Effect of Phenazine Methosulfate on Lipogenesis*

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SUMMARY

The effect of phenazine methosulfate (PMS) on lipogenesis of rat epididymal fat pad tissue was studied. PMS at concentrations of $10^{-4}$ to $10^{-5}$ M stimulates L-lactate oxidation and, most markedly, fatty acid synthesis, without affecting glycerol synthesis. Maximal stimulation of lipogenesis occurs at about $10^{-5}$ M. With tissue of rats fed ad libitum, increase in fatty acid ranged from 300 to 1800%. There was no stimulation of synthesis from pyruvate or glucose. Similar effects of PMS on lipogenesis were also seen with mammary gland slices of lactating rats. It is concluded that there is limited capability for the transfer of cytoplasmic reducing equivalents into mitochondria. The effect of PMS is the result of oxidation of excess cytoplasmic reducing equivalents and regeneration of DPN.

Adipose tissue from the epididymal fat pad of fasted refed rats utilizes lactate and pyruvate equally well for the synthesis of fatty acids (1). Under conditions less favorable for lipogenesis such as with tissue of rats fed a commercial pellet diet containing about 5% fat, pyruvate is better utilized than lactate, and pyruvate serves as a much better substrate for fatty acid synthesis than lactate (1, 2). Pyruvate is metabolized by tissue of fasted rats, but uptake of lactate is virtually abolished (1, 2).

We have shown that the impaired fatty acid synthesis from lactate can be markedly increased by a number of compounds (e.g. aspartate + α-ketoglutarate, α-ketobutyrate) that may serve as hydrogen acceptors for DPNH formed in the cytoplasm.

We have proposed that the block in lactate utilization is caused by the lack of a mechanism for transferring reducing equivalents from the cytoplasm to mitochondria. If the rate of utilization of reduced pyridine nucleotides for biosynthesis is less than the rate of lactate oxidation to pyruvate, DPNH cannot be oxidized, and lactate metabolism is blocked. However, alternate theories to explain the stimulation by added substrates are possible. We report here a very marked stimulation of fatty acid synthesis from lactate, but not from pyruvate, by low concentrations of phenazine methosulfate. Similar findings were observed also with lactating mammary gland. The findings provide strong support for our hypothesis (1).

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EXPERIMENTAL PROCEDURE

Segments of epididymal fat pad tissue (200 to 400 mg) or slices of mammary gland (150 to 200 mg) from rats 14 to 18 days after parturition were used. The tissue was incubated in 2 ml of Krebs-Henseleit bicarbonate buffer in an atmosphere of 95% O2-5% CO2. Substrate concentration was either 5 or 10 mM. Compounds uniformly labeled with 14C were purchased from New England Nuclear or Amersham-Searle (Chicago), and other chemicals, from Sigma. Pyruvate uniformly labeled with 14C was purchased in sealed evacuated tubes containing 5 μCi and only freshly opened material was used. The utilization of the substrate and formation of lactate and pyruvate were assayed enzymically. Incorporation of 14C into CO2, fatty acids, and lipid glycerol was determined. The experimental procedures have been previously described (1).

RESULTS

In adipose tissue of rats weighing 150 to 200 g, fed ad libitum on a commercial pellet diet, the uptake of lactate ranges from 1 to 5 μmoles per g per 3 hours. The major product is CO2, and only 10 to 15% of the lactate carbon is incorporated into fatty acids. Addition of PMS markedly increased both lactate uptake and incorporation into fatty acids (Fig. 1). Concentrations of 0.001 mM were stimulating, and maximal fatty acid synthesis was attained at between 0.005 and 0.01 mM PMS. Concentrations above 0.02 mM became inhibitory. Similar stimulation was obtained with methylene blue, but at concentrations 2 to 4 times those of PMS.

In Table I, the effects of PMS on the metabolism of lactate, pyruvate, and glucose are compared. Glucose oxidation to CO2 was increased up to concentrations of 0.05 to 0.1 mM, but incorporation into fatty acids was not affected. The stimulation of the CO2 production is caused by a large increase in the pentose cycle and will be described in detail elsewhere. The stimulation of lactate oxidation ranged from 2- to 5-fold, and of fatty acid synthesis, from 3- to 18-fold. The extent of stimulation depends probably to a large extent on the nutritional status of the tissue and is most pronounced when lactate metabolism is greatly impaired. At optimal concentrations of PMS when incorporation into fatty acids from lactate was increased many fold, pyruvate metabolism was not affected. With these concentrations, fatty acid synthesis from lactate became nearly equal to that from pyruvate. The fatty acid synthesis is approximately equal to that from glucose without added insulin.

In tissue from rats fasted for a prolonged period (2 days), 1 The abbreviation used is: PMS, phenazine methosulfate.
TABLE I

Effect of PMS on metabolism of rat adipose tissue

Table pool from 10 rats, 200 mg of tissue per flask, was incubated in 2 ml of buffer. Each value is the average of duplicates. Concentration of substrates, 5 mM. Substrates are uniformly labeled.

| Substrate | PMS | Incorporation into CO₂ Fatty acids Lipid glycerol μM Patoms/g/3 hrs Patoms/g/3 hrs Patoms/g/3 hrs |
|-----------|-----|-----------------|-----------------|-----------------|
| Glucose   | 0   | 22              | 15              | 13              |
|           | 10  | 35              | 17              | 16              |
|           | 50  | 55              | 15              | 15              |
| Pyruvate  | 0   | 56              | 18              | 8               |
|           | 10  | 58              | 16              | 7               |
|           | 50  | 55              | 7               | 5               |
| Lactate   | 0   | 11              | 2               | 1               |
|           | 10  | 32              | 13              | 9.5             |
|           | 50  | 37              | 7               | 2.5             |

TABLE II

Effect of PMS on pyruvate and lactate metabolism in fasted and fasted refed rats

Rats were fasted for 2 days and refed for 2 days. Concentration of substrates was 5 mM in experiments with tissue from fasted rats and 10 mM in experiments with tissue from refed rats. PMS, when present, 0.01 mM.

| Condition | PMS | Pyruvate | Lactate |
|-----------|-----|----------|---------|
|           |     | Uptake   | CO₂ Fatty acids Glycerol | Uptake Pyruvate formed | CO₂ Fatty acids Glycerol |
|           | μmol/g/3 hrs | μmol/g/3 hrs | μmol/g/3 hrs | μmol/g/3 hrs | μmol/g/3 hrs |
| Fasted    | -   | 14       | 5.4     | 21     | 0.5     | 4.0     | 0.5     | 0.1     | 1       | 1       | 0       |
|           | +   | 11       | 3.5     | 20     | 0.2     | 2.2     | 10      | 4.5     | 13      | 0.3     | 2.9     |
| Fasted and refed | -   | 64       | 8.0     | 80     | 79      | 2.5     | 63      | 1.6     | 76      | 103     | 2.2     |
|           | +   | 67       | 4.4     | 80     | 81      | 3.0     | 59      | 6.0     | 78      | 100     | 1.9     |

FIG. 1. Effect of phenazine methosulfate concentration on lactate metabolism by rat adipose tissue.

TABLE III

Effect of PMS on metabolism of mammary gland

Mammary gland of one rat 19 days after parturition. Slices, 200 mg, were incubated with 2 ml of buffer. Concentration of substrates was 10 mM. Averaged for duplicate incubations, which agreed closely.

| PMS | Glucose | Pyruvate | Lactate |
|-----|---------|----------|---------|
|     | CO₂ Fatty acids Lipid glycerol | CO₂ Fatty acids Lipid glycerol | CO₂ Fatty acids Lipid glycerol |
| μM  | Patoms/g/3 hrs Patoms/g/3 hrs | Patoms/g/3 hrs | Patoms/g/3 hrs | Patoms/g/3 hrs |
| 0   | 100      | 164      | 26      | 113     | 11      | 5.2     | 32      | 2.7     | 1.9     |
| 5   | 165      | 102      | 26      | 131     | 12      | 3.8     | 94      | 44      | 4.2     |
| 25  | 186      | 161      | 27      | 130     | 7.8     | 3.0     | 63      | 22      | 2.8     |

PMS restored oxidation of lactate to the level of pyruvate (Table II). On the other hand, in tissue of fasted rats refed for 2 days on a high carbohydrate diet, PMS was without any effect (Table II). Under these conditions fatty acid synthesis from lactate exceeds that from pyruvate.

Stimulation of lactate metabolism of lipogenesis by PMS occurred also in slices of mammary gland of lactating rat. The tissue has a large capacity for fatty acid synthesis; however, it incorporates glucose and other substrates also in amino acids and lactose. In mammary gland, lactate is oxidized to some extent, but fatty acid synthesis is negligible. A detailed study of mammary gland metabolism will be reported elsewhere. However, PMS stimulates fatty acid synthesis from lactate 15 to 20 times at concentrations that have no effect on fatty acid synthesis from glucose and pyruvate (Table III). The fatty acids from lactate in the presence of PMS exceeded that from pyruvate 2- to 4-fold but were much less than from glucose.

DISCUSSION

We have previously shown (3) that in rat adipose tissue all the reducing equivalents for lipid synthesis from glucose are generated in the cytoplasm. Balance of reducing equivalents indicated that very little if any of the reducing equivalents are transferred into mitochondria. If fatty acid synthesis is limited as in the fasted animal, the hydrogen equivalents generated from glucose via the glyceraldehyde dehydrogenase are used up in the formation of lactate and glycerol. A limited outflow of reducing...
equivalents from cytoplasm to mitochondria may occur in the presence of dinitrophenol (4). This transfer is limited by the supply of oxalacetate formed by citrate cleavage. Excess oxalacetate, occurring in the presence of dinitrophenol, is reduced to malate, which presumably penetrates the mitochondrial membrane. Under normal conditions such malate is decarboxylated by the malic enzyme to supply TPNH.

The findings with glucose and the results with lactate (1) lead us to the conclusion that rat adipose tissue lacks a shuttle for the transfer of reducing equivalents from cytoplasm to mitochondria. This hypothesis is greatly strengthened by the current experiments with PMS. The dye acts as a hydrogen acceptor from DPNH and is reoxidized by the dissolved oxygen in the medium. Thus reoxidation of DPNH is uncoupled from reductive biosynthesis. Within a fairly narrow range of PMS concentration, the degree of uncoupling is sufficient to dispose of the “excess” of reducing equivalents and regenerate DPN at a proper rate. At higher PMS concentrations competition between dye and reductive biosynthesis occurs, with inhibition of biosynthesis. Rat adipose tissue contains mitochondrial α-glycerophosphate oxidase (5) and a very active cytoplasmic dehydrogenase. In spite of this, the α-glycerophosphate shuttle does not operate. While such a shuttle is a major pathway in insect flight muscle (6), its operation in mammalian tissue is not established.

These studies illustrate the value of comparative studies of lactate and pyruvate to elucidate the pathways of transport of reducing equivalents. The role of such transport in metabolic regulation has not yet received sufficient attention.

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