BIOLOGY OF REPRODUCTION 26, 445–455 (1982)

Metabolism of Round Spermatids from Rats: Lactate as the Preferred Substrate[1]

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ABSTRACT

Round spermatids were prepared from rat testes and incubated with various substrates (glucose, fructose, pyruvate, lactate and acetate) to measure utilization of substrates and production of ATP in the presence of saturating levels of each substrate. By both criteria lactate is the preferred substrate by a factor of 3 or 4. Production of more than half of the ATP with lactate as substrate is prevented by addition of an inhibitor of α-ketoacid dehydrogenase (5-methoxyindole-2-carboxylic acid). Pyruvate and lactate are interconverted and pyruvate inhibits production of ATP from lactate. Synthesis of ATP with lactate and with pyruvate is inhibited by rotenone, rutamycin or 2,4-dinitrophenol. Utilization of glucose is limited by aldolase activity. These findings suggest that exogenous lactate is oxidized by lactate dehydrogenase followed by pyruvate dehydrogenase and Krebs' cycle enzymes under conditions which do not allow pyruvate to inhibit lactate dehydrogenase. ATP is synthesized through electron transport. Post-mitochondrial supernate from spermatids showed that high concentrations of pyruvate (>1 mM) inhibit lactate dehydrogenase with pyruvate as substrate and that with lactate as substrate, pyruvate behaves as a competitive inhibitor of lactate dehydrogenase. Evidently lactate is the preferred substrate for round spermatids and energy production is most efficient when this substance is present in high concentrations and pyruvate is present in low concentrations. Reasons are given for suggesting that Sertoli cells may provide the relatively large amounts of lactate required by round spermatids.

INTRODUCTION

It has been known for some time that the testis shows an unusual dependence on glucose as a source of energy (Waites and Setchell, 1964). Earlier reports from this and other laboratories suggested that spermatids may be at least partly responsible for the high utilization of glucose by the testis (Davis and Firlit, 1965; Means and Hall, 1968). Moreover, glucose has been shown to protect spermatids from the inhibitory influence of body (as opposed to scrotal) temperature on protein synthesis (Davis and Firlit, 1965; Nakamura and Hall, 1976). While studying transport of glucose by fractions of germ cells in vitro (Hall and Nakamura, 1981), we were struck by the poor utilization of glucose by isolated spermatids in vitro. Since the metabolism of mature sperm is complex and differs in a number of important respects from that of other mammalian cells (Melrose and Terner, 1953; Van Dop et al., 1977) it was decided to study the utilization of various substrates by round (immature) spermatids in vitro.

MATERIALS AND METHODS

Preparation and Incubation of Cells

The following studies were performed with immature or round spermatids prepared by centrifugal elutriation from rats aged 40 days, as described previously (Nakamura et al., 1978). This reference describes yields, characterization of the cells and the methods used to isolate the cells. Spermatids (10^6 cells) were incubated in phosphate buffered saline (0.3 ml) (Nakamura et al., 1978) with additions described in the text. Incubation was stopped by addition of perchloric acid. Cells were homogenized in a glass/teflon homogenizer and centrifuged at 2,500 × g for 10 min at 4°C. The supernate was taken to pH 6.5 with KOH (2N). The neutralized supernate was used for measurement of ATP and glycolytic intermediates.

Assays of ATP and Glycolytic Intermediates

The following methods were used to assay the intermediates: glucose (Bergmeyer et al., 1974);

Accepted December 17, 1981.
Received November 5, 1981.
[1]This investigation was supported by Grant PCM-801-5511, awarded by the National Science Foundation.
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fructose (Bernt and Bergmeyer, 1974); glucose-6-phosphate and fructose-6-phosphate (Long and Mickal, 1974); fructose-1,6-diphosphate, dihydroxyacetone phosphate and glyceraldehyde-3-phosphate (Racker, 1974); 1,3-diphosphoglyceric acid (Negelein, 1974); 3-phosphoglyceric acid (Czok, 1974); pyruvate, phosphoenol pyruvate and 2-phosphoglyceric acid (Czok and Lamprecht, 1974); lactate (Gutman and Wahlefeld, 1974) and ATP (Jaworek et al., 1974). Each of these assays was validated by adding known amounts of the substances concerned to testicular tissue that had been previously exhaustively extracted to remove the various compounds to be tested. The references given to the methods used indicate the precision, accuracy and sensitivity of each assay. We have used the assays within the linear range and well above the lowest detectable amounts in each case. We have confirmed the claims made by the authors for each of these assays. Although some variation in the levels of metabolites was seen from 1 preparation of spermatids to another, within 1 preparation duplicate determinations showed little variation.

Metabolism of 1-[^{14}C] Pyruvate and U-[^{14}C] Lactate

Spermatids were incubated at 37°C in plastic tubes (75 mm, Fisher Scientific) which were sealed with plastic caps from which a piece of filter paper (Whatman No. 5) 2.0 × 0.5 cm was suspended. The filter paper was soaked in KOH (2N); this did not affect the pH of the incubation medium. Following incubation, the filter paper was placed in a scintillation vial and ^{14}C was measured by liquid scintillation spectrometry.

Subcellular System

Spermatids were homogenized in 4 vol of 0.02 M Tris-Cl buffer, pH 7.4, in a Waring blender for 2 min and then allowed to stir for 1 h. The extract was centrifuged at 13,000 × g for 15 min. The supernate was dialyzed against the same Tris buffer for 24 h. This cell extract was used as the source of lactate dehydrogenase activity.

Chemicals

Chemicals were obtained from the following sources: rotenone, 2,4-dinitrophenol, glucose, lactate, pyruvate and other glycolytic intermediates: Sigma Chemical Co.; 5-methoxyindole-2-carboxylic acid: Aldrich Chemical; rutamycin: Lilly Labs.; sodium 1-[^{14}C] pyruvate (Lot No. 1310-131; specific activity: 8.0 mCi/mmole) and sodium U-[^{14}C] lactate (Lot No. 1321-209; specific activity: 165.2 mCi/mmole) New England Nuclear.

Statistics

Every experiment presented below was performed 3 times (i.e., 3 separate preparations of spermatids). Unless otherwise stated, each condition was performed in duplicate for each experiment (i.e., 2 identical tubes) and measurements were made in triplicate on each tube. Paired t tests were performed on the 3 experiments. Only 1 representative experiment is shown in each case. The t tests were performed on the means of duplicate tubes (6 readings) by comparing 2 conditions (treated and control) within each experiment (paired t test) for the 3 experiments.

RESULTS

Utilization of Substrates in Vitro

Table 1 shows that round spermatids use glucose, pyruvate and lactate but little fructose during incubation for 1 h. Utilization of lactate was twice that of pyruvate and 3.9 times that of glucose. In experiments not shown here we observed that for these substrates the concentration used (1.0 mM) was saturating and that in no case was greater utilization of these substrates seen with lower or higher concentrations. In additional studies (not shown), lactate was found to be the preferred substrate when the medium used was designed to imitate tubular fluid (Evans and Setchell, 1978) and in medium containing various concentrations of rat serum (1% to 10% v/v).

Influence of Substrates on the Concentration of ATP in Spermatids

It can be seen from Fig. 1 that during incubation without added substrate, spermatids used 0.76 nmoles of ATP per 10^6 cells per h (Fig. 1: zero time – none). Addition of lactate to the medium resulted in a net gain in ATP of 0.15 nmoles per 10^6 cells per h over values at zero time. The other substrates added did not maintain initial levels of ATP. Pyruvate replaced some of the ATP used during incubation

TABLE 1. Utilization of substrates by round spermatids in vitro.[a]

Substrate	Consumption (nmoles/h/10^6 cells)[b]
Glucose	3.30 ± 0.50
Fructose	0.42 ± 0.02
Pyruvate	5.57 ± 0.62
Lactate	11.9 ± 1.05

[a] Round spermatids (10^6 per flask) were incubated in phosphate buffered saline for 1 h with the substrates shown, which were initially present at a concentration of 1.0 mM in each case. Following incubation, the concentration of substrate was measured in the medium.

[b] Mean and range of triplicate determinations on duplicate tubes.

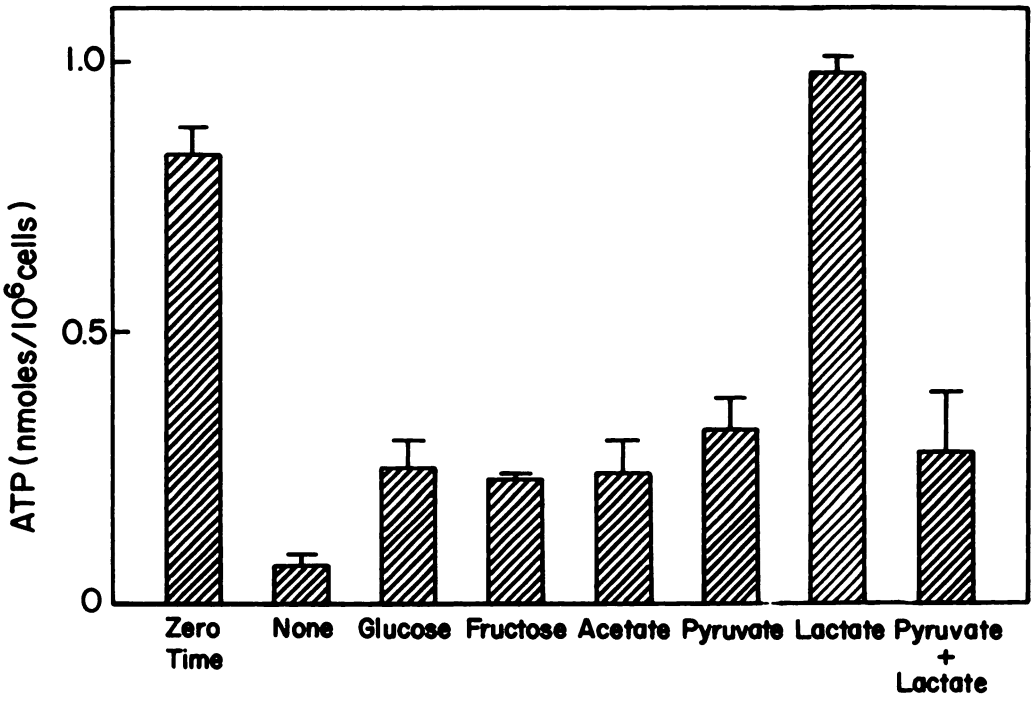

FIG. 1. Production of ATP from various substrates by spermatids. Round spermatids were incubated for 1 h (except the zero time control) with the substrates shown, which were added to a final concentration of 10 mM in each case, including pyruvate + lactate, in which each substrate was present at 10 mM. Following incubation, the concentration of ATP was measured after homogenization of cells. The values shown are means and ranges of triplicate determinations on each of duplicate tubes.

and also caused striking inhibition of the effect of lactate. Glucose and fructose also replaced some of the ATP used during incubation (Fig. 1), but these substrates did not inhibit the effect of lactate (not shown). With inositol as substrate (0.1 to 10 mM) no demonstrable net synthesis of ATP was observed (not shown).

Inhibitory Effect of Pyruvate on Utilization of Lactate by Spermatids

The effect of pyruvate on lactate metabolism was further examined in the experiment presented in Table 2, which shows that pyruvate inhibits utilization of lactate when pyruvate is present over a wide range of concentrations. Evidently the decreased production of ATP from lactate caused by pyruvate (Fig. 1) is associated with decreased utilization of lactate. Table 3 shows that the effects of lactate and pyruvate on net production of ATP are concentration dependent, although with pyruvate a plateau is reached at less than 0.1 mM.

TABLE 2. Effect of pyruvate on the utilization of lactate by round spermatids.[a]

Addition	Lactate consumption (nmoles/h/10^6 cells)
...	7.7 ± 0.07
Pyruvate 0.1 mM	7.47 ± 0.13
Pyruvate 1.0 mM	0.28 ± 1.28
Pyruvate 10.0 mM	−1.47 ± 1.05

[a]Spermatids were incubated with and without the concentrations of pyruvate shown for 1 h in phosphate buffered saline containing lactate 1.0 mM. Following incubation, the concentration of lactate in the medium was measured as described under *Materials and Methods*. Values shown are means and ranges for triplicate determinations on each of duplicate tubes. The values were obtained by subtracting the concentrations of lactate found at the end of the incubation from that present initially (1.0 mM) and were corrected for cell number. The negative value indicates net production of lactate.

TABLE 3. Effect of substrate concentration on net production of ATP by round spermatids.[a]

Addition	ATP (nmoles/10^6 cells) 15 min	40 min
Experiment 1		
...	0.56 ± 0.02	0.41 ± 0.01
Lactate 0.01 (mM)	0.55 ± 0.02	0.41 ± 0.01
Lactate 0.10 (mM)	0.63 ± 0.01	0.42 ± 0.01
Lactate 1.0 (mM)	0.86 ± 0.02	0.75 ± 0.07
Lactate 10.0 (mM)	1.22 ± 0.03	1.29 ± 0.07
Experiment 2		
...	0.52 ± 0.01	0.41 ± 0.01
Pyruvate 0.01 (mM)	0.56 ± 0.01	0.41 ± 0.05
Pyruvate 0.10 (mM)	0.78 ± 0.03	0.61 ± 0.04
Pyruvate 1.0 (mM)	0.70 ± 0.01	0.63 ± 0.02
Pyruvate 10.0 (mM)	0.74 ± 0.01	0.69 ± 0.01

[a]Round spermatids (10^6 cells/0.2 ml of phosphate buffered saline) were incubated for the times and with the additions shown. Following incubation, ATP concentrations were measured after homogenization of the cells. Values shown are means and ranges for triplicate determinations on each of duplicate tubes. At zero time values were: Experiment 1, 0.97 ± 0.01; and Experiment 2, 0.96 ± 0.04.

The response to addition of pyruvate at various times after addition of lactate is shown in Fig. 2. The effect of lactate is quickly inhibited by pyruvate, so that the final concentration of ATP at 60 min is essentially the same as that seen with pyruvate alone. We also observed that addition of acetate, acetylcarnitine and Krebs' cycle intermediates (citrate, α-ketoglutarate, fumarate, malate and oxaloacetate) to spermatids together with lactate (10 mM) did not alter the consumption of lactate by these cells as judged by disappearance of lactate from the medium. Moreover, addition of these agents to the subcellular system *(Materials and Methods)* did not inhibit the conversion of lactate to pyruvate (data not shown). Spermatids, like ejaculated sperm, contain the enzyme acetylcarnitine transferase (Van Dop et al., 1977).

The effect of pyruvate on production of ATP as a function of concentration of this substrate is shown in Fig. 3. It can be seen that increasing the concentration of pyruvate causes no more than minimal increase in the production of ATP in the absence of lactate. The effect of pyruvate on the production of ATP with lactate present is clearly shown in the figure. As the concentration of pyruvate is increased, production of ATP falls almost to levels seen without added substrate (Fig. 3).

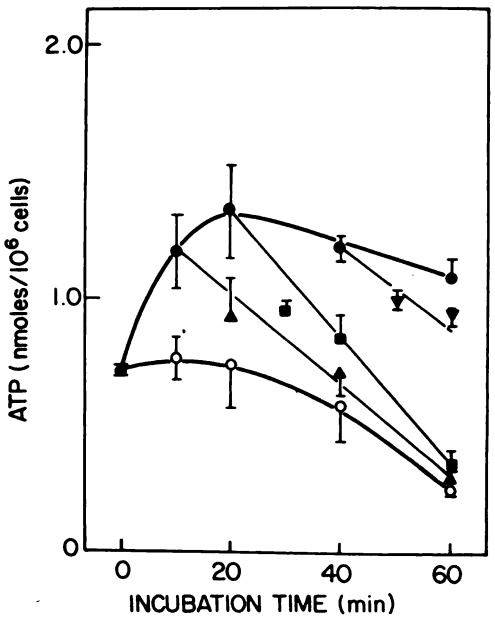

FIG. 2. Net production of ATP by round spermatids with lactate with and without pyruvate. Round spermatids were incubated with lactate (10 mM) for 60 min. In some flasks, pyruvate (10 mM) was added at 10, 20 and 40 min. In all cases the total incubation time was 60 min. Following incubation the concentration of ATP was measured. Values shown are means and ranges for triplicate determinations on each of duplicate tubes. Symbols used are: ○ — pyruvate as substrate, ● — lactate as substrate; no pyruvate, ▲ — lactate as substrate; pyruvate added at 10 min, ■ — lactate as substrate; pyruvate added at 20 min, and ▼ — lactate as substrate; pyruvate added at 40 min.

Interconversion of Pyruvate and Lactate

Pyruvate and lactate are interconverted in