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A bioenergetic model of gossypol action: effects of gossypol on adult rat spermatogenic cells

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REYES, JUAN, LAURA BORRIERO, AND DALE J. BENOS. *A bioenergetic model of gossypol action: effects of gossypol on adult rat spermatogenic cells.* Am. J. Physiol. 254 (Cell Physiol. 23): C564–C570, 1988. —Rat spermatogenic cells have glycolytic rates of 124 ± 36 nmol lactate · mg protein^{-1} · h^{-1}. Maximum glycolytic rates in the absence of oxidative phosphorylation do not exceed the basal rates by >40%. From the rates of oxygen consumption and lactate production it can be calculated that <8% of the total ATP produced by spermatogenic cells is provided by glycolysis. These properties of spermatogenic cells would render them highly susceptible to cytotoxicity due to the action of agents like gossypol that impair mitochondrial oxidative phosphorylation or to conditions that produce testicular hypoxia. The main effect of gossypol on spermatogenic cell energy metabolism is to uncouple mitochondrial oxidative phosphorylation. In the absence of glucose, this uncoupling action of gossypol would change the NAD^+/NADH redox coupling and, consequently, could modify metabolic fluxes through the lactic dehydrogenase catalyzed reaction.

lactate production; lactate dehydrogenase catalyzed reaction

THE PROPOSAL ADVANCED by Liu in 1957 (15), namely, that gossypol, a component in crude cotton oil, was the substance responsible for the cotton oil-induced male infertility detected in the Chinese province of Kiangsu in the 1930s, triggered research on the possible cellular mechanisms of gossypol action (see Ref. 23 for a review). The hypothalamus-hypophysis-gonad hormonal axis does not appear to be altered under gossypol treatment (5, 6, 13, 23). Several cellular enzymes such as Ca^{2+}-adenosinetriphosphatase (ATPase) (4), mitochondrial enzymes (29), glycolytic enzymes (32), LDH-C4 (14), adenylate cyclase (22), arachidonate lipooxygenases (9), and ribonucleotide reductase (18) can be inhibited by gossypol. Therapeutic levels of gossypol in the plasma of rats treated with antifertility-producing doses of this compound are <10 μM (10, 30). However, at these concentrations, the level of gossypol-induced inhibition of most of these enzymes is too low to account for spermatogenic cell toxicity. Thus the cellular actions of gossypol that seem to be pharmacologically relevant are *1*) uncoupling of spermatogenic cell mitochondria (e.g., Ref. 33); *2*) inhibition of plasma membrane calcium transport (4); *3*) inhibition of LDH-C4 (14); *4*) inhibition of arachidonate lipooxigenases (8); and *5*) inhibition of ribonucleotide reductase (17).

The main shortcoming of all the gossypol-induced infertility models is that these cellular actions of gossypol are specific in terms of inducing spermatogenic cell cytotoxicity, while sparing most other cells in the organism.

At infertility-producing doses, gossypol has been shown to selectively affect the meiotic and postmeiotic spermatogenic cells (11, 21, 33). One of the prominent histological characteristics of gossypol action on spermatozoa and spermatogenic cells is the morphological changes induced in the cell mitochondria. Swelling and vacuolization of mitochondria are well observed in the histological preparations of the testis of gossypol-treated rats (11, 21).

In the following paper, we focus on the uncoupling hypothesis of gossypol-induced cytotoxicity. We have studied aspects of the energy metabolism of adult rat spermatogenic cells and the metabolic effects produced by gossypol. Our results indicate that isolated adult rat spermatogenic cells have glycolytic rates of 124 ± 36 nmol lactate · mg protein^{-1} · h^{-1} and consume oxygen in the presence and absence of glucose at 228 ± 27 nmol/mg protein^{-1} · h^{-1}. Furthermore, the maximum glycolytic capacity of rat spermatogenic cells is 170 nmol lactate · mg protein^{-1} · h^{-1}. From these results, we calculate that 92% of the ATP production is derived from oxidative phosphorylation.

It appears that the net effect of gossypol on rat spermatogenic cell energy metabolism can be explained as a uncoupling of spermatogenic cell mitochondria. According to the criteria developed previously (24), we conclude that rat spermatogenic cells can be classified as a cell type susceptible to gossypol-induced cytotoxicity.

MATERIALS AND METHODS

Spermatogenic cell preparation. Spermatogenic cells were prepared from 35–40-day-old rat testicles, using a collagenase-trypsin digestion procedure as described by Bellve et al. (2). The enriched Krebs-Henseleit buffer used for the spermatogenic cell preparation contained (in mM) 5 glucose, 0.1 pyruvate, 1 lactate, and 0.5 inosine (pH 7.4) and was gassed with 95% O_2-5% CO_2. Cell

viability and membrane intactness were routinely checked using a trypan blue exclusion test (>95% trypan blue exclusion) and succinate stimulation of oxygen consumption (<5% stimulation). Through the use of morphological criteria in air-dried and -stained smears, the resulting cell population was composed of 90–95% meiotic and postmeiotic spermatogenic cells (c.f., Ref. 12). The spermatogenic cell population presents 5×10^6 cells/mg protein. In control experiments, the cell suspension was incubated for 3 h at 34°C in an air-5% CO_2 atmosphere to allow Sertoli cells to attach to the culture dishes (e.g., Ref. 8). Approximately 3–4% of the total cells remained attached to the dishes of which 20–40% presented the abundant cytoplasmic inclusions and irregular nuclear morphology characteristic of Sertoli cells. This treatment did not modify the rates of lactate production from glucose or the oxygen consumption in the presence or absence of gossypol in the remaining cell population (data not shown).

Measurements of lactate and pyruvate production. The spermatogenic cell suspension (3–5 mg protein/ml) was preincubated at 34°C with a 95% O_2-5% CO_2 atmosphere in Krebs-Henseleit bicarbonate (KHB) buffer supplemented with 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethenesulfonic acid (HEPES) at pH 7.4 for 10 min. After this time, different substrates and metabolic modifiers were added. Aliquots of the cell suspension were removed at predetermined times and the cells were centrifuged at 14,500 *g* for 30 s. The supernatant was removed for lactate or pyruvate measurements and the pellet was resuspended in 0.1 M NaOH, 0.1% Triton X-100 for protein determination (3). Lactate and pyruvate was estimated spectrophotometrically as NADH appearance or disappearance, respectively, in an enzymatic assay as described by Lowry and Passonneau (16).

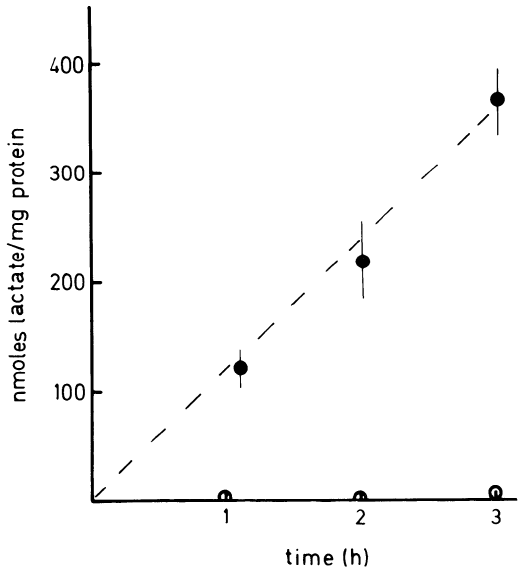

FIG. 1. Rat spermatogenic cells (3–4 mg protein/ml) were preincubated with continous stirring in Krebs-Henseleit medium supplemented with 10 mM HEPES (pH 7.4) for 5 min at 34°C and gassed with 95% O_2-5% CO_2. At zero time, glucose was added to a final concentration of 10 mM. Lactate produced by control (○) or glucose-metabolizing cells (●) was determined as NADH in an enzymatic assay. Bars represent ±SD from duplicate experiments in 4 different cell preparations.

Oxygen consumption measurements. Oxygen consumption (Q_{O_2}) measurements were performed polarographically in a sealed glass chamber (0.9 ml) surrounded by a water jacket held at 34°C. Isolated spermatogenic cells were preincubated for 10 min in modified KHB at 34°C with 95% O_2-5% CO_2 supply before being transferred to the Q_{O_2} chamber. Q_{O_2} values were obtained from the slope of the O_2 tension vs. time record between 0.5 and 2 min after each experimental addition.

Lactate dehydrogenase measurements. Lactate dehydrogenase (LDH) measurements were made in 100 mM sodium phosphate buffer (pH 7.0), 2 μM rotenone, 30 μM NADH, and 0.2 or 2 mM pyruvate. The reaction was initiated by addition of pyruvate to a cell supernatant or a frozen and thawed cell suspension. Absorbance of NADH at 340 nm was monitored continuously with a strip chart recorder, and LDH activity was calculated from the slope of the observed decrease in NADH with time. Absolute values of LDH activity were 8.5 and 6.0 $\mu mol \cdot mg\ protein^{-1} \cdot h^{-1}$ for 0.2 and 2 mM pyruvate, respectively.

RESULTS

Glucose-dependent pyruvate and lactate production by spermatogenic cells. The time course of aerobic lactate production by rat spermatogenic cells incubated in KHB buffer at 34°C with and without glucose is shown in Fig. 1. Lactate production in the absence of glucose was <10 $nmol \cdot mg\ protein^{-1} \cdot h^{-1}$, even in the presence of the uncoupler carbonyl cyanide *m*-chlorophenylhydrazone (CCCP). Addition of glucose increased the rate of aerobic lactate production to 124 $nmol \cdot mg\ protein^{-1} \cdot h^{-1}$. CCCP stimulated the glucose-dependent lactate production by ~40% (see Table 1). Quantitatively, similar results (30–40% stimulation of lactate production) were obtained using 0.1 μM antimycin (not shown). Under similar conditions, pyruvate production from glucose by spermatogenic cells was <10 $nmol \cdot mg\ protein^{-1} \cdot h^{-1}$. The initial rate of lactate production was a function of the glucose concentration in the incubation medium (Fig. 2). Half-maximal stimulation of lactate production was ob-

TABLE 1. *Aspects of energy metabolism of rat spermatogenic cells*

Substrate	Q_{O_2}, % of endogenous Q_{O_2}	Lactate Production, $nmol \cdot mg\ protein^{-1} \cdot h^{-1}$
Endogenous	100 (228 ± 27 $nmol \cdot mg\ protein^{-1} \cdot h^{-1}$)	5 ± 4
+ CCCP (1 μM)	115 ± 10	20 ± 11
Glucose (10 mM)	102 ± 1	124 ± 36
+ CCCP	170 ± 1	171 ± 38
Pyruvate (2 mM)	150 ± 9	295 ± 48
+ CCCP	200 ± 15	170 ± 30
Lactate (6 mM)	140 ± 12	
Succinate (1 mM)	102 ± 2	

Values are means ± SD of several duplicate experiments performed in 3–4 different cell preparations. Isolated spermatogenic cells in suspension were incubated at 34°C in Krebs-Ringer-bicarbonate solution gased with 95% O_2-5% CO_2 and in presence or absence of different substrates.

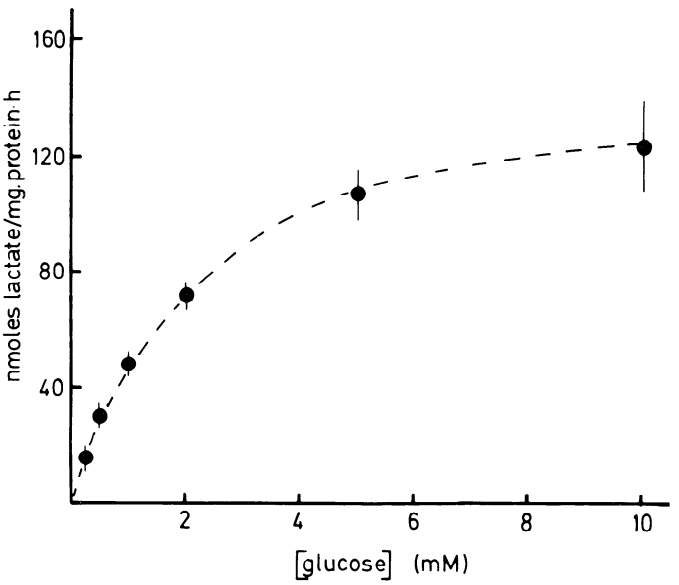

FIG. 2. Concentration dependence of lactate production from glucose by spermatogenic cells. Other experimental conditions are similar to those in Fig. 1.

tained at ~1.4 mM glucose.

Oxygen consumption of rat spermatogenic cells. Spermatogenic cells consumed oxygen at a basal rate of 228 ± 27 nmol · mg protein^{-1} · h^{-1} in the absence of externally added substrates (Table 1). Addition of CCCP in these conditions only stimulated Q_{O_2} by 15%, indicating a possible limitation in reducing equivalent delivery to the mitochondrial electron chain from endogenous substrates. Addition of glucose did not modify significantly the rate of Q_{O_2}. However, glucose metabolism seemed to provide reducing equivalents to the mitochondria. This was evidenced by a 50% increase in Q_{O_2} when oxidative phosphorylation was uncoupled by CCCP in the presence of glucose. Pyruvate and lactate increase the rate of Q_{O_2} by ~50 and 40%, respectively. The concentration dependence of the Q_{O_2} change induced by pyruvate and lactate is shown in Fig. 3. Half-maximal stimulation of Q_{O_2} was obtained at 0.1–0.2 mM pyruvate and 1.2–1.3 mM lactate.

ATP production by spermatogenic cells. The rate of ATP production by the glycolytic and oxidative phosphorylation pathways in rat spermatogenic cells can be estimated from lactate production and Q_{O_2} measurements, assuming a stoichiometry of 1 ATP-lactate and 6 ATP-O_2 (1). The calculated rates of ATP production at different glucose concentrations are shown in Table 2. The percentages of ATP production from glycolysis and oxidative phosphorylation (shown in parenthesis in Table 2) indicated the predominantely oxidative characteristics of spermatogenic cell energy metabolism at all glucose concentrations.

Pyruvate-lactate interconversion in spermatogenic cells. The rate of conversion of pyruvate to lactate by spermatogenic cells was a function of the pyruvate concentration in the incubation medium. Figure 4 shows the time course of lactate production by rat spermatogenic cells at four different extracellular pyruvate concentrations. From the initial rates, taken up to 10 min after pyruvate addition, we estimated a half-maximal, pyruvate-induced lactate production at ~1.7 mM pyruvate. The reverse conversion, i.e., pyruvate production from lactate, can be estimated as pyruvate extrusion at 10 mM lactate. The initial rate of pyruvate production at 10 mM lactate was estimated to be ~50 nmol · mg protein^{-1} · h^{-1}.

*Effect of gossypol on glucose-dependent lactate production and oxygen consumption.*The effect of gossypol on glucose-dependent lactate production is shown in Fig. 5. Gossypol stimulated lactate production in a dose-dependent manner up to levels comparable to the increase in lactate production induced by CCCP, a classic uncoupler of oxidative phosphorylation. At gossypol concentrations >50 μM, inhibition of lactate production could be observed. Gossypol stimulated the Q_{O_2} of spermatogenic cells in the presence of glucose (Fig. 6) up to values comparable to the CCCP-stimulated Q_{O_2} in spermatogenic cells (c.f., Table 1).

Action of gossypol and other mitochondrial effectors on pyruvate-lactate interconversion in spermatogenic cells. Gossypol inhibited the initial rate of pyruvate to lactate conversion by rat spermatogenic cells (Fig. 7*A*). Figure 7*B* shows that this inhibition was dependent on gossypol concentration and that the half-maximal inhibition of pyruvate to lactate conversion rate occurred at ~3–5 μM gossypol. CCCP also inhibited the initial rates of pyruvate to lactate conversion. At 1 μM, CCCP inhibited the rate of lactate production from pyruvate by 40–45%. Sodium cyanide (0.5 mM) stimulated the initial rate of lactate production from pyruvate by an average of 15% (3 cell preparations).

The effect of gossypol on the rate of pyruvate production from lactate was the opposite to the effect on lactate production from pyruvate. Figure 8 shows that the initial rate of pyruvate production from lactate was increased by gossypol in a dose-dependent manner.

Direct measurements of LDH activities after freezing and thawing of the rat spermatogenic cell suspension showed that gossypol (up to 25 μM) and CCCP (up to 2 μM) inhibited spermatogenic cell LDH activity by an average of 10 and 4%, respectively.

DISCUSSION

The studies reported in the literature on the metabolic properties of spermatogenic cells, with their requirements of lactate or pyruvate for structural and functional integrity in vitro (12), have indicated a diminished dependency on glycolytic flux and an enhanced dependency on Krebs cycle metabolism for their energy requirements. Mita and Hall (19), Nakamura et al. (20), and Grootegoed et al. (7) have demonstrated that long-term maintenance of high intracellular levels of ATP can only be achieved when lactate or pyruvate are present in the incubation medium of meiotic and postmeiotic spermatogenic cells. Our results with adult rat spermatogenic cells are in agreement and extend the experimental findings mentioned above.

As shown in Table 1, no endogenous substrates (e.g., glycogen, alanine) appear to be converted to lactate at

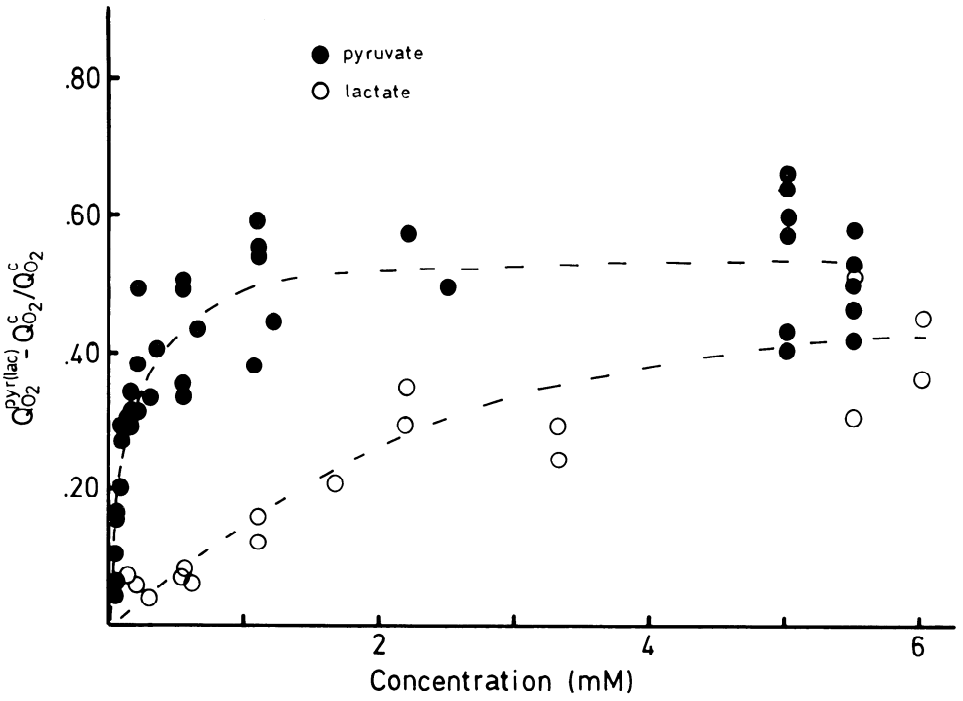

FIG. 3. Concentration dependence of fractional increase in oxygen consumption (Q_{O_2}) of rat spermatogenic cells induced by pyruvate (●) or lactate (○). Superscripts c and pyr represent Q_{O_2} in control and pyruvate-containing medium.

TABLE 2. *Calculated ATP production by rat spermatogenic cells*

Substrate, mM	ATP Production, nmol·mg protein^{-1}·h^{-1}		
	Oxidative phosphorylation	Glycolysis	Total
Glucose			
0	1,368 (99.6)	5 (0.4)	1,373
0.25	1,368 (98.9)	15 (1.1)	1,383
0.5	1,368 (97.9)	30 (2.1)	1,398
1.0	1,368 (96.6)	48 (3.4)	1,416
2.0	1,368 (95.0)	71 (5.0)	1,439
5.0	1,368 (92.1)	117 (7.9)	1,485
10.0	1,368 (91.7)	124 (8.3)	1,492
Pyruvate			
5.0	2,052 (100)		2,052
Lactate			
5.0	1,888 (100)		1,888

Rates of aerobic ATP production by rat spermatogenic cells were calculated from rates of oxygen consumption and lactate production at 34°C in 95% O_2-5% CO_2 atmosphere. Stoichiometries of 6 ATP/O_2 and 1 ATP/lactate were used in calculations (Ref. 1). Percentage of total ATP production given by oxidative phosphorylation and glycolysis at each substrate concentration are in parenthesis.

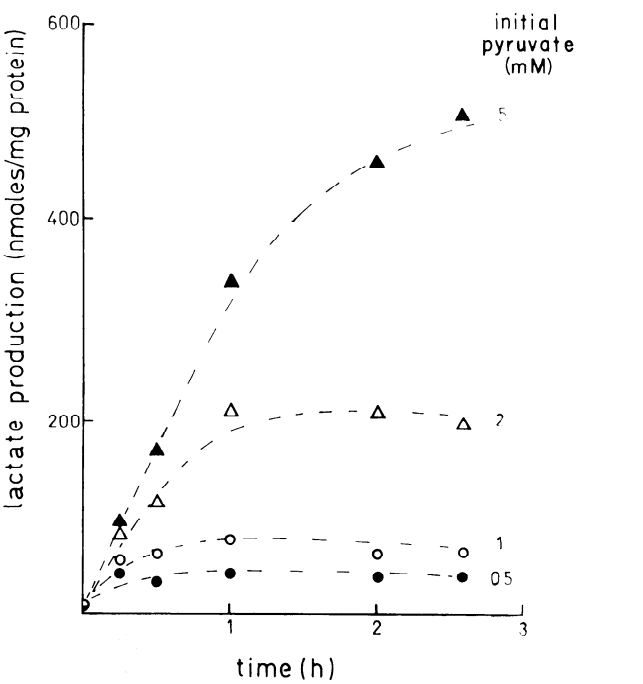

FIG. 4. Rat spermatogenic cell lactate production from pyruvate. Cells (3–4 mg protein/ml) were preincubated for 5 min in Krebs-Henseleit buffer with 10 mM HEPES (pH 7.4) for 5 min at 34°C and gassed with 95% O_2-5% CO_2. Pyruvate at 0.5 (●), 1.0 (○), 2.0 (△), and 5.0 (▲) mM initial concentration was added at zero time. Samples were withdrawn at predetermined times for lactate determination.

any appreciable rates in rat spermatogenic cells. The glucose dependence of lactate production shows (Fig. 2) that it follows a hyperbolic curve. The half-maximal stimulation of lactate production is obtained at 1.4 mM glucose. Extrapolation of these results to the in vivo situation indicates that, given the probable concentrations of glucose in the adluminal compartment of the seminiferous tubule (~0.2 mM, Ref. 27), the glycolytic flux in spermatogenic cells would only contribute 1% of the total ATP production. Exposure to glucose does not modify the rate of Q_{O_2} of rat spermatogenic cells (see also Ref. 12). Hence, there is no evident Crabtree effect in spermatogenic cells, in contrast to that shown by other testicular cells in culture (24). The small stimulation of Q_{O_2} induced by CCCP (15%) strongly suggests that, in the absence of exogenous substrates, spermatogenic cells are utilizing oxidative phosphorylation at close to maximum rates and that the rate-limiting step in the process could be the reducing equivalent delivery to the mitochondrial electron chain. This conclusion is supported by the observation that in the presence of glucose or pyruvate, CCCP does stimulate Q_{O_2} 70 and 40%, respec-

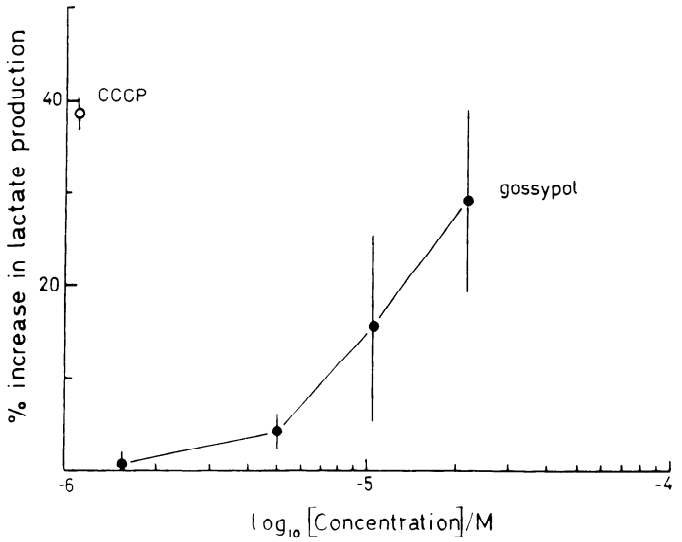

FIG. 5. Percentage increase in lactate production by rat spermatogenic cells induced by gossypol (●) and CCCP (○). Spermatogenic cells were incubated with 10 mM glucose in absence or presence of different concentrations of gossypol or 1.1 μM CCCP. Samples were withdrawn after 30, 60, and 120 min for lactate determination. Bars represent ± SD of duplicate experiments done in 3 different cell preparations.

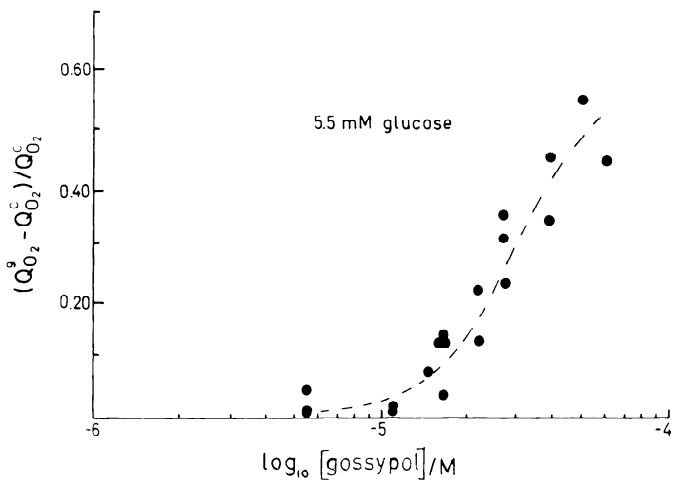

FIG. 6. Fractional increase in oxygen consumption (Q_{O_2}) of rat spermatogenic cells in presence of 5.5 mM glucose. Cells were preincubated 5 min in Krebs-Henseleit buffer supplemented with 10 mM HEPES (pH 7.4) and gassed with 95% O_2-5% CO_2. Cells then were transferred to Q_{O_2} chamber and glucose was added immediately. Gossypol was added 1 min after glucose. Superscripts g and c identify Q_{O_2} in presence and absence of gossypol, respectively.

tively. Pyruvate and lactate stimulate Q_{O_2} in a dose-dependent manner (Fig. 3). There is no available estimation on what the lactate and/or pyruvate concentrations are in the adluminal compartment. However, considering the ability of Sertoli cells to produce lactate at rates of ~600 nmol · mg protein^{-1} · h^{-1} (24, 26), similar or higher values than the seminiferous tubule lactate could be expected (~0.5–1 mM, Ref. 27). Thus at least 30% of the Q_{O_2} of spermatogenic cells in vivo could be provided by the metabolism of exogenous pyruvate or lactate.

The rates of ATP production by spermatogenic cells can be calculated from the rates of lactate production and Q_{O_2}. These estimated values (Table 2) indicate that, at physiological concentrations of glucose, 92–98% of the ATP production of spermatogenic cells is provided by the cell oxidative phosphorylation and only 2–8% by glycolytic ATP production. A small contamination with highly glycolytic Sertoli cells (see MATERIALS AND METHODS) would make this glycolytic-oxidative phosphorylation balance of ATP production more dramatically in favor of oxidative phosphorylation in spermatogenic cells. From our results, and assuming no changes in the ATP-consuming cell functions in the absence or presence of added substrates, it can be predicted that lactate and pyruvate would increase the ATP levels in spermatogenic cells, in agreement with previously published results (7, 12, 19, 20). In the absence of oxidative phosphorylation (antimycin or uncouplers), glycolysis could only provide 12% of the basal total ATP requirements of rat spermatogenic cells.

The effects of gossypol on lactate production and Q_{O_2} (Figs. 5 and 6) are consistent with the idea that the net effect of gossypol on spermatogenic cell energy metabolism is to uncouple spermatogenic cell mitochondria. There is no evidence of a net inhibition of rat spermatogenic cell glycolysis by gossypol, as occurs in human spermatozoa (32).

Reyes et al. (24) postulated that if gossypol decreased the efficiency of ATP production by oxidative phosphorylation, then the cells susceptible to gossypol-induced cytotoxicity would be those having low glycolytic capacity and a energy metabolism heavily dependent on oxidative phosphorylation. The metabolic properties of the spermatogenic cells classify them as having predominantly oxidative phosphorylation-dependent energy metabolism as well as a very low glycolytic capacity, in good agreement with a bioenergetic model of gossypol action.

The inhibition of pyruvate to lactate conversion by gossypol could be interpreted as a possible direct inhibition of spermatogenic cell LDH-C4 as described by Lee et al. (14). However, our results indicate that there is an alternative and more likely explanation. Both gossypol and CCCP produce up to 45–50% inhibition of pyruvate to lactate conversion by spermatogenic cells (Fig. 7). These two compounds produce little or no effect on spermatogenic cell LDH activity measured in a permeabilized cell suspension with either 0.2 or 2 mM pyruvate concentrations. Furthermore, gossypol stimulates lactate to pyruvate conversion in spermatogenic cells (Fig. 8). It seems unlikely, then, that the observed inhibition of pyruvate to lactate conversion could be due to a direct "unidirectional" inhibition of the spermatogenic cell LDH enzyme. Both gossypol and CCCP share the property of being uncouplers of oxidative phosphorylation (25), and, hence, both have the ability to decrease ATP production and increase NADH consumption by the mitochondrial electron chain. The effect of 0.5 mM sodium cyanide was to stimulate by 15% the pyruvate to lactate conversion in spermatogenic cells and to remove the gossypol-induced inhibition of pyruvate to lactate conversion (data not shown; see also Ref. 28). Therefore, the effect of gossypol on pyruvate to lactate conversion does not seem to be mediated by a decrease in cell ATP production. Furthermore, the initial rate of lactate to pyruvate conversion by spermatogenic cells is stimulated

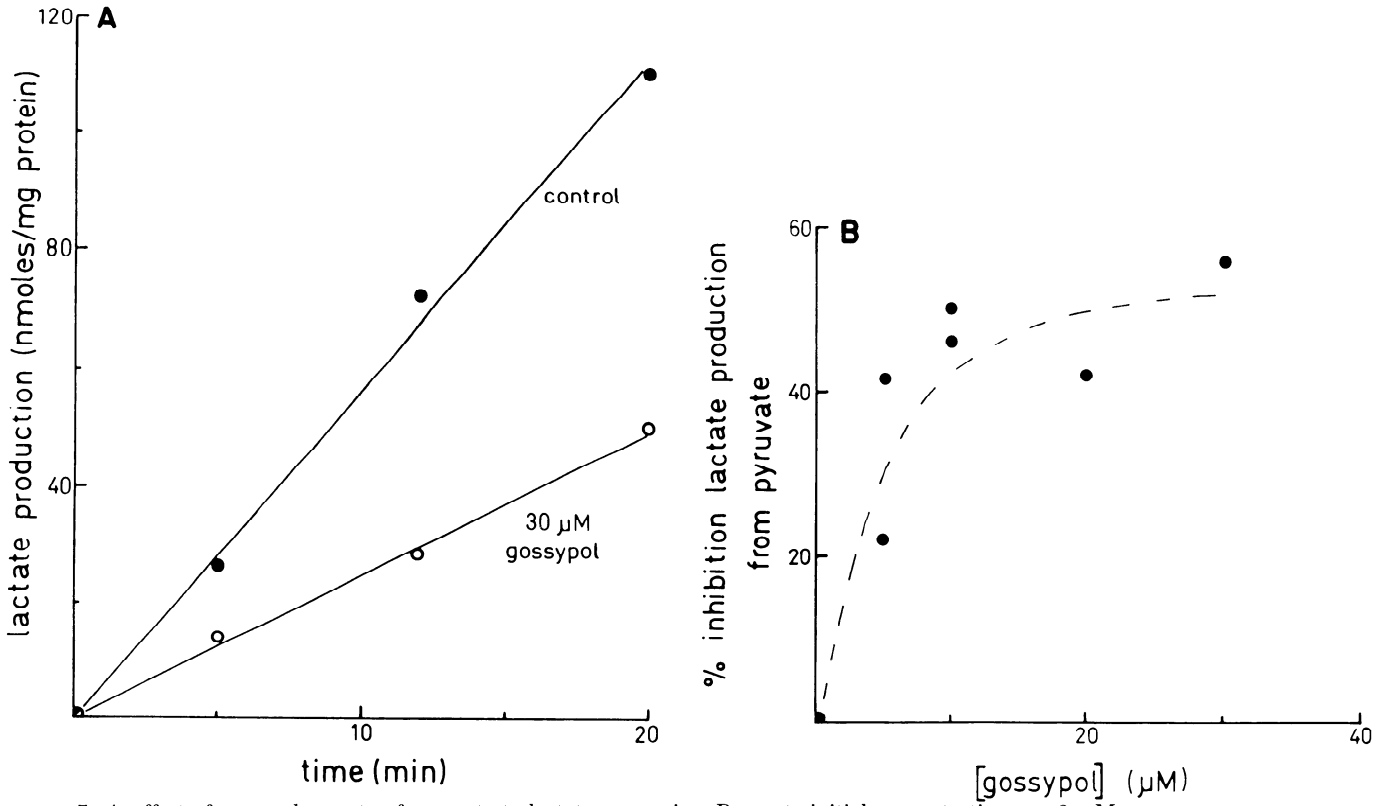

FIG. 7. *A*: effect of gossypol on rate of pyruvate to lactate conversion. Pyruvate initial concentration was 2 mM. *B*: concentration dependence of gossypol inhibition of lactate production from pyruvate in rat spermatogenic cells. Other experimental conditions are same as in Fig. 4.

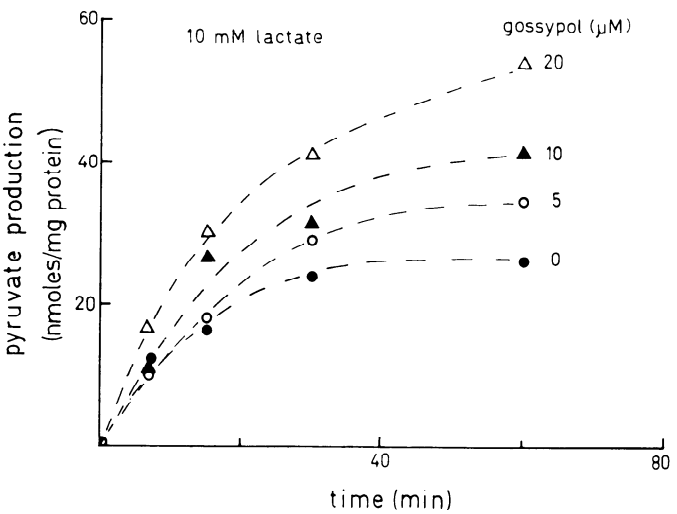

FIG. 8. Time and gossypol dependence of pyruvate production from 10 mM lactate by rat spermatogenic cells. Other experimental conditions are same as in Fig. 4.

by gossypol. These results are consistent with two hypothesis where the effects of gossypol, CCCP, and cyanide on pyruvate-lactate interconversion are mediated by changes in NAD^+/NADH or concentrations. First, these changes could be the consequence of either NAD^+ increases produced by uncoupler-stimulated NADH oxidation or NADH increases due to cyanide inhibition of NADH oxidation. Second, the changes induced in NADH/NAD^+ could mediate an increase in the metabolic fluxes that oxidize pyruvate (e.g., pyruvate dehydrogenase) with subsequent changes in intracellular pyruvate concentration. No experimental evidence is presented in this paper in favor of either of these two hypotheses. Both hypotheses are consistent with the fact that LDH-C4 is partially located in the mitochondrial matrix of meiotic and postmeiotic spermatogenic cells (18, 28). This LDH compartmentalization could lead to a close relation between LDH-C4 activity and mitochondrial NAD^+-NADH, lactate, and pyruvate pools. Gossypol increases lactate to pyruvate conversion in spermatogenic cells (Fig. 8) and, at the same time, increases lactate production from Sertoli cells in vitro (24). These facts make it unlikely that gossypol could be impairing spermatogenesis in vivo by spermatogenic cell "starvation", i.e., by lowering lactate or pyruvate production by Sertoli cells and/or decreasing the utilizability of the available lactate by the spermatogenic cells.

The redox state and the NAD^+/NADH coupling could also affect spermatogenic cell function and differentiation through ADP-ribosylations and ADP-(*poly*)-ribosylations. These covalent modification of proteins play an important role in metabolic regulation, chromatin condensation, DNA synthesis, and growth (33). Thus far, to the best of our knowledge, the regulatory role of ADP ribosylations and ADP-(*poly*)-ribosylations is yet an unexplored subject in spermatogenic cells and could be postulated as one of the putative mechanisms of gossypol action.

Thus we draw six conclusions from this study. First, rat spermatogenic cells rely heavily on oxidative phos-

phorylation for their ATP production. Second, the glycolytic capacity of rat spermatogenic cells is very low and cannot provide the necessary rate of ATP production to meet the cell demands. Third, pharmacological agents, like gossypol, that can partially impair the efficiency of oxidative phosphorylation, ATP production, or pathological conditions producing testicular hypoxia, could, in principle, halt spermatogenesis by meiotic and postmeiotic cell toxicity. Fourth, there is no evidence for a net inhibition of in situ spermatogenic cell LDH induced by acute gossypol exposure. Fifth, the NAD^+/NADH appears to control partially the flux through the LDH catalyzed reaction in spermatogenic cells. Sixth, the uncoupling-mediated effect of gossypol on the redox state of NAD^+/NADH is another possible mechanism through which gossypol could be affecting spermatogenic cell function.

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