Utilization in vitro and in vivo of glucose and glycerol by rat lung

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SCHOLZ, RICHARD W., BRIAN M. WOODWARD, AND RODNEY A. ROHADES. Utilization in vitro and in vivo of glucose and glycerol by rat lung. Am. J. Physiol. 223(4): 991-996. 1972.—Glucose-U-14C and glycerol-2-14C utilization by lung tissue in vitro and in vivo was investigated in normal and fasted (72 hr) rats. In the in vitro studies, glycerol oxidation to CO₂ and incorporation into various lipid components by lung slices was increased as the medium glycerol concentration was increased from 1 to 25 mM. The addition of 5 mM glucose to the incubation medium decreased the apparent utilization of glycerol, suggesting that glucose and glycerol share a common metabolite pool in rat lung. Fasting rats for 72 hr reduced glucose utilization by lung slices, whereas glycerol utilization was unaffected. In the in vivo studies, glucose incorporation into lung lipids was reduced significantly in the fasted rats as compared with fed controls. The incorporation in vivo of glycerol-2-14C into lung lipids was increased significantly in the fasted rats at a time when plasma glycerol levels were increased threefold over control levels. The results suggest that rat lung is capable of altering its metabolism to maintain biosynthesis of its essential lipid components during food deprivation.

METHODS

Animals. Male Long-Evans hooded rats averaging approximately 250-300 g were used in all experiments. This strain of rat was selected because of its low incidence of respiratory disease (25). Upon receipt the animals were placed into individual stainless steel cages with raised wire floors and subsequently were fed a standard pellet diet for an acclimation period of 2 weeks. The rats were maintained in a controlled environment; 22°C, 50% relative humidity, and a 12-hr light-dark cycle, respectively. Fasted rats were deprived of food for 72 hr. All rats were offered water ad libitum.

In vitro techniques with lung slices. The procedures for the procurement of lung tissue, preparation of tissue slices, incubation conditions, and procedures for extracting, isolating, and counting the radioactivity in the various lipid fractions and 14CO₂ were as described previously (24). In vivo techniques with rat lung. The rats were anesthetized with sodium pentobarbital, 3 mg/100 g body wt ip, and the hair on one leg was removed with clippers. Tracer levels of glucose-U-14C (5 μc, specific activity 15.6 μc/μmole) or glycerol-2-14C (7.5 μc, specific activity 13.6 μc/μmole) were administered via a saphenous vein in a volume of 0.3 ml of 0.85% NaCl. The rat was placed in a glass jar through which CO₂-free air was drawn at a rate of 540 ml/min. The expired CO₂ was collected for 15 min in three glass-impinging tubes arranged in series, each containing 20 ml of 1 N NaOH. After 15 min the rat was removed from the jar and killed by exsanguination and thoracotomy. The lungs were perfused with calcium-free Krebs-Ringer bicarbonate buffer, pH 7.4, via the right ventricle while the heart was still actively beating. The heart and lungs were removed en bloc and the lungs were
trimmed of extraneous tissue, blotted, and weighed to the nearest 10 mg. The entire lung was minced and then homogenized on ice in 10 ml of chloroform-methanol (2:1 v/v) with an all glass tissue homogenizer. The time lapse from removing the rat from the glass jar to homogenizing the minced lung generally was 5 min. The isolation and quantitation of the various lung lipid components were as described previously (24).

**Plasma glucose determination.** Blood was collected from additional control and fasted rats into centrifuge tubes containing sodium fluoride and potassium oxalate. Plasma was separated by centrifugation and glucose levels were determined enzymatically using glucose oxidase and peroxidase (2).

**Plasma glycerol determination.** Glycerol was determined enzymatically in rat plasma with glycerol kinase and α-glycerophosphate dehydrogenase by the method of Wieland (29) as modified by Larsen (18).

**Plasma free fatty acid determination.** An automated procedure (5) of the original methods of Antonis (1) and Itaya and Ui (15) was used for determining plasma free fatty acid levels.

**Radioactivity counting conditions.** Liquid scintillation counting of radioactivity in the various lung lipid fractions and in the 14CO2 collected during incubation of lung slices was performed as described previously (24). Counting efficiency was determined by the channels-ratio method. Radioactivity in the 14CO2 collected in 1 n NaOH during the in vivo studies was determined by placing 1 ml aliquots into scintillation vials and adding 15 ml of Bray's solution (3). Counting efficiency was determined by internal standardization with NaH14CO3. All radioactivity counting was performed in a Nuclear-Chicago liquid scintillation spectrometer.

**Statistical treatment of data.** Comparisons of two treatment means for significant differences were made with the Student t test.

**Materials.** Glucose U-14C and glycerol 2-14C were purchased from New England Nuclear Corp. The radioactivity from each compound was found to migrate as a single spot when subjected to thin-layer chromatography with ethanol-25% NH4OH-H2O (100:16:12, by vol) and corresponded to authentic glucose and glycerol, respectively. Enzymes and cofactors used for the plasma glucose and glycerol determinations were purchased from Sigma Chemical Company. All other reagents were of reagent grade quality.

**RESULTS**

The time course of glycerol-2-14C oxidation to CO2 and its incorporation into total lipid and phospholipid fractions of rat lung slices is shown in Fig. 1. After an initial lag period, the incorporation of glycerol into these fractions was linear with time for at least 3 hr. Subsequent incubations were for 2 hr, unless indicated otherwise.

The oxidation of glycerol-2-14C to CO2 and its incorporation into the total lipid fraction of lung slices as a function of glycerol concentration are shown in Fig. 2. The data show an increased utilization of glycerol by rat lung slices as the concentration of glycerol in the incubation medium was increased from 1 to 25 mM. The utilization of glycerol at an incubation medium concentration of 1 mM was linear with time for 90 min and the rate of utilization shown in Fig. 2 was determined during this time. The addition of 5 mM glucose to the incubation medium reduced the apparent utilization of glycerol by lung slices at each level of glycerol, suggesting that glycerol and glucose share a common metabolite pool in rat lung. The distribution of total lung lipid radioactivity (Fig. 2) between the phospholipid and neutral lipid fractions is shown in Fig. 3. The data show that glycerol preferentially was incorporated into the phospholipid fraction, as compared with the neutral lipid fraction, of lung lipids at each concentration of glycerol in the incubation medium. The addition of 5 mM glucose to the medium reduced the apparent incorporation of glycerol into lung phospholipids at each level of glycerol; however, this effect was less apparent for the neutral lipid fraction.

The utilization of glucose-U-14C and glycerol-2-14C by lung slices of fed and fasted rats is shown in Table 1. With both glucose and glycerol as lung lipid precursors, greater than 70% of the total lipid radioactivity was present in the phospholipid fraction. Fasting rats for 72 hr reduced significantly (P < 0.03) glucose utilization by rat lung slices as assessed by its oxidation to CO2 and incorporation into the total lipid fraction of lung slices.
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FIG. 3. Glycerol-2-\(^{14}\)C incorporation into phospholipid (PL) and neutral lipid (NL) fractions by rat lung slices as a function of glycerol concentration in medium with (+) or without (−) addition of 5 mM glucose.

TABLE 1. Utilization of glucose-\(^{14}\)C and glycerol-2-\(^{14}\)C by lung slices of fed and fasted rats

<table>
<thead>
<tr>
<th>Radioactive Fraction</th>
<th>Glucose Incorporation, nmole/100 mg per 2 hr</th>
<th>Glycerol Incorporation, nmole/100 mg per 2 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fed ad lib.</td>
<td>Fasted 72 hr</td>
</tr>
<tr>
<td>Total lipid</td>
<td>71.3±8.9</td>
<td>42.1±9.1†</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>51.6±6.9</td>
<td>30.9±6.1†</td>
</tr>
<tr>
<td>Neutral lipid</td>
<td>12.3±1.1</td>
<td>5.6±0.5†</td>
</tr>
<tr>
<td>Free fatty acid</td>
<td>0.90±0.15</td>
<td>0.29±0.06†</td>
</tr>
<tr>
<td>Nonsaponifiable</td>
<td>1.21±0.25</td>
<td>0.31±0.15†</td>
</tr>
<tr>
<td>CO(_2)</td>
<td>287±19.9</td>
<td>189.5±11.0†</td>
</tr>
</tbody>
</table>

Values are means ± SE of 3 observations each. Lung slices from individual rats were incubated in replicate. *Lung slices weighing approximately 100 mg were incubated for 2 hr at 37°C in 4 ml of calcium-free Krebs-Ringer bicarbonate buffer, pH 7.4, containing 2 µC of glucose-\(^{14}\)C, and 20 µmoles of glucose. †Lung slices were incubated for 2 hr at 37°C in 4 ml of calcium-free Krebs-Ringer-bicarbonate buffer, pH 7.4, containing 2 µC of glycerol-2-\(^{14}\)C, 20 µmoles of glycerol, and 20 µmoles of glucose. ††Statistically different from the corresponding value for control rats fed ad libitum, \(P<0.05\).

into the various lipid fractions. Glycerol utilization by lung slices, unlike glucose utilization, was not reduced in the fasted rats as compared with the fed controls.

The distribution of the phospholipid radioactivity following incubations with glucose-\(^{14}\)C and glycerol-2-\(^{14}\)C (Table 1) between the glycerol and fatty acid moieties is shown in Table 2. The data show considerable lipogenesis from glucose in lung slices, with approximately 40% of the total phospholipid radioactivity present in the fatty acid moiety. Fasting decreased significantly \((P<0.05)\) glucose incorporation into lung phospholipids, although the reduction proportionally was greater for the fatty acid fraction as compared with the glycerol fraction. These results are consistent with our earlier observations (24). Lipogenesis from glycerol was negligible as most of the phospholipid radioactivity was present in the phospholipid glycerol fraction.

The results on the utilization of glucose-\(^{14}\)C and glycerol 2-\(^{14}\)C in vivo by fed and fasted rats are presented in Table 3. Fasting for 72 hr resulted in significant reductions \((P<0.05)\) in glucose-\(^{14}\)C incorporation in vivo into various lung lipid components and oxidation to CO\(_2\) when compared with the fed controls. These findings agree favorably with the in vitro data (Table 1). The in vivo incorporation of glycerol-2-\(^{14}\)C into the total lipid, phospholipid, and neutral lipid fractions was increased significantly \((P<0.05)\) in lung tissue of fasted rats as compared with the fed controls. These findings are in contrast to the in vitro data (Table 1) that show no differences in glycerol utilization between fed and fasted rats. The oxidation in vivo of glycerol-2-\(^{14}\)CO\(_2\) was reduced significantly \((P<0.05)\) in fasted rats as compared with the fed controls (Table 3).

The distribution of the phospholipid radioactivity from the in vivo studies between the glycerol and fatty acid moieties is shown in Table 4. With glucose-\(^{14}\)C as tracer, approximately 75% of the lung phospholipid radioactivity in fed rats appeared in the glycerol moiety. Fasting for 72 hr abolished lipogenesis from glucose as essentially all the radioactivity was present in the glycerol moiety of lung phospholipids. With glycerol-2-\(^{14}\)C as tracer, greater than 85% of the lung phospholipid radioactivity in fed rats appeared in the glycerol moiety. Lipogenesis from glycerol-2-\(^{14}\)C in vivo was negligible in rat lung.

The data in Table 5 indicate marked differences between fed and fasted rats in plasma levels of potential lung phospholipid precursors. The data show that fasted rats have a significant reduction \((P<0.05)\) in plasma glucose and significant increases \((P<0.05)\) in plasma glycerol, plasma

TABLE 2. Distribution of phospholipid radioactivity between glycerol and fatty acid moieties after incubation of lung slices with glucose-\(^{14}\)C and glycerol-2-\(^{14}\)C

<table>
<thead>
<tr>
<th>Radioactive Fraction</th>
<th>Glucose Incorporation, nmole/100 mg per 2 hr</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fed ad lib.</td>
<td>Fasted 72 hr</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>51.6±6.9</td>
<td>30.9±6.1†</td>
</tr>
<tr>
<td>Phospholipid glycerol</td>
<td>32.3±3.3</td>
<td>20.2±4.2†</td>
</tr>
<tr>
<td>Phospholipid fatty acids</td>
<td>21.1±3.7</td>
<td>8.7±3.5†‡</td>
</tr>
</tbody>
</table>

Values are means ± SE of 5 observations each. Refer also to Table 1 legend.

TABLE 3. Utilization of glucose-\(^{14}\)C and glycerol 2-\(^{14}\)C in vivo by fed and fasted rats

<table>
<thead>
<tr>
<th>Radioactive Fraction</th>
<th>Glucose Incorporation, dpm X 10^3/g per 15 min</th>
<th>Glycerol Incorporation, dpm X 10^3/g per 15 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fed ad lib.</td>
<td>Fasted 72 hr</td>
</tr>
<tr>
<td>Total lipid</td>
<td>1.68±0.13</td>
<td>1.05±0.28†</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>0.06±0.14</td>
<td>0.59±0.15*</td>
</tr>
<tr>
<td>Neutral lipid</td>
<td>0.35±0.01</td>
<td>0.29±0.05*</td>
</tr>
<tr>
<td>Free fatty acid</td>
<td>0.03±0.00*</td>
<td>0.007±0.002†</td>
</tr>
<tr>
<td>Nonsaponifiable</td>
<td>0.08±0.008</td>
<td>0.05±0.005†</td>
</tr>
<tr>
<td>CO(_2)</td>
<td>213±34.8</td>
<td>50±28.4*</td>
</tr>
</tbody>
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Values are means ± SE of 5 observations each. Rats were injected intravenously with either 5 µC of glucose-\(^{14}\)C or 7.5 µC of glycerol-2-\(^{14}\)C and were killed after 15 min. Subsequent procedures are described in the Materials section. *Statistically different from the corresponding value for control rats fed ad libitum, \(P<0.05\). †Values for radioactive CO\(_2\) represent dpm X 10^3/g per 15 min.
fraction revealed that fasting decreased in particular glu-
cose incorporation into phospholipid fatty acids. These
results agree with our earlier studies with rat lung slices
and by the whole lung in vivo. Plasma glucose also was
depressed significantly (P < 0.05) in the starved rats as
compared with the fed controls. Although measurements of
glucose turnover were not undertaken in the present studies,
it is assumed that glucose utilization is reduced markedly
in the fasting state. Since an equal amount of glucose-UH4C
was administered intravenously to both the fed and fasted
rats, the specific activity of the labeled glucose would be
considerably higher for the fasted rats. Therefore the re-
duction in glucose utilization by the starved rats may be
even greater than is apparent from the isotope data.

The results on the utilization of glycerol by rat lung in
vivo suggest that its incorporation into lung lipids is in-
creased in the fasting state at a time when its oxidation to
CO2 by the whole animal is decreased. Glycerol utilization
by rat lung slices was not altered by the 72-hr period of
starvation. These results may suggest discrepancies be-
cause of a lack of knowledge concerning metabolite fluxes and pool
sizes within specific tissues. A further problem associated with
the interpretation of the in vivo results is that fluctuations in circulating levels of glucose, glycerol, and fatty
acids also occur, depending upon the physiological state of
the animal (Table 5).

In general, the relative utilization by rat lung of glucose
in the in vitro and in vivo studies was similar. Starving
rats for 72 hr decreased significantly (P < 0.05) glucose
incorporation into phospholipid fatty acids by lung slices
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Interpretation of the in vivo studies with glycerol-2-\textsuperscript{14}C is also made difficult because of the extremely rapid turnover rate of plasma glycerol in the intact animal (10). It is likely that a portion of the glycerol-2-\textsuperscript{14}C administered intravenously was converted to glucose. That glycerol-2-\textsuperscript{14}C per se, rather than glucose, was metabolized by lung tissue initially in the in vivo studies with glycerol-2-\textsuperscript{14}C becomes apparent when the following points are considered: 1) the absolute level of radioactivity in lung lipids was much higher with glycerol, rather than glucose, as tracer. Although more glycerol radioactivity was administered intravenously than glucose radioactivity (7.5 \( \mu \)C/rat as compared with 5.0 \( \mu \)C/rat), this difference could not explain the higher levels of radioactivity incorporated with glycerol as radioactive tracer. 2) The distribution of radioactivity in lung glycerides differed depending upon the radioactive tracer employed. Approximately 25% of the phospholipid radioactivity was present in the fatty acid fraction 15 min following the intravenous administration of glucose-U\textsuperscript{-14}C. With glycerol-2-\textsuperscript{14}C as tracer, however, nearly all the radioactivity was incorporated into the phospholipid glycerol portion of lung phospholipids. It is emphasized that plasma glycerol levels are increased threefold during the 72-hr starvation period. Since glycerol incorporation into lung lipids was increased in the fasted rats, these results suggest the possibility of an important role of glycerol in the metabolism of this tissue.

The precise biochemical pathways by which glycerol is utilized in rat lung remain unknown at present. We have been unable in this laboratory to detect lung glycerol kinase activity as measured spectrophotometrically (16) or by a more sensitive radioassay (20). It was shown in the present studies that glucose decreased the incorporation of glycerol in vitro into lung lipids. These results suggest that a metabolite pool, perhaps at the level of triose phosphate, is common to both glucose and glycerol in rat lung. Whether L-\( \alpha \)-glycerol phosphate serves as the direct link between glucose and glycerol metabolism in rat lung remains to be investigated.

In addition to glucose, glycerol, and acetate utilization for phospholipid synthesis, mammalian lung also actively incorporates pre-formed fatty acids into its glycericidic components (4, 7, 11, 23, 31). In this context, Heinemann (13) has demonstrated a lipase activity in the perfused rabbit lung. The results of the present experiments demonstrate that rat lung is capable of altering its metabolism of glucose and glycerol during a 72-hr period of starvation. This suggests a capacity for metabolic adaptation whereby the biosynthesis of essential lung lipid components from various precursors is maintained during various physiological states. This suggestion is strengthened further by our (unpublished) observation of unchanged turnover of whole-lung lecithin from fasted rats as compared with fed rats.

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