start of the chase was almost twofold higher in the SOL compared with the FDB and EPT muscles (Table 1). In all muscles, this was ∼1–2.5% of the total TGm pool (assuming a TGm content of ∼4 mol/g wet wt; Ref. 12).

The acylglycerols (TG + DG + MG) constituted the major [14C]palmitate incorporation in SOL (71.5%) and EPT (65%). However, in the FDB, the acylglycerol labeling was a lower percentage of the total (47%). In the FDB, a high percentage of the total labeling was observed in the PL pool, almost twice as much as that

Table 1. Preexperimental labeling of endogenous lipid pools with [14C]palmitate

<table>
<thead>
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<th></th>
<th>SOL</th>
<th>FDB</th>
<th>EPT</th>
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<tbody>
<tr>
<td>TGm</td>
<td>112 ± 6</td>
<td>61 ± 4†</td>
<td>63 ± 4*</td>
</tr>
<tr>
<td>DG</td>
<td>10.9 ± 0.6†</td>
<td>5.9 ± 0.3</td>
<td>13.0 ± 0.8†</td>
</tr>
<tr>
<td>MG</td>
<td>0.94 ± 0.06†</td>
<td>1.4 ± 0.1</td>
<td>0.80 ± 0.06†</td>
</tr>
<tr>
<td>PLs</td>
<td>44 ± 2†</td>
<td>68 ± 2*</td>
<td>32 ± 1†</td>
</tr>
<tr>
<td>FFAs</td>
<td>5.3 ± 0.7</td>
<td>8.4 ± 0.4</td>
<td>9.3 ± 1.2*</td>
</tr>
<tr>
<td>Total</td>
<td>173 ± 9</td>
<td>145 ± 7</td>
<td>118 ± 7</td>
</tr>
</tbody>
</table>

*Significantly different from SOL; †significantly different from FDB.
observed in both SOL and EPT (47 vs. 25 and 27%, respectively).

Effect of Epinephrine on Hydrolysis and Oxidation of Intramuscular Lipids

Endogenous TG m. Without epinephrine, no loss of [14C]palmitate from endogenous TG stores was observed in any muscle at the end of the 1-h chase (Fig. 4). In the SOL, there was a breakdown of the TG pool and loss of [14C]palmitate at all epinephrine concentrations examined (Fig. 4). The hydrolysis of TG in the FDB was significant at 2.5 and 200 nM compared with control, and the trend was also evident at 10 nM (P = 0.066). In contrast, a significant decrease was only observed at the pharmacological concentration of 200 nM in the EPT.

Endogenous DG and MG. Epinephrine did not affect DG hydrolysis in the SOL and FDB, as a small decrease in the 14C label in the DG pool was observed in these muscles with or without epinephrine (3.6 ± 0.5 and 2.0 ± 0.3 nmol/g for SOL and FDB, respectively). In the EPT, larger decreases in endogenous DG were observed at 10 and 200 nM (3.2 ± 1.4 and 5.1 ± 1.2 nmol/g, respectively) compared with 0.1 nM (0.5 ± 1.6 nmol/g).

Epinephrine did not affect the loss of [14C]palmitate from the MG pool in the SOL and EPT (data not shown). No difference was observed in the loss of [14C]palmitate from the MG pool with epinephrine in the FDB except with 200 nM, at which level a small decrease in MG hydrolysis occurred (data not shown).

Endogenous PL and FFA pools. Epinephrine did not affect the hydrolysis of the PL pool compared with control in any muscle (Table 2). In the control condition, all three muscles lost the same amount of [14C]palmitate from the intramuscular FFA pool. In the SOL, epinephrine significantly decreased the amount of [14C]palmitate lost from the FFA pool, although the change was very small. In contrast, no changes were observed in the FFA pool of either FDB or EPT due to epinephrine (Table 2).

Oxidation of endogenous lipid stores (14CO2 production). SOL had approximately two- to threefold higher endogenous lipid oxidation rates, averaging 30–40 nmol/g palmitate oxidized compared with 8–15 nmol/g oxidized in the FDB and EPT (Fig. 5A). No increase in oxidation was observed in any muscle with increasing epinephrine concentration. In the EPT at 200 nM epinephrine, a significant decrease in oxidation was observed compared with control.

Effect of Epinephrine on Exogenous Palmitate Oxidation and Esterification

Oxidation of exogenous fatty acids (3H2O production). SOL oxidized more than twice the amount of exogenous palmitate (~170 nmol/g) compared with both FDB and EPT (~60–80 nmol/g) in the control condition (Fig. 5B). Epinephrine had no effect on exogenous palmitate oxidation in all muscles except at 200 nM in the EPT, at which level a significant decrease was observed.

Esterification of exogenous palmitate into the TGm pool. SOL incorporated twice as much [3H]palmitate (108 ± 11 nmol/g) into the TG pool as the other two muscles (49 ± 2 nmol/g) in the control condition (Fig. 6). There were no changes in incorporation in any of the muscles with epinephrine except for an increase in [3H]palmitate incorporation in the SOL muscle at 200 nM compared with 0.1 and 2.5 nM epinephrine.

Incorporation of exogenous palmitate into endogenous DG, MG, and PL pools. In the control condition, the FDB incorporated ~1.5–2 times the amount of [3H]palmitate into PL than either EPT or SOL (Table 3). Incorporation of the exogenous label into MG and DG pools was similar in all three muscles. However, epinephrine did not affect the incorporation of [3H]palmitate into any of the minor glyceride pools (PL, MG, DG).
Effect of Epinephrine on Net Glycogenolysis

Prechase glycogen concentrations were 129 ± 7 (n = 18), 100 ± 5 (n = 21), and 120 ± 5 (n = 15) mol/g dry wt in the SOL, FDB, and EPT muscles, respectively. SOL demonstrated minimal net changes in glycogen concentration with or without epinephrine during the 60-min chase (Fig. 7). Net glycogen breakdown in the FDB was not significantly greater than control at 2.5 nM epinephrine but was elevated significantly at 10 and 200 nM epinephrine. In contrast, the EPT muscle demonstrated increased net glycogenolysis at all epinephrine concentrations compared with control.

DISCUSSION

This study examined the effect of epinephrine on the metabolism of TG and palmitate in resting rat skeletal muscle. There were several important new observations. First, we observed that HSL was present in all types of skeletal muscle but that the abundance of this enzyme was greater in the oxidative SOL than in more glycolytic muscles. Second, another important new finding was the observation that TGm hydrolysis exhibited markedly different dose-response characteristics from epinephrine in different types of skeletal muscles. Specifically, epinephrine at all physiological concentrations promoted hydrolysis of TGm in the oxidative SOL muscle. In the FDB muscle, TG hydrolysis increased only at the higher concentrations of epinephrine (2.5 and 200 nM); in the glycolytic EPT muscle, it increased only when a pharmacological dose (200 nM) of epinephrine was used. Physiological concentrations of epinephrine did not affect the TGm, DG, MG, or PL synthetic rate or the rate of exogenous and endogenous fat oxidation.

A resting preparation was chosen to isolate the effects of epinephrine from other potential cellular regulators provoked by exercise, which may be involved in TGm metabolism. Hydrolysis and synthesis of TGm and its intermediates were monitored with a dual-label pulse-chase technique as described previously (12, 35). This technique also permitted simultaneous measurements of exogenous and endogenous fat oxidation.

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Table 2. Epinephrine and alterations in [14C]palmitate within endogenous PL and FFA pools between start and end of 1-h chase in SOL, FDB, and EPT muscles

<table>
<thead>
<tr>
<th>Epinephrine Conc, nmol/l</th>
<th>Change in [14C]Palmitate As</th>
<th>SOL</th>
<th>FDB</th>
<th>EPT</th>
<th>SOL</th>
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<td></td>
<td>PL, nmol/g</td>
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<td>FFA, nmol/g</td>
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<tr>
<td>0.1</td>
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<tr>
<td>10</td>
<td>2.3 ± 4.2</td>
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Values are means ± SE; n = 8–9. Negative values indicate a decrease of [14C] in pool. *Decrease in intramuscular [14C]palmitate in endogenous FFA pool during chase is less than control.

Fig. 5. Effect of epinephrine on endogenous and exogenous fat oxidation in resting SOL, FDB, and EPT muscles. C, control or 0 nM epinephrine. a Significantly different from control in same muscle.

Fig. 6. Effect of epinephrine on esterification of [3H]palmitate into triacylglycerol pool in resting SOL, FDB, and EPT muscles. C, control or 0 nM epinephrine. b Significantly different from 0.1 and 2.5 nM epinephrine in same muscle.
Table 3. Epinephrine and esterification of exogenous [3H]palmitate into PL, DG, and MG during 1-h chase in SOL, FDB, and EPT muscles

<table>
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<tr>
<th>Epinephrine Conc., nmol/l</th>
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<th>FDB</th>
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<th>DG, nmol/g SOL</th>
<th>FDB</th>
<th>EPT</th>
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<td>55.4±2.9</td>
<td>32.5±3.4</td>
<td>23.0±2.8</td>
<td>21.9±1.3</td>
<td>21.9±1.8</td>
<td>2.2±0.3</td>
<td>2.1±0.1</td>
<td>1.6±0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8–9.

Concentrations of epinephrine were chosen from within the physiological range (0.1–10 nM) as well as the pharmacological (200 nM).

The muscles chosen for incubation in this study have recently been characterized by us (12) and compared with earlier characterizations of EPT and SOL (3, 41) and FDB (8). Our previous work confirmed that the SOL is predominantly an oxidative muscle with high type 1 fiber content, citrate synthase activity, and cytosolic fatty acid-binding protein concentration. In addition, a recent study reported that SOL muscle exhibits a greater innate ability to synthesize TG than less oxidative muscles (7). In contrast, EPT muscle possesses a high percentage of type IIb fibers and high phosphofructokinase activity and low citrate synthase activity and fatty acid-binding protein content. Previously, and in contrast to earlier work (8), we demonstrated that FDB has low glycolytic and oxidative capacities but contains a large number of type IIc (or transition) fibers (12). It was surmised that the FDB is still in transition in younger rats (~180 g) and therefore has not fully maximized its oxidative or glycolytic potential. Our HSL data are consistent with the potential for lipid metabolism in these muscles, with evidence that the SOL contains approximately twice the amount of lipase protein as the EPT and FDB. These are the first comparative data between skeletal muscles.

Epinephrine and TGm Hydrolysis

The β2-adrenergic receptor population predominates in skeletal muscle, and, since this subtype has the greatest affinity for epinephrine, the present study examined this hormone (25, 30, 32). Jensen et al. (25) have shown that SOL has approximately twofold higher β-receptor density than EPT, which is consistent with the higher rate of TGm hydrolysis observed in this muscle. Their study indicated that receptor affinity was similar between muscle types, and, assuming that the postreceptor events are similar, they postulated that the magnitude of the response would depend on receptor number. Taken together, we can extrapolate from our data that the β-receptor density of FDB must lie somewhere between that of SOL and EPT. SOL demonstrated increased TGm hydrolysis with a very low level of epinephrine (0.1 nM), and no greater loss of [14C] from the TGm pool was observed with higher levels of epinephrine (2.5–200 nM). This may be explained by the fact that our model did not include insulin in the medium, since the experiment was designed to separate the effect of epinephrine from both insulin and exercise. Therefore, it is possible, without the opposing effect of insulin, that even very low levels of epinephrine were lipolytic (23).

Unlike the beating heart, resting muscles do not incorporate enough [14C]palmitate into the TGm pool to be representative of the total pool (12, 35). Saddik and Lopaschuk (35) were able to achieve ~40% labeling of the myocardial TG pool and demonstrated that changes in [14C] reflected changes in TG content. We previously demonstrated that 1–2.5% of the TGm pool was labeled in incubated skeletal muscle, and, although ~40% decreases in [14C] labeling of TGm were observed under certain chase conditions, no measurable changes in TGm content were documented (12). It is therefore difficult to determine the endogenous TGm specific activity and quantify total TGm utilization. We are of the opinion that the incorporated [14C]palmitate is not homogeneously distributed within the total TGm and represents a small subpool that is rapidly turning over (12).

Endogenous and Exogenous Lipid Oxidation

In the present study, we observed that TGm was reduced when muscles were exposed to epinephrine, but unexpectedly there was no concurrent increase in
CO₂ production from the TGₘ. We have no obvious explanation for this finding, although it is important to note that, in other experiments using exactly the same procedures, we were able to measure increases in CO₂ production when TGₘ was reduced (e.g., during muscle contraction; unpublished results). Indeed, the recovery of CO₂ (94%) from radiolabeled bicarbonate indicates that the CO₂ measurement can be obtained reliably. Therefore, it appears that, despite the degradation of TGₘ, this source of FFAs was not metabolized in the present experiments. Because 90% of the oxidized FFAs in resting skeletal muscle are derived from the incubation medium, it may well be that resting muscle is not critically dependent on FFAs derived from TGₘ.

Whether preferential utilization of circulating FFAs indicates selective FFA trafficking and oxidation by mitochondria is unknown. It is possible that the FFAs that were released from the TG pool but not oxidized (~40–60 nmol/g) were spread among the other lipid pools (PL, MG, and DG) as well as the other membrane lipids (cholesterol, cholesterol esters), which were not measured.

Both endogenous and exogenous oxidation rates were at least twofold higher in the SOL compared with either the FDB or EPT and correlated well with the oxidative profiles of the muscles. Exogenous fat oxidation in the SOL was unaffected by epinephrine in the physiological range. However, in the EPT, the oxidation of both endogenous and exogenous lipid was depressed with 200 nM epinephrine, indicating that the decreased oxidation was unrelated to the source of the fat. This supramaximal effect warrants further investigation despite the fact that its impact on normal physiological systems would be limited.

Epinephrine and the Synthesis of Muscle TG and Other Lipid Pools

The activities of enzymes involved in the synthesis of lipids such as glycerol-3-phosphate acyltransferase have been shown to be depressed by phosphorylation in vitro (6) and in cardiac muscle (18). Therefore, a decrease in endogenous lipid synthesis during exposure to epinephrine would be expected. However, in the present study, epinephrine had no effect on the synthesis of any of the intramuscular lipid pools. Either epinephrine did not result in phosphorylation and inactivation of the synthetic enzymes in the incubated skeletal muscles, or any downregulation of synthesis due to phosphorylation was balanced by other factors that would increase TG production. Evidence for increased cycling of TGₘ during exercise (16) would indicate that increases in synthesis are a possibility, even in the presence of higher exercise epinephrine levels. Also, increases in epinephrine have been shown to increase skeletal muscle glucose uptake (17) and thereby provide additional glycerol-3-phosphate as substrate for TG synthesis. Because the synthetic reactions are also regulated by substrate availability, this could be the factor that balanced any potential downregulation in our physiological range of epinephrine. This was also supported by our finding that lipid synthesis in the SOL was actually increased at the pharmacological level of epinephrine.

Epinephrine and Net Glycogen Breakdown

In EPT, epinephrine exposure for 1 h caused net glycogen breakdown at 2.5, 10, and 200 nM, in good agreement with previous work in resting muscle (26, 27, 34). This muscle relies strongly on carbohydrate as a fuel, and, in the physiological range of epinephrine, EPT demonstrated marked net glycogen breakdown over TGₘ utilization despite the fact that the second messenger system for both processes was cAMP mediated. In contrast, the SOL muscle demonstrated no increase in net glycogenolysis with epinephrine, despite the fact that SOL has twice the number of β-receptors as EPT (25). Significant TGₘ hydrolysis occurred in the SOL at all epinephrine concentrations, suggestive of preferential fat breakdown through an unknown mechanism. Another possible explanation for the range of epinephrine-mediated glycogenolytic effects could be posttransformation effectors. For example, inorganic phosphate is an activator of phosphorylase a (9) and may be accumulated to a different extent in the EPT and SOL muscles during incubation. An approximate concentration of inorganic phosphate can be calculated as the difference in phosphocreatine concentration between control and incubated muscles from our previous work (12). From this calculation, EPT had approximately fourfold greater accumulation of inorganic phosphate than SOL, and this could account for increased net glycogen utilization.

Summary and Conclusions

This study examined TG metabolism in resting rat skeletal muscles spanning a range of oxidative capacities. Physiological concentrations of epinephrine stimulated TGₘ hydrolysis in the oxidative SOL muscle and in the FDB. Only the supramaximal concentration of epinephrine stimulated lipolysis in the glycolytic EPT. A very low level of 0.1 nM epinephrine was able to maximally promote TGₘ breakdown in the oxidative SOL in the absence of the anti-lipolytic hormone insulin. This data correlated well with the total amount of HSL protein observed in the three muscles.

TGₘ hydrolysis and oxidation are separately governed events, since there was no change in the endogenous or exogenous oxidation rates in the range from 0 to 10 nM epinephrine. No changes in TGₘ synthesis were observed in this physiological range of epinephrine. The smaller intermediate lipid pools demonstrated no change in synthesis or hydrolysis with epinephrine. A notable exception was the DG pool of the EPT, in which increases in hydrolysis were observed at 10 and 200 compared with 0.1 nM.

Although the stimulations of muscle glycogen and TG breakdown are both epinephrine-mediated events, there appears to be a differential selective sensitivity between these two fuels in different muscles. Epinephrine in the oxidative SOL muscle preferentially stimulated TG hydrolysis over glycogenolysis, whereas the
inverse was observed in the glycolytic EPT muscle. The mechanism(s) for this is unknown but could be explained in part by the posttransformational activation of phosphorylase a.

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