Effect of epinephrine on myocardial triglyceride and free fatty acid utilization

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KREISBERG, Robert A. Effect of epinephrine on myocardial triglyceride and free fatty acid utilization. Am. J. Physiol. 210(2): 385-389, 1966. In the isolated perfused rat heart, palmitate produced a 90% suppression of glucose oxidation which was partially reversed by epinephrine. Epinephrine increased C4O2 production from glucose-U-Cl4 sixfold in the presence of palmitate and twofold in its absence, but did not increase the oxidation of circulating chylomicron tripalmitin-C14 or palmitate-1-C14. Titratable fatty acid (FFA) uptake was less than the palmitate-C14 uptake with the result that there was an exaggerated decrease in perfusate FFA specific activity. Recovery of chylomicron tripalmitin-C14 and palmitate-1-C14 in myocardial triglyceride doubled, possibly as a result of increased exchange between endogenous triglyceride fatty acid and perfusate FFA. Increased glycerol release indicated that endogenous lipid turnover and utilization were enhanced. Hydrolysis and utilization of circulating triglyceride did not correlate with that predicted from studies of myocardial lipoprotein lipase. These experiments suggest that eninephrine: 1) specifically stimulates glucose utilization, 2) increases the utilization of endogenous lipid, and 3) indirectly influences myocardial FFA uptake in the intact animal by virtue of its ability to elevate circulating levels of FFA rather than by a direct effect on myocardial extraction.

METHODS

The perfusion apparatus, techniques, and analytical methods have been previously described (13, 14). Male albino Sprague-Dawley rats (Charles River Laboratories), weighing 190-270 g, allowed food and water ad lib., were used in all experiments. For all perfusions, sodium ethylenediaminetetraacetate (5 X 10^-4 M) was added to the Krebs bicarbonate-Ringer buffer. This has been demonstrated to potentiate the effects of epinephrine, presumably by preventing its degradation by heavy metals, without altering basal glucose uptake and oxidation (25) or oxidation of palmitate-1-C14 (unreported observations). Glucose U-C14 was obtained from New England Nuclear Corporation, Boston, Massachusetts. Control data recorded in this paper are the same as those presented in the preceding paper.

RESULTS

Chylomicron tripalmitin-C14 metabolism. Epinephrine (0.2 µg/ml) did not significantly alter the conversion of chylomicron tripalmitin-C14 (TP-C14) to C14O2 (Table 1). The recovery of TP-C14 in perfusate FFA decreased from 21 ± 1.5 to 17 ± 0.6 µmoles/g heart, dry wt. The total TP-C14 disappearing from the perfusate was not altered. TP-C14 disappearance is differentiated from myocardial uptake since a large proportion of that which
disappears as triglyceride is recovered in the perfusate. Table 1 shows Myocardial fatty acid uptake (the sum of C14 recovered in CO2, myocardial lipids, and intermediates) increased by 30% from 18.10 ± 1.76 to 25.1 ± 1.73 μmoles/g heart, dry wt, most of which was recovered in myocardial triglyceride (P < .01), although recovery in phospholipid also increased significantly (P < .01). The difference in the recovery of C14 between the two groups cannot account for the differences in TP-C14 uptake.

**Palmitate-1-C14 metabolism.** The effect of epinephrine on palmitate-1-C14 metabolism is shown in Table 2. FFA uptake, determined by titration, decreased from 32 ± 1 to 27 ± 1 μmoles/g heart, dry wt, in the presence of epinephrine, whereas palmitate-1-C14 uptake determined by the decrease in perfusate C14 counts remained unchanged. The perfusate FFA specific activity decreased 32% (vs. 16% for control hearts). Conversion of palmitate-1-C14 to C14O2 decreased significantly. Palmitate-1-C14 recovery in myocardial triglyceride and phospholipid increased from 0.0 ± 0.25 to 6.77 ± 0.53 and from 1.50 ± 0.05 to 3.11 ± 0.10 μmoles/g heart, dry wt, respectively. This increase is comparable with that observed with TP-C14 under similar conditions (Table 1). Palmitate-1-C14 recovery in the intermediate fraction decreased from 2.19 ± 0.14 to 1.20 ± 0.08 μmoles/g heart, dry wt.

**Glycerol release.** In the presence of glucose and palmitate-1-C14, 2.50 ± 0.10 μmoles glycerol/g heart, dry wt per hour were released by the perfused heart. After 15 min of preperfusion with oxygenated buffer containing 5 nm glucose, 5 X 10^-4 M EDTA, 1% albumin, and 0.6 mm chylomicron tri- paimitin, and epinephrine, as indicated. Values shown in each group are the means ± SE of 6 hearts. Label recovery is expressed as a percentage of the initial radioactivity available. The number of hearts in each group is indicated in parentheses. *P < .05 vs. control. †P < .01 vs. control.

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**Glucose metabolism.** The effect of chylomicron triglyceride and palmitate on glucose uptake and lactate production is shown in Table 3. Hearts of control rats removed glucose at a rate of 80 ± 5 μmoles/g dry wt per hr. Lactate production during this interval was 10 ± 4 μmoles/g dry wt, and represented approximately 15% of the glucose uptake. Epinephrine produced a threefold increase in glucose uptake and markedly elevated lactate production. Both chylomicron triglyceride and palmitate suppressed glucose uptake 50% while producing a moderate elevation of lactate production. With palmitate, C14O2 production from glucose-U-C14 decreased 90-95% from 43 ± 5 to 2.97 ± 0.25 μmoles/g heart.

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**TABLE 1. Myocardial tripalmitin-C14 utilization:**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control (12)</th>
<th>Epinephrine (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfusion conditions</td>
<td>C14 disappearance</td>
<td>G1 in perfusate</td>
</tr>
<tr>
<td>1C14</td>
<td>48.5 ± 0.9</td>
<td>46 ± 1</td>
</tr>
<tr>
<td>21.1 ± 0.6</td>
<td>17 ± 0.9 *</td>
<td></td>
</tr>
<tr>
<td>Incorporation of C14</td>
<td>Triglyceride</td>
<td>Phospholipids</td>
</tr>
<tr>
<td>5.97 ± 0.73</td>
<td>1.93 ± 0.08</td>
<td>1.07 ± 0.06</td>
</tr>
<tr>
<td>12.16 ± 0.33</td>
<td>2.46 ± 0.13</td>
<td>1.01 ± 0.05</td>
</tr>
<tr>
<td>C14O2 production</td>
<td>8.33 ± 0.57</td>
<td>9.42 ± 0.81</td>
</tr>
<tr>
<td>Total myocardial C14 uptake</td>
<td>18.10 ± 1.76</td>
<td>25.1 ± 1.73 *</td>
</tr>
<tr>
<td>Label recovery</td>
<td>85 ± 5</td>
<td>91 ± 4</td>
</tr>
</tbody>
</table>

After 15 min of preperfusion, hearts were perfused for 60 min with Krebs bicarbonate-Ringer buffer containing 5 nm glucose, 5 X 10^-4 M EDTA, 1% albumin, and 0.6 mm chylomicron tri- paimitin. Epinephrine was added to a concentration of 5 x 10^-5 M. Values represent the mean ± SE and are expressed as μmoles/g heart, dry wt. FFA specific activity decreased go-95% from 43 ± 5 to 2.97 ± 0.25 μmoles/g.

**TABLE 2. Myocardial palmitate-1-C14 utilization:**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control (6)</th>
<th>Epinephrine (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfusion conditions</td>
<td>C14 uptake</td>
<td>FFA specific activity, % decrease</td>
</tr>
<tr>
<td>1C14</td>
<td>32 ± 1</td>
<td>27 ± 1</td>
</tr>
<tr>
<td>33 ± 2</td>
<td>31 ± 1</td>
<td></td>
</tr>
<tr>
<td>Incorporation of C14</td>
<td>Triglyceride</td>
<td>Phospholipid</td>
</tr>
<tr>
<td>3.00 ± 0.25</td>
<td>5.90 ± 0.05</td>
<td>3.22 ± 0.10</td>
</tr>
<tr>
<td>6.27 ± 0.55</td>
<td>11 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>C14O2 production</td>
<td>15.32 ± 1.14</td>
<td>12.24 ± 0.87</td>
</tr>
</tbody>
</table>

After 15 min of preperfusion hearts were perfused for 60 min with buffer containing 5 nm glucose, 5 X 10^-4 M EDTA, 1% albumin, 0.5 mm palmitate, and palmitate-1-C14 (0.1 μc/ml). Epinephrine, in a concentration of 0.2 μg/ml, was added at the beginning of the experiment. Values represent the mean ± SE and are expressed as μmoles palmitate-1-C14/g heart, dry wt, incorporated into heart lipids, CO2, or disappearing from the perfusate. Glycerol is recorded as pmoles/g heart, dry wt, release in 60 min. The number of hearts in each group is indicated in parentheses. *P < .01 vs. control. †P < .05 vs. control.

**TABLE 3. Effect of epinephrine on glucose-U-C14 oxidation in the presence of palmitate or chylomicron tripalmitin**

<table>
<thead>
<tr>
<th>Palmitate, 0.2 mm</th>
<th>Chylomicron, 0.6 mm</th>
<th>Glucose</th>
<th>Lactate production</th>
<th>CO2 production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitate, 0.2 mm</td>
<td>Chylomicron, 0.6 mm</td>
<td>Glucose</td>
<td>Lactate production</td>
<td>CO2 production</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>80 ± 5</td>
<td>10 ± 4</td>
<td>43 ± 5</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>230 ± 10</td>
<td>94 ± 11</td>
<td>79 ± 5</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>48 ± 8</td>
<td>33 ± 5</td>
<td>5.97 ± 0.25</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>245 ± 13</td>
<td>21 ± 4</td>
<td>100 ± 3</td>
</tr>
</tbody>
</table>

Following 15 min of preperfusion with oxygenated buffer containing 5 nm glucose, hearts were perfused for 60 min in a recirculation apparatus containing 20 ml buffer with 5 nm glucose, 5 X 10^-4 M EDTA, 1% albumin and palmitate, chylomicron tripalmitin, and epinephrine, as indicated. Values shown in each group are the means of 6 rats ± SE and are expressed as μmoles glucose equivalents per g heart, dry wt.
lipid utilization

levels of cytoplasmic DPNH due to predominance of inhibition of pyruvate decarboxylation (I-tate formation was similar to that observed by William-
son (25). There are several interesting features of its cose in the presence of free fatty acids. The addition of effect on the metabolism of circulating lipid and on glu-\npere elevation in lactate production.

Despite enhanced oxidation, the theoretical contribu-
tion of glucose to the fuel of myocardial respiration is calculated to be less than 15%. Glycogen may have made

dry wt, thus confirming the observations of Shipp et al. (22). Epinephrine elevated glucose uptake and lactate production to rates that were equal to or greater than those observed in the absence of circulating lipid. The elevation of glucose uptake in the absence of palmitate suggested that epinephrine might have specifically stim-
ulated glucose utilization. In Fig. 1, the effect of epi-
nephrine on glucose-U-C^{14} oxidation in the presence and absence of palmitate is shown. Thirty minutes after the addition of epinephrine, C^{14}O_{2} production had increased six- to sevenfold over control rates in the presence of palmitate but only twofold in its absence. Although epinephrine had a relatively greater effect on glucose-
U-C^{14} oxidation in the presence of palmitate, C^{14}O_{2} production was only 25% of that observed when glucose alone was the substrate.

**DISCUSSION**

Chylomicron triglyceride and fatty acids were equally effective in decreasing glucose uptake by the perfused rat heart although not to levels observed in diabetes (13). The inhibitory effects of fatty acid on glucose uptake and glycolysis are thought to be mediated partially by elevation of citrate levels within the cell and inhibition of phosphofructokinase (9, 17), and partially from the inhibition of pyruvate decarboxylation (19). Elevated levels of cytoplasmic DPNH due to predominance of lipid utilization (16) may contribute to the moderate elevation in lactate production.

The effect of epinephrine on glucose uptake and lac-
tate formation was similar to that observed by William-
son (25). There are several interesting features of its effect on the metabolism of circulating lipid and on glu-
cose in the presence of free fatty acids. The addition of epinephrine in vitro did not increase C^{14}O_{2} production from either chylomicron tripalmitin-C^{14} or palmitate-1-
C^{14}. The studies of Williamson have demonstrated that 0.2 μg/ml epinephrine produced near maximal changes in oxygen consumption (6), glucose uptake, and oxida-
tion (25). If Williamson’s values for oxygen consumption can be applied to the present study, the contributions of both palmitate-1-C^{14} and chylomicron tripalmitin-C^{14} to the fuel of respiration of the perfused rat heart is calcu-
lated to have decreased from 96% to 18% on addition of epinephrine. This was unexpected since, under similar experimental conditions, Williamson had demonstrated that epinephrine increased glucose oxidation and oxygen consumption twofold and it might have been assumed that the relative contribution of all substrates to the fuel of respiration would remain unchanged. Epinephrine preferentially stimulated glucose-U-C^{14} oxidation in the presence of palmitate, although C^{14}O_{2} production was still significantly less than that observed when glucose alone was the substrate. The effect of epinephrine on myocardial glucose transport and phosphorylation has been thought to be secondary to changes in contractility (25), since epinephrine decreased glucose uptake and phosphorylation by rat diaphragm. Although Willi-

EMINEPHRINE AND MYOCARDIAL LIPID METABOLISM

**FIG. 1.** Effect of epinephrine on C^{14}O_{2} production from glucose-U-C^{14} in the presence and absence of palmitate. Hearts were perfused with buffer containing 5 mM glucose, 5 × 10^{-3} M EDTA, 1% albumin, 0.5 mM palmitate, and 0.1 μg/ml glucose-U-C^{14}. Epinephrine was added after 15 or 30 min of perfusion to give a circulating concentration of 0.2 μg/ml. Values are the means of 12 hearts until the addition of epi-
nephrine; thereafter, the means of 6 hearts. □ Represents control hearts, ○ represents epinephrine-
treated hearts.
up part of the deficit, yet the bulk of myocardial fuel must still have been derived from endogenous lipid (23, 26). Endogenous lipid utilization was enhanced as indicated by increased glycerol release. Since the myocardium utilized glycerol to a limited extent, glycerol release can only qualitatively reflect endogenous lipid turnover (13) and therefore cannot be used to calculate the quantitative contribution of endogenous lipid to the fuel of respiration as proposed by Randle et al. (18).

The increased recovery of palmitate-1-\(^{14}C\) in myocardial lipid without increased uptake is difficult to explain. However, since epinephrine stimulates endogenous lipid turnover and elevation of tissue FFA in rat diaphragm and heart (8), it is possible that the exchange between fatty acids derived from endogenous triglyceride and those in the medium was enhanced. This phenomenon occurs normally to a limited extent in the perfused rat heart (4, 22). The exaggerated decrease in palmitate-1-\(^{14}C\) specific activity is compatible with such an exchange and would offer an explanation for the increased recovery of palmitate-1-\(^{14}C\) in myocardial lipid without involving a net increase in FFA transport. If such were the case, a decreased specific activity of tissue FFA and their CoA derivatives would be expected and \(^{14}CO_2\) from labeled perfusate FFA would be decreased even though the actual \(^{14}CO_2\) production may have remained unchanged or increased. The diminished \(^{14}CO_2\) produced by epinephrine and also observed with hearts of alloxan-diabetic and starved rats in the preceding paper may be partially due to this phenomenon. However, this does not reconcile the difference between these data and those of Gousios and Felts (11) and Gold et al. (10). A 30% increase in \(^{14}CO_2\) production from palmitate-1-\(^{14}C\) has been demonstrated with both epinephrine and norepinephrine in the perfused rabbit heart (11). Since a recirculatory apparatus was not used, changes in fatty acid concentration were determined by A-V differences of lipid-bound radioactivity and changes in specific activity may not have been appreciated. Comparison with the results obtained from the in situ perfusion of dog hearts (10) is more difficult since the action of norepinephrine on peripheral FFA release also has to be considered. Despite a fall in palmitate-1-\(^{14}C\) specific activity due to the peripheral lipolytic action of norepinephrine and in \(^{14}CO_2\) production, the fraction of arterial palmitate-\(^{14}C\) appearing as \(^{14}CO_2\) increased, leading to the conclusion that norepinephrine increased free fatty acid oxidation (10). Also, because of methodology, a norepinephrine-induced exchange between myocardial and perfusate fatty acids would not be appreciated. It is of interest to note that these observations were made in the absence of any increase in oxygen consumption.

As with the hearts of diabetic rats, the uptake of chylomicron triglyceride did not correlate with that which might be expected from the levels of LPL observed following chronic administration of epinephrine to rats in vivo (1). The discrepancy may be due to the relatively brief in vitro nature of the present experiments since administration of a single dose of epinephrine in vivo also did not alter LPL content (1). Yet LPL content can be influenced acutely as demonstrated by the twofold increase following 60 min of exercise in rats (15). Rat heart has been shown to contain more than one lipase (2) and, since glycerol release was increased acutely, it is possible that epinephrine’s effect on myocardial lipolytic activity was mediated through activation of a specific lipase, similar to its mechanism of action on adipose tissue (24). A role for lipoprotein lipase in endogenous lipid turnover cannot be excluded, as it has been demonstrated that 70% of myocardial LPL is microsomal bound (1). Not only was there no increase in circulating triglyceride uptake, but hydrolysis was also unchanged. This may be due to the relatively low concentration of chylomicron triglyceride (equivalent to 50 mg/100 ml available in the perfusate), the high lipolytic activity of rat heart (5), or the release of lytic enzyme into the perfusate (3). In this regard, it would be of interest to determine whether hydrolysis of perfusate chylomicron triglyceride would continue after removal of the hearts from the perfusion apparatus.

In conclusion, the data suggest that epinephrine specifically increases glucose oxidation and endogenous lipid turnover in the isolated perfused rat heart but does not stimulate oxidation of exogenous triglyceride or fatty acid. Since fatty acid utilization by liver (21), skeletal muscle (7), and heart (4) is related to concentration, it is likely that epinephrine increases myocardial FFA uptake by virtue of its ability to elevate circulating FFA, rather than by a direct effect on myocardial lipid extraction. A similar conclusion has been reached by Gold and his co-workers (10).

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REFERENCES


