Effect of epinephrine on myocardial triglyceride and free fatty acid utilization

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Robert A. Kreisberg. 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 C14O2 production from glucose-U-C14 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 epinephrine: 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.

The increased metabolic demand produced by epinephrine in the perfused rat heart is accompanied by a twofold increase in oxygen consumption (6) and glucose oxidation (25), indicating that the relative contribution of glucose to the fuel of respiration is unchanged and that lipid remains the main energy source. The effect of epinephrine on the utilization of circulating lipid by the myocardium has not been clearly defined, although increased utilization of circulating triglyceride has been suggested from studies of myocardial lipoprotein lipase (LPL) in rats treated chronically with epinephrine (1). Recently Gousios and Fefts (11) and Gold et al. (10) have reported that epinephrine and norepinephrine increased myocardial extraction and oxidation of palmitate-1-C14.

These studies and those in the preceding paper were designed to study the utilization of circulating triglyceride and fatty acids (FFA) in conditions in which myocardial LPL is known to be elevated. In addition, the effect of lipid on the epinephrine-induced stimulation of glucose oxidation was also investigated.

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 × 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
TABLE 1. Myocardial tripalmitin-C<sup>14</sup> utilization: effect of epinephrine

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control (12)</th>
<th>Epinephrine (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfusion time (min)</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>C&lt;sup&gt;14&lt;/sup&gt; disappearance</td>
<td>50.5 ± 0.9</td>
<td>46 ± 1</td>
</tr>
<tr>
<td>C&lt;sup&gt;14&lt;/sup&gt; in perfusate</td>
<td>21.1 ± 1.0</td>
<td>17 ± 0.5*</td>
</tr>
<tr>
<td>Incorporation of C&lt;sup&gt;14&lt;/sup&gt; into heart as:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td>5.97 ± 0.73</td>
<td>12.10 ± 0.33†</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>1.93 ± 0.08</td>
<td>2.45 ± 0.13†</td>
</tr>
<tr>
<td>Intermediates</td>
<td>1.07 ± 0.06</td>
<td>1.01 ± 0.05</td>
</tr>
<tr>
<td>C&lt;sub&gt;14&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt; production</td>
<td>8.33 ± 0.57</td>
<td>9.42 ± 0.81</td>
</tr>
<tr>
<td>Total myocardial C&lt;sup&gt;14&lt;/sup&gt; 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 mm glucose, 5 x 10<sup>-8</sup> M EDTA, 1% albumin, and 0.5 mm chylomicron tripalmitin-C<sup>14</sup>. Epinephrine was added to a concentration of 0.2 μg/ml. Values represent the mean ± SE and are expressed as μmoles/g heart, dry wt, of tripalmitin-C<sup>14</sup> incorporated into heart lipids, CO<sub>2</sub>, and perfusate FFA or disappearing from the perfusate. 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.

Bleomycin and palmitate on glucose uptake and lactate production is shown in Table 2. The effect of epinephrine on plasma lipids and intermediates is 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 C<sup>14</sup> between the two groups cannot account for the differences in TP-C<sup>14</sup> uptake.

**Palmitate-1-C<sup>14</sup> metabolism.** The effect of epinephrine on palmitate-1-C<sup>14</sup> 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-C<sup>14</sup> uptake determined by the decrease in perfusate C<sup>14</sup> counts remained unchanged. The perfusate FFA specific activity decreased 32% (vs. 16% for control hearts). Conversion of palmitate-1-C<sup>14</sup> to C<sub>14</sub>O<sub>2</sub> decreased significantly. Palmitate-1-C<sup>14</sup> recovery in myocardial triglyceride and phospholipid increased from 3.00 ± 0.25 to 6.77 ± 0.53 and from 15.0 ± 0.65 to 3.11 ± 0.10 μmoles/g heart, dry wt, respectively. This increase is comparable with that observed with TP-C<sup>14</sup> under similar conditions (Table 1). Palmitate-1-C<sup>14</sup> 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-C<sup>14</sup>, 2.50 ± 0.10 μmoles glycerol/g heart, dry wt per hour were released by the perfused heart (Table 2). Epinephrine produced a two- to threefold increase in glycerol release in the presence of these substrates.

**Glucose metabolism.** The effect of chylomicron triglycer-
lipid utilization levels of cytoplasmic DPNH due to predominance of inhibition of pyruvate decarboxylation (1). Glucose production 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 elevation in lactate production.

**DISCUSSION**

Chylomicron triglyceride and fatty acids were equally effective in decreasing glucose uptake by the perfused rat heart although not to levels observed in diabetics (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 lactate 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 C14O2 production from either chylomicon tripalmitin-C14 or palmitate-1-C14. The studies of Williamson have demonstrated that 0.2 µg/ml epinephrine produced near maximal changes in oxygen consumption (6), glucose uptake, and oxidation (25). If Williamson’s values for oxygen consumption can be applied to the present study, the contributions of both palmitate-1-C14 and chylomicron tripalmitin-C14 to the fuel of respiration of the perfused rat heart is calculated to have decreased from 36% 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-1-C14 oxidation in the presence of palmitate, although C14O2 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-amson has demonstrated that epinephrine increases the concentrations of all intracellular intermediates of the anaerobic glycolytic pathway (25), the results of the present studies would indicate that this stimulatory effect was not sufficient to completely reverse the inhibition of glyco-lysis produced by palmitate. The data, however, do suggest that epinephrine may have an effect on glucose transport that is distinct from its inotropic action.

Despite enhanced oxidation, the theoretical contribution of glucose to the fuel of myocardial respiration is calculated to be less than 15%. Glycogen may have made
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-C\textsuperscript{14} 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-C\textsuperscript{14} specific activity is compatible with such an exchange and would offer an explanation for the increased recovery of palmitate-1-C\textsuperscript{14} 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 C\textsuperscript{14}O\textsubscript{2} from labeled perfusate FFA would be decreased even though the actual CO\textsubscript{2} production may have remained unchanged or increased. The diminished C\textsuperscript{14}O\textsubscript{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 C\textsuperscript{14}O\textsubscript{2} production from palmitate-1-C\textsuperscript{14} 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-C\textsuperscript{14} specific activity due to the peripheral lipolytic action of norepinephrine and in C\textsuperscript{14}O\textsubscript{2} production, the fraction of arterial palmitate-C\textsuperscript{14} appearing as C\textsuperscript{14}O\textsubscript{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


