Biochimica et Biophysica Acta, 1015 (1990) 243–247
Elsevier

BBABIO 43120

Quantification of aerobic energy turnover in epididymal bull spermatozoa

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(Received 12 September 1989)

Key words: Respiration; Motility; Oxidative phosphorylation; Caffeine; Spermatozoa; Epididymal spermatozoa; (Bull)

Turnover rates of oxidative energy metabolism were measured as oxygen consumption in untreated and caffeine-stimulated epididymal bull spermatozoa respiring with lactate. Incubation of spermatozoa with 1 mM caffeine led to an increase in respiration of approx. 60%. The rate of uncoupled respiration and the vanadate-insensitive part of oxygen consumption were not affected by caffeine. The small effect of ouabain on respiration (−10%) indicated a minor contribution of Na^+/K^+-ATPase to the ATP consumption. The major part of ATP turnover was caused by motility shown by the strong linear correlation between respiration and motility in untreated and caffeine-treated spermatozoa. In comparison with ejaculated spermatozoa investigated in a previous study, epididymal cells exhibited the same rates of uncoupled and ouabain-sensitive respiration. The efficiency of transforming mitochondrially-produced ATP into cell motion was the same in epididymal and ejaculated spermatozoa. The ATP-producing capacity of sperm mitochondria was utilized in untreated epididymal, in caffeine-stimulated epididymal and in ejaculated spermatozoa, by 20–25%, 40–45% and 45–50%, respectively. The results showed that the capacity of mitochondrial. ATP formation remains unchanged after ejaculation and is utilized to a higher extent by stimulated motility. Treatment with caffeine affected epididymal spermatozoa in a similar manner.

Introduction

Bull spermatozoa are known to acquire their ability of progressive motion during their passage through the epididymis [1,2]. Isolated from the caudal part, spermatozoa show a forward motility which is increased in intensity and quantity of motile cells after ejaculation [2]. In ejaculated spermatozoa, the rate of respiration is higher than in epididymal cells [3]. It is well known that this activation of respiration is a result of the higher energy demand required for increased motility. It was not investigated, however, whether the capacity for ATP production is increased simultaneously. Furthermore, it is not known whether there is an activation of other energy-requiring processes which contribute to the cellular energy turnover, besides motility. The present paper aims to study the contribution made by motility and other processes to the aerobic energy turnover in epididymal bull spermatozoa. This is why, the influence of specific effectors on respiration was measured in order to estimate the share of energy-requiring processes in cellular energy metabolism. A similar experimental approach was made in studies of the energy metabolism in reticulocytes and Ehrlich ascites tumour cells [4,5]. To obtain spermatozoa in different states of activity, untreated and caffeine-stimulated cells were compared as described by Lardy and co-workers [6,7]. Caffeine is known to stimulate sperm motility and respiration due to its inhibiting effect on cyclic nucleotide phosphodiesterase [8,9]. The results demonstrated that only 20–25% of the capacity of mitochondrial ATP production was utilized in untreated epididymal spermatozoa, whereas caffeine was capable of raising this percentage to a level typical of ejaculated cells.

Materials and Methods

Spermatozoa were prepared by retrograde flushing from the distal part of bovine caudal epididymis within 1 h of slaughter. The cells were washed twice by centrifugation for 6 min at $600 \times g$ using 120 mM NaCl and

Abbreviation: FCCP, carbonyl cyanide *p*-trifluoromethoxy-phenylhydrazone.

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50 mM Hepes (pH 7.4) as medium. The spermatozoa were incubated under gentle shaking at 37 °C in a medium containing 10 mM lactate, 140 mM NaCl, 6 mM KCl, 1.5 mM $MgCl_2$, 20 mM Tris and 10 mM Hepes (pH 7.4).

Concentrations of spermatozoa were assayed by using a spermatocrit procedure and calculated in μl packed cells, with 1 μl equal to $13.9 \cdot 10^9$ spermatozoa [10]. Respiration was determined polarographically at 37 °C adopting an oxygen solubility as described by Reynafarje et al. [11]. The motility of spermatozoa was assayed by a turbidimetric method and calculated as percentage of a total number of cells moving per min in the light path of an optical cuvette [12].

Lactic acid and rotenone were obtained from Sigma (St. Louis). Oligomycin was produced by Calbiochem (San Diego) and FCCP by Boehringer (Mannheim). Sodium monovanadate was purchased from Merck (Darmstadt). Ouabain and caffeine were obtained from Arzneimittelwerk (Dresden). All other chemicals were of analytical grade.

Results and Discussion

In the experiment presented in Fig. 1, the oxygen uptake of untreated spermatozoa respiring with lactate was inhibited by vanadate. As seen from the respiration rate (trace B) registered simultaneously, the new stationary rate of respiration occurred after approx. 1.5 min. Vanadate is known to inhibit extramitochondrial ATPases [13,14]. Hence, it follows that the lowering of respiration after addition of vanadate was due to inhibited extramitochondrial ATP consumption. The rate of vanadate-inhibited respiration was strongly stimulated by the uncoupler FCCP which was added at an optimal concentration determined by parallel titration experiments in order to obtain the maximum rate of respiration. Consequently, this rate is considered as the total capacity of mitochondrial respiration. The traces of the second incubation demonstrated that 1 mM caffeine stimulated respiration by approx. 60% as compared with untreated spermatozoa. On the other hand, vanadate-inhibited and FCCP-stimulated respiration rates remained practically unchanged in the presence of caffeine. For comparison, Fig. 2 shows these effects on respiration found in several cell preparations. The rates measured were plotted versus the respiration of untreated samples. The respiration rate of untreated cells was found to differ considerably, and the question arose whether the effects of vanadate, caffeine or FCCP depend on the initial state of the cells. This was true of the case when caffeine was present alone, the increase caused by caffeine was approx. 1 nmol O_2 per min, per μl cells. However, the uncoupled as well as the vanadate-inhibited respiration were found to be independent of the initial state of respiration of untreated cells and also

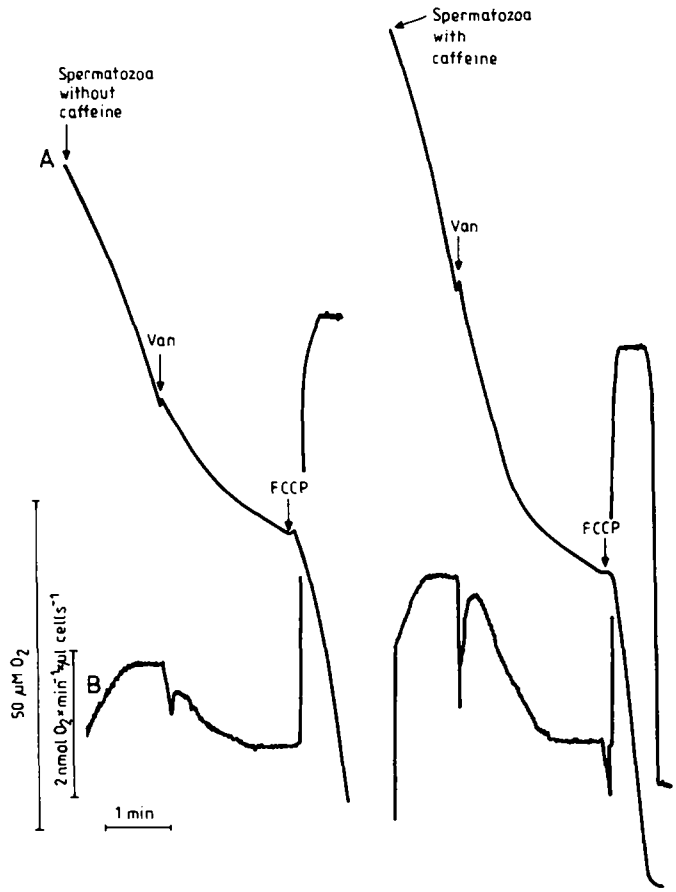

Fig. 1. Effect of vanadate and FCCP on respiration of untreated and caffeine-treated spermatozoa. Spermatozoa (15.6 μl cells per ml) were preincubated for 5 min at 37 °C in the absence or presence of 1 mM caffeine. Additions were: 0.22 mM vanadate (Van) and 0.6 μM FCCP. Traces A refer to oxygen concentrations, traces B to respiratory rates which were recorded simultaneously as first derivatives of trace A.

caffeine did not have any further effect, showing that the stimulation of respiration by caffeine was not due to an increased mitochondrial capacity for substrate oxidation. The sensitivity to vanadate indicated that the effect of caffeine is related to extramitochondrial ATP consumption.

Besides dynein ATPase, Na^+/K^+-ATPase is inhibited by vanadate [13,14] and may contribute to the vanadate-sensitive ATP turnover. The specific share of this enzyme in the vanadate-sensitive respiration was determined with ouabain. The data listed in Table I show that ouabain inhibited the respiration of both untreated and caffeine-treated cells to an extent which was comparable. From these data, a relation between the ouabain effect and the initial respiration rate could not be found.

The small contribution of Na^+/K^+-ATPase to the extramitochondrial ATP consumption of untreated, and especially of caffeine-treated spermatozoa, suggested that the major portion of vanadate-sensitive respiration

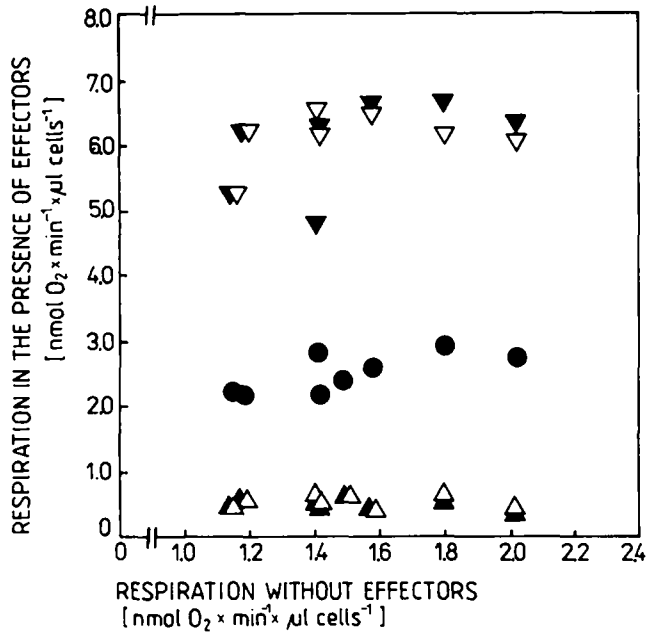

Fig. 2. Effect of caffeine, vanadate and FCCP on respiration of untreated spermatozoa. For various sperm preparations, rates of respiration measured in the presence of effectors were plotted versus the rates in the absence of any effectors. The cells (20.0–27.5 µl cells per ml) were preincubated for 5 min with effectors; in samples with caffeine the additional effectors were added 3 min prior to measurement. Open and closed symbols refer to samples in either absence or presence of caffeine: △, ▲ vanadate: ▽, ▼ FCCP; ● caffeine; concentrations as in Fig. 1.

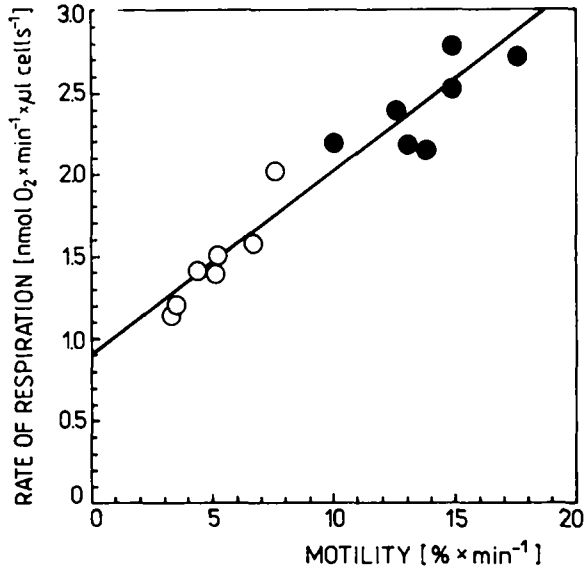

Fig. 3. Relationship between respiration and motility. After 5 min of preincubation without (○) or with 1 mM caffeine (●), samples were taken for parallel measurements of respiration and motility. The data were obtained from the same experiments as shown in Table I. Motility rates represented means of three runs. Parameters ± S.D. of the regression line: $V_{resp} = 0.901 \pm 0.097 + (0.110 \pm 0.009) \times V_{mot}$; correlation coefficient: 0.961.

was linked to motility. Since specific inhibitors of motility are not known, the contribution of this process to cellular energy turnover was estimated on the basis of the relation between respiration and motility. Motility was quantitatively assayed with a turbidimetric method recording the percentage of cells moving into the light path of a photometer cuvette per min [12]. Fig. 3 shows the respiratory rates versus the rates of motility, indicating that different initial rates of respiration corresponded with the motility rates ranging from 3.3 to 7.6% per min. Caffeine stimulated motility to rates of 10.0 of 17.6% per min. The relation between respiration and motility could be described by a common regression line, including both untreated and caffeine-stimulated cells. This relation allowed to conclude that caffeine increases the turnover rate of dynein ATPase without changing the efficiency with which this enzyme utilizes the energy delivered by respiration. This efficiency results from the slope of the regression line and can be expressed as oxygen equivalent of ATP consumed for motility. It amounted to 0.110 ± 0.009 nmol O_2 per µl cells per percent. This value does not differ

TABLE I

Effect of ouabain on respiration of epididymal bull spermatozoa

Data are derived from 7 to 8 sperm samples as shown in Fig. 2. The concentration of ouabain was 1 mM.

Sperm preparation	Without caffeine				Plus 1 mM caffeine			
	rate of respiration (nmol O_2/min per µl cells)			inhibition	rate of respiration (nmol O_2/min per µl cells)			inhibition
	− ouabain	+ ouabain	Δ	(%)	− ouabain	+ ouabain	Δ	(%)
1	1.15	0.99	−0.16	14	2.19	2.01	−0.18	8
2	1.19	0.95	−0.24	20	2.14	2.00	−0.14	7
3	1.41	1.39	−0.02	1	2.72	2.56	−0.16	6
4	1.42	1.26	−0.16	11	2.54	2.43	−0.11	4
5	1.49	1.25	−0.24	16	2.36	2.17	−0.19	8
6	1.58	1.37	−0.21	13	2.15	2.15	0	0
7	2.02	1.79	−0.23	11	2.78	2.56	−0.22	8
$\bar{x}$ ± S.D.			−0.180 ± 0.079	12.3 ± 5.9			−0.143 ± 0.072	5.9 ± 3.0

from the one obtained for ejaculated bull spermatozoa by vanadate titration (0.127 ± 0.012 nmol O_2 per μl cells per percent [15]), taking into account that vanadate inhibits, besides dynein ATPase, also Na^+/K^+-ATPase. The intercept of the regression line was 0.901 nmol O_2 per min, per μl cells, and represented the oxygen consumption which is not linked to motility. The major part of this amount has to be attributed to the vanadate-insensitive respiration, which was found to be approx. 0.50 nmol O_2 per min, per μl cells in untreated as well as in caffeine-stimulated cells (cf. Fig. 2). The remaining difference was mainly due to the activity of Na^+/K^+-ATPase corresponding to approx. 0.2 nmol O_2 per min, per μl cells (cf. Table I). The mean value of the sum of both rates (0.665 ± 0.124 nmol O_2 per min, per μl cells) did not significantly differ from the intercept of the regression line.

In a previous study, the respiratory rates of ejaculated spermatozoa were investigated under comparable conditions [15,16]. These rates are listed together with the rates of both untreated and caffeine-stimulated epididymal cells in Table II. Supplemented to these data are the rates measured in the presence of oligomycin and of rotenone. The former blocks the mitochondrial generation of ATP, and the latter completely inhibits the mitochondrial respiration when lactate is the substrate [15]. As compared with ejaculated cells, the total oxygen consumption of untreated epididymal spermatozoa amounted to about 50% only. Both epididymal and ejaculated spermatozoa, however, had the same capacity of mitochondrial respiration as indicated by the maximum rate observed in the presence of FCCP. Strictly speaking, this rate has to be corrected by the rotenone-insensitive respiration, which was found to be only 3% of the uncoupled rate. This identical capacity of respiration in epididymal and ejaculated spermatozoa is noteworthy because the former were obtained from unselected slaughter house material and the latter from high-quality semen. Therefore, one could reasonably speculate that the different motility rates of spermatozoa in individual ejaculates are caused by differences in the activity of the dynein-tubulin system rather than by the capacity of mitochondrial ATP synthesis. The fact that the rates of the ouabain-sensitive respiration in epididymal and in ejaculated spermatozoa are comparable suggests that the Na^+/K^+-ATPase contributes to the ATP turnover to the same extent.

The vanadate- and oligomycin-insensitive rates of respiration were found to be the same, indicating that the total cellular ATP turnover was linked to extramitochondrial ATP-consuming processes. The oligomycin-insensitive respiration was partly due to rotenone-insensitive, non-mitochondrial oxygen consumption [17,18]. The major part of the former was discussed to be caused by the uncoupled respiration in damaged cells [15]. This rate was significantly higher in ejaculated spermatozoa. From the present data, however, it could not be decided whether the higher rate was caused by the portion of damaged cells or by the degree of uncoupling in these cells.

Comparison of the actual respiration rates with uncoupled rates of the three preparations listed in Table II indicated that the capacity of respiration was by no means exhausted by the cellular energy demand. However, the uncoupled respiration is no direct measure of the capacity of mitochondria to produce ATP. This information is not available from intact cells. In epididymal spermatozoa permeabilized with digitonin, the respiration could be stimulated by adding ADP to max. 4.9 nmol O_2 per min, per μl cells in the presence of lactate plus malate, which enabled similar rates of uncoupled respiration as measured in intact cells. This value served to calculate the capacity of mitochondrial ATP production equalling 27 nmol ATP per min per μl

TABLE II

Respiration rates of epididymal and ejaculated spermatozoa with various effectors of cellular energy turnover

Incubations were carried out as described in Fig. 2. Given are means ± S.D. of (n) sperm samples

Effector	Concentration	Respiration rate (nmol O_2/min per μl cells)		
		epididymal spermatozoa	epididymal spermatozoa + caffeine	ejaculated spermatozoa
without		1.47 ± 0.29 (7)	2.42 ± 0.27 (7)	2.87 ± 0.46 (14)*
FCCP	0.60 μM	6.36 ± 1.36 (11)	6.06 ± 0.47 (6)	6.60 ± 1.42 (6)
Vanadate	0.22 mM	0.49 ± 0.10 (7)	0.49 ± 0.09 (7)	0.79 ± 0.19 (11) *
Ouabain	1.00 mM	1.29 ± 0.28 (7)	2.27 ± 0.24 (7)	2.62 ± 0.68 (7)
Oligomycin	0.88 μM	0.59 ± 0.14 (6)	0.50 ± 0.08 (7)	0.71 ± 0.01 (3) *
Rotenone	5.60 μM	0.18 ± 0.05 (6)	0.20 ± 0.06 (7)	0.19 ± 0.01 (4)
ATP turnover [a]		6.0	11.4	12.6

* Difference significant to caffeine-treated epididymal spermatozoa (t-test; $p < 0.05$)

[a] Calculated from the oligomycin-sensitive respiration using P/O = 3.

cells, taking into account the resting rate of respiration in the absence of ADP phosphorylation, and a P/O ratio of 3. The ATP turnover rates calculated for intact cells in the same way (Table II) indicated that the mitochondrial capacity is utilized by 20–25% in untreated epididymal, by 40–45% in caffeine-stimulated epididymal, and by 45–50% in ejaculated spermatozoa.

We concluded from the present data and previous results obtained from ejaculated spermatozoa, that, after ejaculation, the capacity of spermatozoal respiration and mitochondrial ATP production remains unchanged, but is utilized to a higher extent by the stimulated motility. A comparable stimulation of epididymal spermatozoa could be produced by caffeine. Besides motility, no other processes contributing significantly to the cellular energy turnover were affected by caffeine in vitro, or by constituents of the male accessory sex gland fluids upon ejaculation.

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