Fructose 2,6-bisphosphate and the control of glycolysis in bovine spermatozoa

Benoît Philippe, Guy G. Rousseau* and Louis Hue

Hormone and Metabolic Research Unit, University of Louvain Medical School and International Institute of Cellular and Molecular Pathology, UCL 7529, Avenue Hippocrate 75, B-1200 Brussels, Belgium

Received 15 March 1986

Epididymal bovine sperm contain fructose-1,6-bisphosphatase activity which is inhibited by AMP and by fructose 2,6-bisphosphate. Sperm phosphofructokinase displays kinetic characteristics that are typical of the F-type and it is stimulated by fructose 2,6-bisphosphate. The concentration of sperm fructose 2,6-bisphosphate remained unaffected at 1–2 μM when the glycolytic rate was either increased by glucose, caffeine or antimycin, or decreased by α-chlorohydrin or 6-chloro-6-deoxyglucose.

Fructose-1,6-bisphosphatase *Phosphofructokinase* *Glucose* *Gossypol* *α-Chlorohydrin* *(Epididymis)*

1. INTRODUCTION

Epididymal bull sperm provide an interesting model for studying the control of glycolysis because glucose metabolism is simpler than in other tissues. There is no glycogen synthesis, no pentose phosphate pathway, and no formation of metabolites such as uronic acids [1]. The discovery in liver [2] of Fru-2,6-P2 makes it important to determine whether this key regulator of glycolysis is present in epididymal sperm and whether it is involved in controlling sperm glycolysis. Fru-2,6-P2 stimulates PFK and inhibits FBPase [3]. We have therefore investigated the kinetics of these enzymes in epididymal bull sperm and their sensitivity to Fru-2,6-P2. While sperm PFK has been studied [4,5], there is no available evidence for the presence of FBPase in these cells [6]. To our knowledge, this paper provides the first demonstration of the occurrence of FBPase and Fru-2,6-P2 in sperm.

* To whom correspondence should be addressed

Abbreviations: Fru-2,6-P2, fructose 2,6-bisphosphate; PFK, phosphofructokinase (EC 2.7.1.11); FBPase, fructose-1,6-bisphosphatase (EC 3.1.3.11)

2. MATERIALS AND METHODS

2.1. *Preparation and incubation of sperm cells*

Testes with epididymides attached were kept on ice and used within 2 h of killing the bulls. Sperm obtained from the cauda epididymis by retrograde flushing of the proximal vas deferens with ice-cold 0.15 M NaCl were collected by centrifugation (300 × *g*, 10 min), washed twice with Krebs-Henseleit buffer [7] and resuspended in the same buffer for incubation. 2–4 ml of cell suspension containing 2–10 × 10^8 cells/ml were incubated in 20-ml glass vials at 37°C in a rotary shaker with continuous gassing with O_2/CO_2 (19:1). After a 30-min preincubation, glucose was added and lactate production was measured during 30 min of incubation. Lactate production was linear over this incubation period and within the range of cell density used. Norbornene, which is the incubation buffer used in [6], was also tested but discarded when it was found to be an inhibitor of lactate production (table 1). Lactate was measured [8] in neutralized perchlorate extracts. For the measurement of Fru-2,6-P2 [9], 1-ml samples were taken, treated with 0.05 ml of 1 M NaOH and heated at 80°C for 10 min [10].

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

2.2. *Purification and assay of PFK and FBPase*

PFK was purified from frozen sperm samples and frozen rat gastrocnemius. After homogenisation (about 5×10^{10} cells or 1 g of muscle in 4 ml) with an Ultra-Turrax in 50 mM Tris-HCl, 50 mM NaF, 1 mM dithiothreitol, 1 mM ATP-Mg, pH 7.6, and centrifugation (10000 × *g*, 30 min), PFK was precipitated (50% ammonium sulfate) and purified by chromatography on ATP-agarose [11]. PFK activity was assayed as described in [12]. FBPase was partially purified and assayed as in [13].

3. RESULTS AND DISCUSSION

3.1. *Kinetic properties of PFK and sensitivity to Fru-2,6-P2*

The influence of positive and negative effectors on the kinetics of PFK was studied, and a comparison was made between the sperm and muscle enzymes. Whereas the saturation curves with the two substrates fructose 6-phosphate and ATP were rather similar for the two enzymes, sperm PFK was much less sensitive than muscle PFK to allosteric stimulation by AMP and glucose 1,6-bisphosphate (fig.1). This suggests that sperm PFK is of the poorly regulated F-type which is found in fibroblasts [14] and in ascites tumor cells [15]. This conclusion is at variance with immunological studies which have shown that the major PFK isozymes present in human testis were of the M- and L-type with a small contribution of the F-type [5]. The cellular heterogeneity of testis, or sperm maturation during transit to cauda epididymis, or else species differences, may explain this discrepancy.

Despite the lack of stimulation by glucose 1,6-bisphosphate, sperm PFK was stimulated by Fru-2,6-P2 (fig.2) as is the case for the F-type isozyme of ascites tumor cells [15]. Stimulation of rat spermatid PFK by Fru-2,6-P2 has been described [16]. In the presence of physiological concentrations of substrates and effectors, the K_a for Fru-2,6-P2 was about 0.15 μM for the sperm enzyme and several-fold larger for the muscle enzyme (fig.2). Under the same conditions and in the presence of 0.1 μM Fru-2,6-P2, sperm PFK was fully stimulated by 20 mM P_i while the muscle enzyme was hardly affected (not shown).

Fig.2 also illustrates the inhibitory effect of gossypol, a male contraceptive, on the activity of muscle and sperm PFK. Sperm PFK was less sensitive than muscle PFK and the inhibition could be partially relieved by Fru-2,6-P2.

3.2. *Sperm FBPase*

In contrast to an earlier report [6], FBPase activity could be detected and partially purified from bovine sperm. The activity was inhibited by AMP and Fru-2,6-P2 (fig.3) as is the case for the liver enzyme. There was a synergism between the two inhibitors, 90% or more inhibition being achieved with 10 μM AMP in the presence of 0.5 μM Fru-2,6-P2. Gossypol (50 μM) was found to inhibit FBPase by more than 50% (not shown). From the V_{max} of PFK and FBPase (0.17 and 0.06 μmol/min

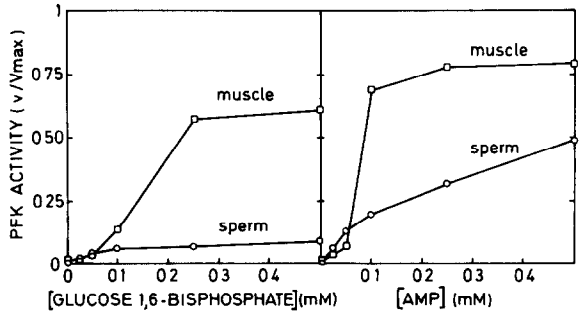

Fig.1. Effect of glucose 1,6-bisphosphate and AMP on muscle and sperm PFK activity. PFK activity was measured in the presence of 0.5 mM fructose 6-phosphate, 1 mM ATP-Mg and the indicated concentrations of glucose 1,6-bisphosphate or AMP.

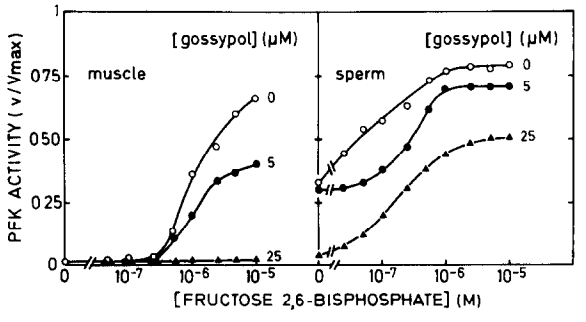

Fig.2. Effect of Fru-2,6-P2 and gossypol on muscle and sperm PFK activity. PFK activity was measured in the presence of 0.1 mM fructose 6-phosphate, 2.5 mM ATP-Mg, 0.05 mM AMP, 5 mM P_i and the indicated concentrations of Fru-2,6-P2 and gossypol.

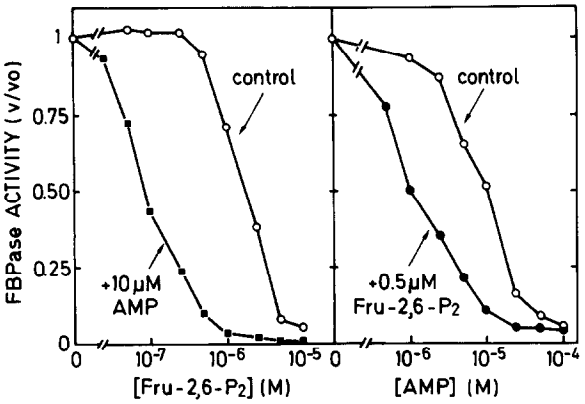

Fig.3. Effect of Fru-2,6-P2 and AMP on sperm FBPase activity. FBPase activity was measured in the presence of 0.025 mM fructose 1,6-bisphosphate (vo) and the indicated concentrations of Fru-2,6-P2 and AMP.

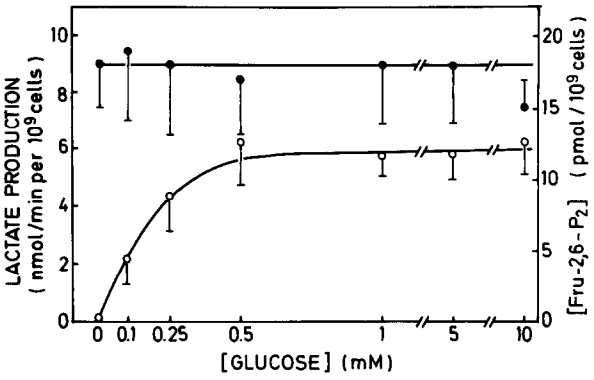

Fig.4. Lactate production (○) and Fru-2,6-P2 content (●) of bovine sperm incubated with increasing glucose concentrations. The data are means (± SE) for at least 4 different cell preparations.

per mg of sperm protein) one concludes that sperm cells contain about 3-times more PFK than FBPase activity. The presence in sperm of a specific FBPase could explain the occurrence of the cycle between fructose 6-phosphate and fructose 1,6-bisphosphate that was observed in bovine sperm under conditions of low glycolytic rate [6].

3.3. *Lack of correlation between glycolytic rate and Fru-2,6-P2 concentration*

Experiments were performed to determine whether Fru-2,6-P2 is present in sperm and, if so, to relate its concentration with various glycolytic rates. Incubation of sperm with increasing concentrations of glucose caused a dose-dependent stimulation of lactate production (fig.4). Fru-2,6-P2 was detected in these cells even in the absence of glucose, but its concentration remained unchanged despite the increase in glycolytic rate. Similar results were obtained with fructose as the glycolytic substrate (not shown). In addition, there was no change in Fru-2,6-P2 concentration (table 1) whether lactate production was inhibited by α-chlorohydrin, 6-chloro-6-deoxyglucose or norbornene, or whether it was stimulated by caffeine or antimycin. These substances are inhibitors and stimulators of sperm glycolysis [17,18]. Neither insulin, which increases heart Fru-2,6-P2 concentration [19], nor dibutyryl cyclic AMP, which decreases liver Fru-2,6-P2 concentration [3], had any similar effect on the concentration of this modulator in sperm (not shown).

From published estimates of spermatozoal cell volume [6] one can calculate that the concentration of Fru-2,6-P2 in sperm is about 1–2 μM, i.e. not as high as in liver from fed rats but similar to that in heart muscle [3,19]. This concentration is sufficient to fully activate sperm PFK and inhibit FBPase (figs 2,3). However, the actual concentration of free Fru-2,6-P2 is probably lower due to binding to these two enzymes.

Unlike the case in liver, Fru-2,6-P2 concentration is rather insensitive to regulatory influences in sperm perhaps as a result of compartmentation. There is evidence for a role of Fru-2,6-P2 in the control of glycolysis in rat liver [3,20], pancreatic islets [21], adipocytes [22], enterocytes [10], and hepatoma cells [11], and in chick embryo fibroblasts [23]. In contrast, the present data suggest that epididymal sperm glycolysis can change irrespective of fluctuations in Fru-2,6-P2 concentration.

These data pertain to the search for a reversible male contraceptive which could control sperm maturation in the epididymis by inhibiting glycolysis [24]. Glucose metabolism is also obligatory to initiate both the acrosome reaction and the whiplash motility associated with fertilizing ability [25]. The reversible antifertility action of chlorinated sugars has been ascribed to an immobilization of epididymal sperm as a result of in-

Table 1

Effect of various agents on lactate production and Fru-2,6-P2 content of bovine sperm

Addition	Fru-2,6-P2 content	Lactate production
None	100	100
5 mM α-chlorohydrin	74	0
1 mM 6-chloro-6-deoxyglucose	98	46
20 mM norbornene	96	60
10 mM caffeine	85 ± 4	172 ± 15
0.01 mM antimycin	97 ± 4	324 ± 20

Sperm cells were incubated with 5 mM glucose for 30 min at 37°C. Under control conditions, Fru-2,6-P2 concentration was 12.7 ± 1.4 nmol/10^9 cells and lactate production was 5.9 ± 1.5 nmol/min per 10^9 cells for 4 different experiments. The data are expressed as percent of control and are means of duplicate experiments with one cell preparation or means (± SE) for 3 different cell preparations

hibition of sperm glycolysis [17]. The male contraceptive gossypol acts, at least in part, through an analogous mechanism [26]. The sensitivity of muscle PFK towards gossypol described here is interesting in view of the fatigue and muscle weakness reported in men taking gossypol as a contraceptive [27].

ACKNOWLEDGEMENTS

We thank T. Lambert for typing the manuscript. L.H. is Maître de Recherches of the Belgian Fonds National de la Recherche Scientifique. This work was supported in part by the Fonds de la Recherche Scientifique Médicale (Belgium) and by the Ford, Mellon, and Rockefeller Foundations.

REFERENCES

[1] Hammerstedt, R.H. (1980) in: Reproduction and Contraception (McKerns, K.W. ed.) pp.353–391, Plenum, New York.
[2] Van Schaftingen, E., Hue, L. and Hers, H.G. (1980) Biochem. J. 192, 897–901.
[3] Hers, H.G. and Hue, L. (1983) Annu. Rev. Biochem. 52, 617–653.
[4] Hoskins, D.D. and Stephens, D.T. (1969) Biochim. Biophys. Acta 191, 292–302.
[5] Kahn, A., Meienhofer, M.C., Cottreau, D., Lagrange, J.L. and Dreyfus, J.C. (1979) Hum. Genet. 48, 93–108.
[6] Hammerstedt, R.H. and Lardy, H.A. (1983) J. Biol. Chem. 258, 8759–8768.
[7] Krebs, H.A. and Henseleit, K. (1932) Hoppe-Seyler's Z. Physiol. Chem. 210, 33–66.
[8] Hohorst, H.J. (1963) in: Methods of Enzymatic Analysis (Bergmeyer, H.U. ed.) pp.266–270, Academic Press, New York.
[9] Van Schaftingen, E., Lederer, B., Bartrons, R. and Hers, H.G. (1982) Eur. J. Biochem. 129, 191–195.
[10] Louis, J., Philippe, B. and Hue, L. (1985) Biochem. J. 232, 622–623.
[11] Loiseau, A., Rousseau, G.G. and Hue, L. (1985) Cancer Res. 45, 4263–4269.
[12] Van Schaftingen, E., Jett, M.F., Hue, L. and Hers, H.G. (1981) Proc. Natl. Acad. Sci. USA 78, 3483–3486.
[13] Corredor, C., Bosca, L. and Sols, A. (1984) FEBS Lett. 167, 199–202.
[14] Meienhofer, M.C., Cottreau, D., Dreyfus, J.C. and Kahn, A. (1980) FEBS Lett. 110, 219–222.
[15] Bosca, L., Aragon, J.J. and Sols, A. (1982) Biochem. Biophys. Res. Commun. 106, 486–491.
[16] Nakamura, M., Suzuki, A., Okinaga, S. and Arai, K. (1984) Dev. Growth Differ. 26, 11–16.
[17] Jones, A.R. (1983) Aust. J. Biol. Sci. 36, 333–350.
[18] Garbers, D.L., Lust, W.D., First, N.L. and Lardy, H.A. (1971) Biochemistry 10, 1825–1831.
[19] Rider, M.H. and Hue, L. (1984) FEBS Lett. 176, 484–488.
[20] Hue, L., Sobrino, F. and Bosca, L. (1984) Biochem. J. 224, 779–786.
[21] Sener, A., Van Schaftingen, E., Van de Winkel, M., Pipeleers, D.G., Malaisse-Lagae, F., Malaisse, W.J. and Hers, H.G. (1984) Biochem. J. 221, 759–764.
[22] Rider, M.H. and Hue, L. (1985) Biochem. J. 225, 421–428.
[23] Bosca, L., Rousseau, G.G. and Hue, L. (1985) Proc. Natl. Acad. Sci. USA 82, 6440–6444.
[24] Voglmayr, J.K. (1976) in: Regulatory Mechanisms of Male Reproductive Physiology (Spilman, C.H. et al. eds) pp.101–113, Excerpta Medica, Amsterdam.
[25] Fraser, R.L. and Quinn, P.J. (1981) J. Reprod. Fertil. 61, 25–35.
[26] Wichmann, K., Käpyaho, K., Sinervirta, R. and Jänne, J. (1983) J. Reprod. Fertil. 69, 259–264.
[27] Prasad, M.R.N. and Diczfalusy, E. (1982) Int. J. Androl. 5 (suppl.), 53–70.