MODIFICATION OF THE METABOLISM OF RAT EPIDIDYMAL SPERMATOZOA BY SPERMINE

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SUMMARY: Spermine (1 to 10 mM) markedly enhanced (2- to 7-fold) the formation of labelled lactate from radioactive fructose in rat epididymal spermatozoa in vitro. The combination of spermine and calcium (1 mM), the latter ion inconsistently stimulating the formation of carbon dioxide and lactate from fructose, resulted in a striking synergistic stimulation of the fructolysis in epididymal spermatozoa. The production of lactate from fructose in the presence of spermine and calcium increased 5- to 30-fold over that in normal Ringer solution. Spermidine, but not putrescine, also stimulated the formation of lactate from fructose in epididymal spermatozoa. While stimulating the aerobic fructolysis the polyamines inhibited the formation of radioactive CO_2 from (2-^{14}C)pyruvate thus apparently interfering with the reactions of the tricarboxylic acid cycle. In this respect, however, oxidized spermine {N,N´-bis(3-propionaldehyde)-1,4-diaminobutane} was far more effective. Spermine (and spermidine) being normal constituents of the secretions of male accessory sexual glands in most mammals could conceivably belong to the factors responsible for the "intensely glycolytic" nature of ejaculated spermatozoa.

Although the presence of unusually high concentrations of polyamines, notably spermine, in mammalian semen has been known for almost three centuries (1) no specific physiological function has been established for this polyamine. While a great variety of biological effects, if not necessarily physiological functions, of polyamines have been described in a number of system involving both prokaryotic and eukaryotic organisms (2) there appear to be no systematic studies on the possible effects of polyamines on the metabolism of mammalian spermatozoa. The few reports describing the influence of added spermine on the motility and survival of mammalian postejaculatory spermatozoa are slightly confusing and the effects of polyamines can be considered neither clearly beneficial nor entirely harmful (3).

When mammalian spermatozoa move through the male reproductive tract to finally reach the place of fertilization in the female reproductive tract they are confronted by profound changes in their immediate environments during this passage. The first fundamental change takes

place when the quiescent epididymal spermatozoa get contact with the secretions of the male accessory sexual glands, i.e. at the time of ejaculation. The exposure of sperm cells to seminal plasma initiates the motility and greatly enhances sperm enrgy metabolism (1). The factors resposible for the activation of mammalian spermatozoa are not fully understood although it appears that cyclic nucleotides are involved in the initiation of motility (4) and in the stimulation of respiration (4) and fructolysis (5) as well as in the senescence changes (6) in aged ejaculated spermatozoa of a variety of mammalian species.

Our present results indicate that the polyamines (spermine and spermidine), occuring at high concentrations in rat prostate (7), can modify the metabolism of rat epididymal spermatozoa by markedly enhancing the rate of fructolysis with a concomitant inhibition of the tricarboxylic acid cycle.

MATERIALS AND METHODS

Caudal epididymal spermatozoa from adult Wistar rats (250-300 g) were milked into prewarmed (37°C) Ringer solution containing NaCl (123 mM), KCl (5 mM), $MgSO_4$ (1 mM) and human albumin (0.3 %) (obtained from the Blood Service of Finnish Red Cross, Helsinki, Finland) buffered with 37 mM Tris-HCl (pH 8.0). In experiments involving the use of (^{14}C)pyruvate the Ringer solution also contained 2 mM glucose.

Uniformly labelled (^{14}C)fructose (specific radioactivity 100 mCi/mmole), (1-^{14}C)pyruvate (sp.act. 8.92 mCi/mmole) and (2-^{14}C)pyruvate (sp.act. 10 mCi/mmole) were purchased from the Radiochemical Centre (Amersham, England).

Putrescine,spermidine and spermine (as their hydrochloride salts) were purchased from Calbiochem (San Diego, Calif., U.S.A.). Cadaverine was obtained from Nutritional Biochemicals Corp. (Cleveland, Ohio, U.S.A.) and 1,3-diaminopropane from Fluka AG (Buchs SG, Switzerland). The stock solutions of polyamines were neutralized before use.

Oxidized spermine {N,N´-bis(3-propionaldehyde)-1,4-diaminobutane} was prepared enzymically with the aid of partially purified amine oxidase from bovine blood plasma, and isolated from the incubation mixture essentially as described earlier (8). The activity of amine oxidase was measured by the method of Aarsen and Kemp (9) using o-dianisidine as the chromogen.

The formation of radioactive lactate from (U-^{14}C)fructose was measured by the method of Hoskins and Patterson (10).

The collection and counting of $^{14}CO_2$ formed from radioactive fructose or pyruvate were performed as routinely used for decarboxylase assays in this laboratory (11).

RESULTS

When rat epididymal spermatozoa were incubated at 37°C in the presence of uniformly labelled (^{14}C)fructose small but easily detectable amounts of radioactive lactate were formed. Labelled fructose was also converted to $^{14}CO_2$, however, the formation of carbon dioxide varied

greatly and the total amount of $^{14}CO_2$ was only a small fraction of that of (^{14}C)lactate (from less than 1 mole% to about 10 mole%).

Table 1 shows that spermine remarkably enhanced the spermatozoal fructolysis. The addition of 1 to 10 mM spermine to the epididymal sperm suspensions resulted in a 2- to 7-fold increase in the formation of labelled lactate from uniformly labelled fructose (Table 1). Table 1 also shows that the inclusion of 1 mM $CaCl_2$ appeared to influence the rate of fructolysis in epididymal spermatozoa. The effect of latter ion, however,

Table 1. Effect of polyamines and Ca^{++} on the conversion of (U-^{14}C)fructose to labelled CO_2 and lactate. Rat epididymal spermatozoa (11.5 to 17.6 x 10^8 cells) were incubated in the presence of additions indicated for 15 min at 37°C whereafter (U-^{14}C)fructose was added (final concentration 0.25 mM) and the incubation continued for further 60 min.

	Addition	Concentration (mM)	Conversion of (U-^{14}C)fructose to $^{14}CO_2$ pmoles (%)	^{14}C-lactate pmoles (%)
Expt. 1.				
	None	-	25 (100)	270 (100)
	Spermine	5	29 (120)	1 050 (390)
	$CaCl_2$	1	45 (180)	980 (360)
	Spermine +	5		
	$CaCl_2$	1	210 (840)	7 840 (2 900)
Expt. 2.				
	None	-	1.3 (100)	180 (100)
	Spermine	1	2.0 (150)	760 (420)
		5	3.0 (230)	1 150 (640)
		10	1.0 (80)	1 360 (760)
	$CaCl_2$	1	2.2 (170)	240 (130)
		5	4.0 (310)	220 (120)
	Spermine +	5		
	$CaCl_2$	1	2.0 (150)	1 990 (1 100)
Expt. 3.				
	None	-	3.3 (100)	540 (100)
	Spermine	5	5.2 (160)	1 180 (220)
	Spermidine	5	5.3 (160)	1 000 (190)
	Putrescine	5	10.3 (310)	590 (110)
	$CaCl_2$	1	7.5 (230)	450 (80)
	Spermine +	5		
	$CaCl_2$	1	45.2 (1 400)	2 820 (520)

was inconsistent varying from slight inhibition to more than 3-fold stimulation of the lactate production (Table 1).

A combination of spermine (5 mM) and Ca^{++} (1 mM) resulted in an apparent synergistic stimulatory effect on the formation of lactic acid from fructose in epididymal spermatozoa. The dramatic stimulation of lactate formation in the presence of spermine an Ca^{++} varied from 5- to 30-fold over the control values (Table 1).

Although spermine greatly enhanced the formation of lactate from (U-^{14}C)fructose the production of radioactive CO_2 either remained unchanged or only slightly increased in the presence of the polyamine (Table 1). Unlike spermine and spermidine, which also enhanced the formation of lactate, calcium consistently stimulated the formation of carbon dioxide from radioactive fructose (Table 1). Only in one experiment (Expt. 3) the combination of spermine and calcium produced a greater enhancement in the formation of $^{14}CO_2$ than of (^{14}C)lactate.

Assuming that a major portion of the $^{14}CO_2$ evolved from radioactive fructose occured via pyruvate, the finding indicating that the formation of carbon dioxide was not always increased to the same extent as did lactate suggests that polyamines actually might have inhibited the further oxidation of pyruvate.

Fig. 1 illustrates that spermine at concentrations 5 mM or higher indeed inhibited the formation of radioactive carbon dioxide from (2-^{14}C)pyruvate virtually without any influence on the oxidation of

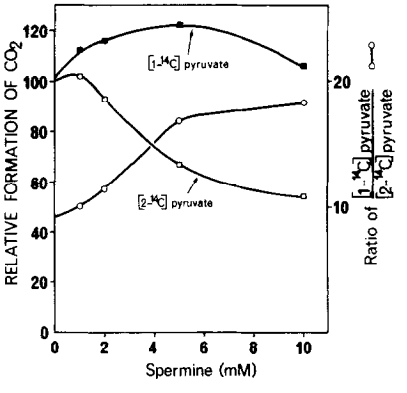

Fig. 1. Effect of spermine on the oxidation of selectively labelled pyruvate in rat epididymal spermatozoa. The spermatozoa (3.6 x 10^8 cells) were incubated at 37°C for 30 min in the presence of varying concentrations of spermine. After this prior incubation radioactive pyruvate (final concentration 0.1 mM) was added and the incubation continued for further 30 min.

(1-^{14}C)pyruvate. The release of radioactive carbon dioxide from (2-^{14}C)pyruvate under aerobic conditions mainly reflects the activity of tricarboxylic acid cycle whereas radioactive CO_2 derived from (1-^{14}C)pyruvate (carboxyl labelled) is partly due to the oxidation of pyruvate in the tricarboxylic acid cycle, partly due to the pyruvate dehydrogenase system (formation of acetyl CoA) and also due to a dismutase reaction where two molecules of pyruvate are converted to one molecule of each lactate, acetate and carbon dioxide. The latter reaction supposedly also occurs in mammalian spermatozoa (12, 13, 14).

As shown in Table 2, the effect of 5 mM 1,3-diaminopropane was comparable with that of spermine whereas 5 mM spermidine, putrescine or cadaverine did not inhibit the formation of CO_2 from (2-^{14}C)pyruvate. At the concentration of 15 mM all the amines exhibited a clear inhibition

Table 2. Effect of different amines, oxidized spermine, malonate and vigorous mechanical shaking on the oxidation of radioactive pyruvate in rat epididymal spermatozoa. The spermatozoa (3.2 or 7.4 x 10^8 cells) were incubated with the additions indicated for 30 min at 37°C whereafter pyruvate (final concentration 0.1 mM) was added and the incubation continued for further 30 min. Oxidized spermine, {N,N´-bis(3-propionaldehyde)-1,4-diaminobutane}.

Addition	Concentration (mM)	Formation of $^{14}CO_2$ from (1-^{14}C)pyruvate pmoles (%)	(2-^{14}C)pyruvate pmoles (%)
Expt. 1.			
None	-	9 480 (100)	890 (100)
Spermine	5	11 590 (130)	600 (67)
Spermidine	5	10 070 (110)	1 040 (120)
Putrescine	5	9 990 (110)	940 (110)
1,3-diaminopropane	5	11 130 (120)	550 (62)
Cadaverine	5	10 260 (110)	870 (98)
Expt. 2.			
None	-	9 120 (100)	1 020 (100)
Spermine	15	7 470 (82)	450 (45)
Spermidine	15	7 990 (88)	580 (57)
Putrescine	15	8 670 (95)	460 (45)
Oxidized spermine	0.5	5 300 (58)	100 (10)
	1.0	1 650 (18)	11 (1)
Malonate	5	12 300 (140)	310 (30)
Vigorous shaking	-	4 570 (50)	22 (2)

of the formation of labelled carbon dioxide from (2-^{14}C)pyruvate (Table 2). Furhermore, malonate (5 mM) expectedly possessed a preferential inhibition of the formation of radioactive CO_2 from (2-^{14}C)pyruvate while even slightly enhancing the evolution of $^{14}CO_2$ from (1-^{14}C)pyruvate (Table 2). As also shown in Table 2, the system responsible for the oxidation of (2-^{14}C)pyruvate to radioactive carbon dioxide seemed to be sensitive to structural damage since vigorous mechanical shaking (on Vortex mixer) of spermatozoa suspension virtually abolished the incorporation of radioactivity from (2-^{14}C)pyruvate to CO_2.

The iminoaldehyde derivative of sperminc {N,N´-bis(3-propinaldehyde)-1,4-diaminobutane', "oxidized spermine"} appeared to be extremely powerful inhibitor of the oxidation of pyruvate in the epididymal spermatozoa (Table 2). Oxidized spermine at concentrations as low as 0.5 to 1.0 mM almost completely abolished the formation of $^{14}CO_2$ from (2-^{14}C)pyruvate whereas the production of radioactive carbon dioxide from (1-^{14}C)pyruvate, albeit inhibited, was considerably more resistent against the action of the iminoaldehyde (Table 2).

It should be mentioned that under the experimental conditions used there was virtually no conversion of polyamines to their oxidized derivatives during the incubation (human serum albumin used in the incubation mixture was found to be devoid of any amine oxidase activity).

Table 3 shows that the addition of rat prostatic secretion (milked from the ventral prostate) to the suspension of epididymal spermatozoa resulted in a similiar preferential inhibition of the formation of labelled CO_2 from (2-^{14}C)pyruvate as did the addition of spermine. This might

Table 3. Effect of spermine and prostatic secretion on the oxidation of radioactive pyruvate in rat epididymal spermatozoa. Epididymal spermatozoa (3.6×10^8 cells) were incubated with additions indicated as described in the legend to Table 2.

Addition	Formation of $^{14}CO_2$ from (1-^{14}C)pyruvate pmoles (%)	(2-^{14}C)pyruvate pmoles (%)
None	8 510 (100)	440 (100)
Spermine (5 mM)	5 210 (61)	190 (43)
Prostatic secretion (10%)	3 250 (38)	70 (16)

conceivably indicate that the modification of the metabolism of the epididymal spermatozoa by prostatic secretion is, at least partly, due to its high content of spermine and spermidine (1, 7).

DISCUSSION

The postulated physiological functions of polyamine putrescine, spermidine and spermine are most often related to the synthesis and accumulation of nucleic acids (for references see 2, 15). There exist only a surprisingly limited number of studies on the influence of polyamines on the individual reactions of intermediary metabolism of any kind of cells (2).

It has been recently reported that spermidine (and spermine), but not putrescine, possess an insulin-like effect on rat adipocytes by greatly enhancing the oxidation of exogenous glucose and concomitantly inhibiting the lipolysis (15, 16).

The mechanism of the action of polyamines on spermatozoal fructolysis remains open. Neither it is known whether the activation occurs as a result of direct interaction of the polyamines with sperm membranes, as it appears to be the case in rat adipocytes (17), or whether spermine specifically stimulates some of the glycolytic enzymes. In any case, however, the stimulation is elicited at physiological concentrations of polyamines found in the rat prostate (7).

The remarkable synergistic stimulation of sperm fructolysis by spermine and calcium is a phenomenon that might be of some physiological importance. Calcium ion appears to be specifically required for the activation of motility in quiescent pre-ejaculatory hamster sperm (18). Ca^{++} is supposed to activate the motionless epididymal spermatozoa by initiating a synthesis of cyclic AMP (18). It appears that the activation *in vivo* of the motility can take place only after ejaculation since hamster epididymal fluid contains high phosphodiesterase activity and too little calcium while the seminal plasma contains sufficient concentrations of the latter ion (18). The mechanism of the synergism of spermine and calcium is not known but it is possible that spermine changes the permeability for Ca^{++} or *vice versa*, or the polyamine is involved in the numerous interactions between calcium and cyclic nucleotides (19). It should be mentioned,however, that under conditions employed in the present experiments we found that cyclic AMP or cyclic GMP (0.5 to 2 mM), in the absence or presence of spermine, invariably were inhibitory to the spermatozoal fructolysis.

Polyamines inhibited the conversion of (2-^{14}C)pyruvate to $^{14}CO_2$ thus apparently interfering with the reactions of the tricarboxylic acid cycle.

In this respect,however, the iminoaldehyde derivative of spermine was far more potent. The spermicidal action of oxidized spermine (and spermidine) has been known since late fifties when Tabor and Rosenthal (20) showed that purified amine oxidase and spermine rapidly immobilized mammalian sperm cells. It also appears that seminal spermine can be oxidized in human semen to iminoaldehydes by a diamine oxidase recently purified from human seminal plasma (21). However,rat prostate,seminal vesicle or their secretions, unlike human prostate and seminal plasma (22), do not contain any measurable diamine oxidase activity (unpublished observation). Thus the physiological significance of oxidized polyamines to the survival and metabolism of rat postejaculatory spermatozoa is doubtful,however, the polyamines as such might conceivably be involved in the metabolic adjustments making the epididymal spermatozoa capable of surviving in the female reproductive tract.

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