Effect of Low Levels of Carbon Dioxide on Aerobic Metabolism of Boar Spermatozoa

Kato, Seishiro
Kanda, Sunao
Ikegami, Junko

(Citation)
神戸大学農学部研究報告,15(1):189-193

(Issue Date)
1982

(Resource Type)
departmental bulletin paper

(Version)
Version of Record

(JaLCDOI)
https://doi.org/10.24546/00227272

(URL)
https://hdl.handle.net/20.500.14094/00227272

神大農研報 (Sci. Rept. Fac. Agr. Kobe Univ.) 15 : 189—193, 1982

EFFECT OF LOW LEVELS OF CARBON DIOXIDE ON AEROBIC METABOLISM OF BOAR SPERMATOZOA

Seishiro KATO*, Sunao KANDA* and Junko IKEGAMI**

(Received for publication on August 10, 1981)

Abstract

The effect of low levels of CO_2 on the aerobic metabolism of boar spermatozoa was investigated. Results obtained are as follows.

(1) Metabolic CO_2 markedly stimulated O_2 uptake of spermarozoa incubated with glucose, fructose, pyruvate or lactate as substrate. The initial level of CO_2 in the gas phase was approximately 0.3% in a flask incubated with metabolic CO_2. However, the stimulatory effect of CO_2 was not enhanced when the CO_2 level was increased to 1-2% ; this suggests that only a small amount of CO_2 is required for maximum metabolism of boar spermatozoa at a pH of 7.0. The response of spermatozoa to CO_2 depended on the presence of potassium (5mM).

(2) Low levels of CO_2 had no apparent effect on glucose utilization of spermatozoa but depressed the rate of lactate production. (3) A large amount of radioactivity which implies the fixation of CO_2 was detected in the acid soluble fraction of reaction mixture when spermatozoa were incubated with $^{14}CO_2$.

Introduction

Stimulation of spermatozoal metabolism by low levels of CO_2 or bicarbonate has been reported for the bull[4,10], ram[14,20], goat[7], rabbit[4,13] and fowl[4]. Spermatozoa of these species have a high capacity of utilizing glycolysable sugars and accumulate large amounts of lactate either under aerobic or anaerobic conditions[12]. Boar spermatozoa, however, have a low glycolysis rate and rapidly become immotile in the absence of oxygen[1]. It thus appears possible that the responce of boar spermatozoa to CO_2 may differ from that observed in other species. The present study was undertaken to investigate this possibility by measuring the metabolic activity of boar spermatozoa in the presence or absence of CO_2.

* Laboratory of Animal Reproduction.
** College of Agriculture, Kyoto University, Kyoto.

Materials and Methods

Semen was collected from four Landrace boars by the manual method and only sperm-rich fraction was used. Calcium-free Krebs-Ringer phosphate solution[3] (pH 7.0), containing 123 mM NaCl, 1 mM $MgSO_4 \cdot 7H_2O$, 5mM KCl, 16 mM sodium phosphate, was the basic diluent used in all studies. In cases when KCl was omitted from the diluent (Table 1), and when bicarbonate was added to the diluent to maintain the pH at 7.0(Table 3), isotonicity was maintained by adjusting the NaCl content. Substrates were added separately to the diluent to give the following amounts per flask : glucose, 10 μmol; fructose, 10 μmol; pyruvate, 20 μmol; lactate, 20 μmol.

Semen samples were centrifuged at 600g for 15 min to remove seminal plasma. The spermatozoa were washed twice using the same volume of diluent as the seminal plasma removed and resuspended in the washing

diluent to give a concentration of $3\text{-}4\times10^8$ cells per ml.

Washed spermatozoal suspensions (2.0 ml each) were incubated for 3 hr at 37°C with various substrates (0.5 ml each) in WARBURG flasks or DIXON-KEILIN flasks. O_2 uptake was measured in the absence of CO_2 and also in the presence of metabolic and exogeneous CO_2 by methods described previously[7]. Aliquots of the spermatozoal suspensions prior to incubation and those of the reaction mixtures after incubation were deprotenized by the addition of 1 volume of 5%(W/V) $ZnSO_4 \cdot 7H_2O$ and 1 volume of 0.3N $Ba(OH)_2$. Lactate was measured in the supernatant by the method of BARKER and SUMMERSON[2] and glucose by the glucose oxidase method[5].

In the experiment designed to examine the possibility that the fixation of CO_2 may occur in the boar spermatozoa, washed cell suspensions were incubated with pyruvate or glucose under the atmosphere contatining $^{14}CO_2$ (20μCi, 12μmol/flask)[8]. Following the incubation, the reaction mixtures were acidified with perchloric acid and centrifuged. The supernatant was neutralized with KOH and assayed for radioactivity in a toluene-dioxane-ethyl cellosolve scintillation mixture. The sperm-free diluent was treated in a similar manner to measure the background radioactive count. Spermatozoal suspensions boiled for 5 min were used as controls.

The number of spermatozoa in each flask was determined using a haemocytometer, and all the experimental measurements were expressed as values on a 10^8 cell basis. Data were analyzed by analysis of variance techniques. DUNCAN's new multiple range test was used to determine differences between means[18].

Results

Interacting Effects of Metabolic CO_2 and Potassium on the Metabolism of Glucose by Spermatozoa

Since the stimulatory effect of CO_2 on the metabolism of ram spermatozoa depends on the presence of potassium[14,20], the possible occurrence of such interactions in boar spermatozoa was first examined. As shown in Table 1, metabolic CO_2 markedly ($p<.01$) increased the O_2 uptake of spermatozoa only in the presence of potassium. Potassium by itself, with or without metabolic CO_2, also had the stimulatory effect ($p<.01$). In contrast, metabolic CO_2 and/or potassium had no significant effect on the rate of glucose utilization. For the production of lactate, the effect of metabolic CO_2 and/or potassium was dramatic. The amount of lactate produced, which was almost negligible in the absence of potassium, increased tremendously in the presence of potassium ($p<.01$). Interestingly, metabolic CO_2 depressed the rate of lactate production significantly ($p<.05$) in the presence on potassium.

Table 1. Effects of metabolic CO_2 and potassium on the metabolism of glucose by boar spermatozoa (n=6)

Gas phase	Potassium (5mM)	O_2 uptake (μl)	Glucose utilized (μg)	Lactate produced (μg)
No CO_2	−	15.0	70	4
	+	25.9	88	37
Metabolic CO_2	−	18.3	83	2
	+	44.2	112	19

After 15 min of equilibration, the spermatozoal suspensions were mixed with substrates and incubation was started. At this time, each flask with metabolic CO_2 contained approximately 0.3% CO_2 in the gas phase and 0.7 μmol bound CO_2 in the reaction mixture. At the completion of incubation, the level of CO_2 in the gas phase rose to approximately 2–3%.

All the experiments to be described below were carried out using the diluent containing potassium.

Effect of Metabolic CO_2 on the O_2 Uptake of Spermatozoa in the Presence of Different Substrates

As shown in Table 2, O_2 uptake of spermatozoa was markedly ($p<.01$) stimuladed by the addition of glucose, fructose, pyruvate or lactate. Metabolic CO_2 significantly ($p<.01$) stimulated the rate of O_2 uptake in the presence of all these substrates. The stimulatory effect was greater with glucose and fructose than with pyruvate and lactate.

Table 2. Effect of metabolic CO_2 on the oxygen uptake of boar spermatozoa in the presence of different substrates: glucose, fructose, pyruvate and lactate

Gas phase	O_2 uptake (μl)				
	Substrate added				
	None	Glucose	Fructose	Pyruvate	Lactate
Experiment 1 (n=5)					
No CO_2	6.7	26.7	27.4	—	—
Metabolic CO_2	6.0	43.9	46.2	—	—
Experiment 2 (n=6)					
No CO_2	7.8	29.0	—	31.4	30.5
Metabolic CO_2	—	46.7	—	45.2	41.9

Effect of Higher Levels of CO_2 on the Metabolism of Glucose by Spermatozoa

The results are shown in Table 3. O_2 uptake of spermatozoa was stimulated in the presence of metabolic CO_2 ($p<.01$). Initial level of CO_2 in the gas phase was approximately 0.3% in a flask incubated with metabolic CO_2. However, the stimulatory effect of CO_2 was not enhanced even when the CO_2 level was increased to 1-2%. Both metabolic and exogeneous CO_2 had no signifcant effect on glycolysis, although there was a tendency that the presence of the gas increased the rate of glucose utilization. On the other hand, lactate production was significantly ($p<.05$) depressed in the presence of CO_2.

Fixation of CO_2 by Spermatozoa

Evidence that the fixation of CO_2 occurs in boar spermatozoa is presented in Table 4. Significant ($p<.01$) amounts of radioactivity were detected in the acid soluble fraction of reaction mixture when spermatozoa were incubated in the presence of $^{14}CO_2$. The accumulation of radioactivity began to slow down after the first 15 min, and reached near plateau at 30 min. Similar amounts of radioactivity were incorporated when either pyruvate or glucose was used as substrate, while very little incorporation was observed when no substrate was present.

Discussion

It is apparent from these experiments that CO_2 acts on boar spermatozoa to stimulate respiration. The level of CO_2 that promotes optimum metabolism of spermatozoa seems to depend on the diluent used[7,20]. It appears,

Table 3. Effect of low levels of CO_2 on the metabolism of glucose by boar spermatozoa (n=8)

Gas phase	Bicarbonate conc. in diluent (mM)	O_2 uptake (μl)	Glucose utilized (μg)	Lactate produced (μg)
No CO_2	0	29.2	104	56
Metabolic CO_2	0	47.9	152	11
1% CO_2	1.18	50.5	124	19
2% CO_2	2.36	49.8	131	24

Table 4. Incorporation of ^{14}C from $^{14}CO_2$ into acid soluble fraction by boar spermatozoa (n=7)

Incubation time (min)	^{14}C incorporated ($\times 10^3$ cpm)			
	Live sperm			Dead sperm
	Pyruvate	glucose	None	Pyruvate
15	17.3	16.6	3.1	0.3
30	24.1	25.7	4.3	0.4
60	26.1	25.9	4.0	0.3
120	23.0	24.2	3.7	0.3
180	25.4	23.0	—	0.5

however, that a low level of CO_2, 0.3%, is sufficient for maintaining high rate of O_2 uptake of boar spermatozoa under conditions employed in the present study. It is interesting to note that CO_2 markedly stimulates respiration of boar spermatozoa without significantly altering the rate of glycolysis. The inhibitory effect of CO_2 on the lactate production suggests that in the presence of CO_2 the rate of conversion of pyruvate to lactic acid hardly exceeds the oxidative capacity of the tricarboxylic acid cycle.

Boar spermatozoa differ from rabbit, goat and ram spermtozoa in their response to CO_2. Thus MURDOCH and WHITE[13] found that CO_2 increased glycolysis and respiration to almost the same extent in rabbit spermatozoa. The same tendency was reported in goat spermatozoa by KATO et al[7]. MURDOCH and WHITE[14] have further shown that in ram spermatozoa CO_2 stimulates glycolysis to a much greater extent than it stimulates respiration. The reasons for these differences in the response to CO_2 among various species are still not clear. MURDOCH and WHITE[14] have suggested that differences in membrane permeability and in the initial rates of tricarboxylic acid cycle reactions may be the factors.

The present study confirms previous reports [14,20] that low concentrations of potassium stimulate aerobic metabolism of spermatozoa. Although the spermatozoa of different species respond differently to CO_2, the present results and those obtained by other workers [14,20] suggest that spermatozoa require adequate concentrations of potassium for CO_2 to exert maximal effects.

CO_2 fixation reactions involving the condensation of C_3 compounds with CO_2 is an important reaction in many cells for the replenishment of C_4 compounds[9]. In recent years, the occurrence of such reactions in spermatozoa was demonstrated for the ram[16], bull[17] and goat[8]. The present results suggest that boar spermatozoa are also capable of fixing CO_2. Thus CO_2 may stimulate respiration by increasing the intracellular levels of tricarboxylic acid cycle intermediates, as HAMNER and WILLIAMS[4] have suggested. The results also indicate that boar spermatozoa still possess a limited synthetic ability even after ejaculation. Although details of CO_2 fixation reactions in boar spermatozoa are still unknown, such synthetic reactions may be important for the maturation of spermatozoa in the epididymis.

At present, it is difficult to estimate the physiological importance of CO_2 in the female genital tract. The secretions of the female genital tract contain substantial amounts of CO_2 and bicarbonate together with adequate amounts of potassium and utilizable substrates such as pyruvate and lactate[4,6,11,13,19]. It has been suggested that the acrosome reaction and capacitation of spermatozoa are metabolically controlled[15]. Carbon dioxide may play a significant role in the regulation of these processes by altering the metabolism of spermatozoa.

References

1) Aaleers, J. G., T. Mann and C. Polge : *J. Reprod. Fert.*, **2**, 42-53, 1961.
2) Barker, J. B. and W. H. Summerson : *J. Biol. Chem.*, **138**, 535-554, 1941.
3) Dixon, M. : *Manometric Methods*, 3rd ed., University Press. Cambridge, 1951.
4) Hamner, C. E. and W. C. Williams : *Proc. Soc. Exp. Biol. Med.*, **117**, 240-243, 1964.
5) Huggett, A. St. G. and D. A. Nixon : *Biochem. J.*, **66**, 12, 1957.
6) Iritani, A., W. P. Gomes and N. L. Van Demark : *Biol. Reprod.*, **1**, 72-76, 1969.
7) Kato, S., A. Iritani and Y. Nishikawa : *Jpn. J. Zootech. Sci.*, **49**, 16-22, 1978.
8) Kato, S., A. Iritani, S. Yoshida and Y. Nishikawa: *Jpn. J. Zootech. Sci.*, **50**, 150-154, 1979.
9) Lachica, R. V. F. : Enzymologia, **34**, 281-298, 1968.
10) Lodge, J. R. and G. R. Salisbury : *J. Dairy Sci.*, **46**, 140-144, 1963.
11) Lutwak-Mann, C. : *Biochim. biophys. Acta*, **58**, 637, 1962.
12) Mann, T. : *The Biochemistry of Semen and of the Male Reproductive Tract*, Methuen, London., 1964.
13) Murdoch, R. N. and I. G. White : *Aust. J, biol. Sci.*, **21**, 961-972, 1968.
14) Murdoch, R. N. and I. G. White : *J. Reprod. Fert.*, **25**, 231-242, 1971.
15) Rogers, B. J. and R. Yanagimachi : *Biol. Reprod.*, **13**, 568-575, 1975.
16) O'Shea, T. and R. G. Wales : *J. Reprod. Fert.*, **14**; 333-334, 1967.
17) Sexton, T. J. and R. J. Flipse : *J. Dairy Sci.*, **54**, 417-419, 1971.
18) Steel, R. G. D. and J. H. Torrie : *Principles and Procedures of Statistics*, McGraw-Hill, New York, 1960.
19) Vishwakarma, P. : *Fertil. Steril.* **13**, 481-485, 1962.
20) Wales, R. G. and R. N. Murdoch : *Aust. J. biol. Sci.*, **24**, 345-354, 1971.
21) White, I. G. : *Aust. J. biol. Sci.*, **6**, 706- , 1953.

豚精子の好気的代謝に及ぼす低分圧の炭酸ガスの影響

加藤征史郎・苅田淳・池上順子

要約

低分圧のCO_2が豚精子の好気的代謝に及ぼす影響につき検討し，つぎのような結果をえた。(1)カリウムイオンの存在下では，グルコース，フラクトース，ピルビン酸および乳酸のいずれを基質とした場合でも，精子の酸素消費は代謝CO_2の蓄積により著しく促進された。しかし，代謝CO_2による精子の呼吸促進はカリウムイオンの不在下では明確でなかった。(2)代謝CO_2を含むフラスコでは，インキュベーション開始時の気相のCO_2分圧は約 0.3%であった。しかし，気相のCO_2分圧を1～2%まで高めても，CO_2による呼吸促進は増強されなかった。このことから，豚精子がpH 7.0で最も活発な好気的代謝を営むのに要するCO_2分圧はかなり低いと考えられる。(3)精子の解糖能については，代謝CO_2または1～2%CO_2によりグルコース消費の増加する傾向がみられたが，この促進は統計的に有意なものではなかった。一方，乳酸生成は低分圧CO_2により有意に抑制された。(4)精子浮遊液を$^{14}CO_2$含有の空気下でインキュベートすると，その酸溶性分画から多量の放射能が検出され，このことから豚精子は射出後においてもCO_2固定能を保持することが推定された。