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Effect of caffeine and of pentoxifylline on the motility and metabolism of human spermatozoa

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Summary. Human spermatozoa were washed and incubated with 6 mM-caffeine or 0·15–1·2 mM-pentoxifylline. Sperm motility was measured by time-lapse photography, the rate of glycolysis by the release of tritiated water from 1 mM-[3-^{3}H]D-glucose and the rate of mitochondrial respiration by the release of $^{14}CO_2$ from 1 mM-[U-^{14}C]-L-lactate or 1 mM-[2-^{14}C]pyruvate. Caffeine stimulated the majority of spermatozoa to convert from the 'rolling' to the 'yawing' mode of progression with a concomitant increase in lateral head displacement from 4·1 ± 0·09 μm (343) to 6·7 ± 0·25 μm (105) (mean ± s.e.m. (number of spermatozoa)). There was a 45% decline in the percentage of progressively motile spermatozoa and a very small decrease in their velocity. Pentoxifylline had only a slight effect on lateral head displacement or percentage motility but produced a significant increase in velocity. Both compounds increased the rate of glycolysis by >40% but elevated the rate of $^{14}CO_2$ production to a smaller extent. The concentrations of ATP and ADP changed very little. We conclude that the glycolytic pathway in human spermatozoa can respond efficiently to changes in energy demand.

Keywords: spermatozoa; metabolism; motility; ATP; man

Introduction

Defective energy metabolism in spermatozoa is a potential cause of male infertility and some inhibitors of sperm energy metabolism (e.g. α-chlorohydrin) are effective male contraceptives in animals (see Ford & Waites, 1986). The concentration of ATP in human semen has been proposed as an index of fertility (Comhaire *et al.*, 1983) although its predictive power was slight in a later study (Irvine & Aitken, 1986). Despite these practical concerns, knowledge about the regulation of energy metabolism in human spermatozoa is sparse. Human spermatozoa can obtain energy both from glycolysis and from mitochondrial oxidative phosphorylation but in comparison to other species they derive a greater share of their ATP production from the former (see Ford & Rees, 1990). However, the way the different pathways respond to changes in energy demand is unknown.

Methyl xanthines, of which caffeine has been most used, can enhance sperm motility, especially in samples of poor quality. They are generally supposed to act through the inhibition of cAMP phosphodiesterase which leads to an increase in the intracellular cAMP concentration (see Garbers & Kopf, 1980; Tash & Means, 1982). However, in bull spermatozoa cellular alkalinization (Vijayaraghavan *et al.*, 1985) or other unknown events (Schoff & Lardy, 1987) may be involved. In bull and boar spermatozoa the increased motility is accompanied by increases in the rate of glycolysis and of mitochondrial respiration, together with a decrease in the concentration of ATP and rises in ADP and AMP (Garbers *et al.*, 1973a, b). In bull spermatozoa the decrease in the cytoplasmic ATP/ADP ratio is the principal factor which controls the increase in the rate of oxidative phosphorylation (Halangk *et al.*, 1985, 1987).

Caffeine also increases the percentage of motile spermatozoa in human semen and, as in other species, the effect is most dramatic with poor samples. Most reports agree that there is no effect on the velocity of the motile spermatozoa (Levin *et al.*, 1981; Aitken *et al.*, 1983; Moussa, 1983), although others claim that velocity is enhanced (e.g. Traub *et al.*, 1982). Other methyl xanthines including pentoxifylline have broadly similar effects (De Turner *et al.*, 1978; Read & Schnieden, 1978) and pentoxifylline has been reported to improve semen quality when taken orally by oligoasthenozoospermic men (Micic *et al.*, 1988).

In this study we have used caffeine and pentoxifylline to stimulate the motility of human spermatozoa so that the response of glycolysis, mitochondrial oxidative phosphorylation and the cellular concentration of adenine nucleotides to an increased energy demand could be studied.

Materials and Methods

Materials. Enzymes and biochemicals including pentoxifylline were purchased from Sigma Chemical Co Ltd, Poole BH17 7NH, Dorset, UK. Caffeine hydrochloride was from Koch-Light Ltd, Haverhill CB9 8PB, Suffolk, UK. [3-^{3}H]D-glucose and [U-^{14}C]L-lactate were obtained from Amersham International plc, Little Chalfont HP7 9NA, Bucks, UK. The tritiated glucose was subjected to at least 2 cycles of evaporation to dryness and dissolution in fresh distilled water to remove traces of tritiated water. [2-^{14}C]pyruvate was from DuPont (UK) Ltd, NEN Products, Stevenage SG1 4QN, Herts, UK. Scintillation counting materials were from Zinsser Analytic (UK) Ltd, Maidenhead SL6 1AP, Berks, UK, or Canberra-Packard Ltd, Pangbourne RG8 7DT, Berks, UK. Reagents for the luminometric analysis of ATP and ADP were bought from LKB Instruments Ltd, Selsdon, South Croydon CR2 8YD, Surrey, UK. Other chemicals were from BDH Chemicals Ltd, Bristol BS5 7PE, UK.

Source and preparation of the spermatozoa. Semen samples were provided by normal donors from the selected panel used by the Donor Insemination Service operated by the Department. Semen was collected by masturbation into warmed (37°C) sterile plastic jars and was allowed to liquefy at 37°C for 20 min to 1 h. Liquefied semen was diluted with BWW medium modified from Aitken (1983) ('modified BWW': 94·8 mM-NaCl, 4·78 mM-KCl, 1·70 mM-$CaCl_2$, 1·19 mM-KH_2PO_4, 1·19 mM-$MgSO_4$, 20 mM-N-[2-hydroxyethyl] piperazine-N'-ethanesulphonic acid (Hepes), 3·6 mM-$NaHCO_3$, 8·2 μM-streptomycin sulphate, 25·2 μM-penicillin G, 3 mg bovine serum albumin/ml (Cohn Fraction V)) and centrifuged (300 g_{av}, 5 min). The sperm pellet was washed twice by resuspension in modified BWW and centrifugation (300 g_{av}, 5 min) and finally resuspended in modified BWW at a concentration of about 100×10^6 spermatozoa/ml. All the washes were done at room temperature (about 20°C). The exact sperm concentration was measured with the Horwell chamber and by estimations of DNA concentration (Puzas & Goodman, 1978).

Metabolic experiments. Incubations were done in 3-ml plastic vials (Luckham Ltd, Burgess Hill RH15 9QN, UK). A well about 8 mm deep, made by cutting the base from a 2-ml 'Eppendorf' centrifuge tube, was affixed about one-third way down the wall using a drop of chloroform as adhesive and a small roll of filter paper was placed in it. Into the tubes, 0·2 μmol (1 μCi) [3-^{3}H]D-glucose plus either 0·2 μmol (0·1 μCi) [U-^{14}C]L-lactate and 0–2·4 μmol caffeine HCl or 0·2 μmol (0·1 μCi) [2-^{14}C]pyruvate and 0–0·24 μmol pentoxifylline was pipetted and sperm suspension equivalent to about 20×10^6 cells and modified BWW were added to give a final volume of 0·20 ml. The tubes were immediately sealed with silicone rubber 'Suba-Seal' caps (Gallenkamp, West Loughborough LE11 0TR, UK). The zero time tubes were acidified by the immediate injection of 100 μl 1 M-perchloric acid and the other tubes were incubated in a shaking water bath at 37°C for the appropriate time before reaction was stopped with 100 μl 1 M-perchloric acid. After acidification 100 μl 2·0 M-KOH were carefully injected into the well and the tubes were left for 20 min in the water bath to ensure that all the $^{14}CO_2$ was trapped. Subsequently the vial was transferred to ice, the filter paper was removed and the well washed with 4×100 μl H_2O. The paper and the washings were transferred to a 6-ml scintillation vial and shaken with 5 ml scintillation fluid (Quickszint 212, Zinsser or Ultima Gold, Packard). The acidified incubation medium was transferred to an Ependorf centrifuge tube, vortexed for 30 sec and then centrifuged at 10 000 *g* for 30 sec. The supernatant was weighed, cooled in ice and neutralized with 25 μl 2·3 M-K_2CO_3 containing 0·7 M-2(N-morpholino) ethane sulphonic acid. $KClO_4$ was removed by centrifugation (10 000 *g*, 30 sec) and the supernatant stored at −20°C.

Estimation of tritiated water production. Distilled water (500 μl) was pipetted into the bottom of a 3-ml plastic vial and 25 μl incubation supernatant were allowed to soak into a small roll of filter paper contained in a side well attached to the wall and the vial was sealed and stood overnight at 37°C. This allowed complete equilibration of the water in the two compartments so that the water in the base came to contain 500/525 or 95% of the tritiated water present in the sample. This water was quantitatively transferred to a scintillation vial and counted.

Estimation of ATP and ADP. The nucleotides were measured with a bioluminescent assay as described in LKB Wallac Application Note 507.

Measurement of sperm motility. The procedure was based on that described by Overstreet *et al.* (1979). To minimize the tendency of washed spermatozoa to stick to the glass, microscope slides and coverslips were immersed in

'Repelcote' (BDH), rinsed in acetone and dried thoroughly before use. Sperm suspension (10 µl) was placed in the centre of a slide, previously warmed to 37°C, and covered with a 22 × 22 mm coverslip. The slide was transferred to a heated microscope stage (Micro Instruments (Oxford) Ltd, Oxford OX1 2HP, UK) set at 37°C. The slide was viewed under dark-field illumination using maximum light intensity and photographed for a 1-sec exposure using Ilford FP4 film. Two or three frames each of a different area of the slide were taken for each suspension. In the caffeine experiments a Zeiss Photomicroscope 2 with a ×16 objective and an 'Optivar' set at 1·25 (time course) or 2·0 (dose–response) was used. In the pentoxifylline experiment an Olympus BH2 microscope equipped with an OM2 camera, a ×10 objective and a ×3·3 photographic eyepiece was used. These changes prevent the comparison of velocity measurements between the experiments because they are affected by the magnification used. At higher magnifications the field of view is smaller and fast moving spermatozoa are more likely to appear as part tracks and not be scored whereas more of the very sluggish spermatozoa (velocity < 10 µm/sec) are recognized as progressively motile. Therefore, measurements at high magnification tend to underestimate the mean velocity. Comparisons within experiments remain valid.

The negatives were analysed by projecting them onto a digitising tablet ('Bit-Pad 2'; Summagraphics, Newbury RG14 5QY, UK) connected to an Opus PC computer programmed to record the XY co-ordinates of the points selected by the cursor and the distance between successive points. The scale was calibrated by projecting an image of the grid of a Horwell Fertility chamber (100 × 100 µm) photographed at the same magnification as the negatives to be analysed. Velocity was defined as the rate of linear progression and was measured by placing the cursor on the beginning and on the end of the track as shown by squares in Fig. 1. Most tracks in these experiments were straight and the 'curviliner' or 'track' velocity (Mortimer, 1986) was not measured. Lateral head displacement (lhd) was defined as the width of the track near to the centre of its length and was measured by placing the cursor over the points indicated by triangles in Fig. 1. Rolling and yawing spermatozoa were distinguished as described by Overstreet *et al.* (1979). Briefly, rolling spermatozoa follow a spiral trajectory rotating on their own axis as they progress. Under dark-field illumination the human sperm head appears brightest when at an angle of about 45° to the plane of view. Thus these tracks appear as a series of sharp images of the sperm head. Yawing spermatozoa do not rotate but follow a comparatively flattened trajectory with the head slewing from side-to-side. Their tracks appear as ribbons following a sinusoidal path (Fig. 1). Spermatozoa with erratic trajectories were classified as unusual. The numbers of spermatozoa with different trajectories were counted separately but the data were combined for the calculation of average velocities and lateral head displacements. For yawing spermatozoa, lateral head displacement was measured about the straight line or simple curve representing the average track rather than the actual track (Fig. 1). Yawing spermatozoa therefore tend to have a large lhd. This interpretation is different from that of Aitken *et al.* (1985). To avoid possible errors due to the non-random selection of spermatozoa our policy was to measure every spermatozoon in a photograph. Occasionally, when >200 spermatozoa were present in a frame, analysis was confined to part of it but the area was chosen before the tracks were examined in detail.

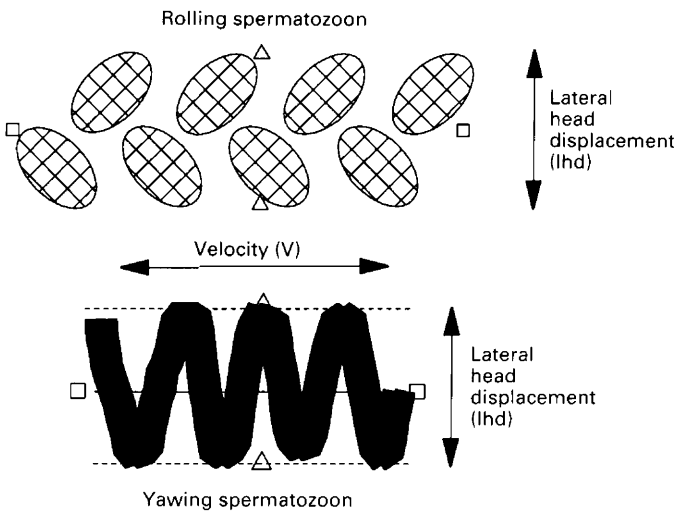

Fig. 1. A schematic outline of the track of a rolling and a yawing spermatozoon to show the measurement of velocity (v) and lateral head displacement (lhd).

Statistical evaluation of the results. The motility data were pooled by adding together the spermatozoa from all the replicates. Thus only one value per group was obtained for % motile and % yawing spermatozoa and differences were tested by a two-way analysis of variance (model: caffeine or pentoxifylline × time) and no interaction sum of squares

was calculated. For the velocity and lateral head displacement measurements the data for each individual spermatozoon were included in the data set and a three-way analysis of variance (replicates × caffeine or pentoxifylline × time) was done using the GLM procedure of the SAS software package (SAS Institute Inc, Cary, NC 27511, USA). The probabilities shown are based on the type 3 sum of squares. The metabolic data were also analysed by analysis of variance but as the group sizes were more nearly equal the ANOVA procedure of SAS was used. Differences between individual treatments were tested by Tukey's Studentized Range Test. Differences between individual cells in the tables were not tested.

Results

Motility measurements

The most dramatic effect of 6 mM-caffeine was to increase the proportion of spermatozoa exhibiting the yawing mode of progression. The effect was apparent immediately after the addition of caffeine but increased with time and >50% of the progressively motile spermatozoa were yawing after 2 h (Table 1). The lateral head displacement (lhd) of the spermatozoa was significantly increased by caffeine ($P < 0.0001$, Analysis of Variance) and there was a significant ($P < 0.0001$) interaction between caffeine and time, with the drug having a greater effect after 2 h incubation. The lhd also increased with time in the control group but to a smaller extent (Table 1). The concentration of progressively motile spermatozoa remained stable during 2 h incubation under control conditions but decreased by almost 50% in the presence of caffeine. The velocity of the spermatozoa increased by about 20% after incubation for 2 h ($P < 0.0001$) but caffeine had no significant effect on this parameter (Table 1). There were significant differences in both velocity and lhd between the 5 replicates but the effect of caffeine was consistent.

Table 1. The effect of 6 mM-caffeine on the motility of human spermatozoa at 37°C and at 0 and 2 h of incubation (pooled data from 4–5 experiments)

	Control		6 mM-caffeine	
	0 h	2 h	0 h	2 h
No. of spermatozoa	425	505	356	300
% progressively motile spermatozoa	64	68	68	35
% of progressive spermatozoa yawing	5	5	18	52·7
Mean ± s.e.m. velocity (μm/sec)	46 ± 0·89	55 ± 0·7	45 ± 0·8	53 ± 1·4
Mean ± s.e.m. lateral head displacement (μm)	3·9 ± 0·10	4·1 ± 0·09	4·8 ± 0·15	6·7 ± 0·25

Significance of treatment effects (P): analysis of variance

	Replicates	*Caffeine*	*Time*	*Caffeine × time*
Velocity	0·0001	0·109	0·0001	0·79
lhd	0·0001	0·0001	0·0001	0·0001

After exposure to 6 mM-caffeine the frequency distribution of lhd among the spermatozoa remained unimodal but the most frequent range of lhd increased from 3–4 to 6–8 μm (Fig. 2).

A dose–response experiment was done to confirm that the effect of caffeine on lhd was not a pathological response associated with the decline in the percentage of progressively motile spermatozoa. The lhd was increased by concentrations of caffeine as low as 0·75 mM which had no effect on the % progressively motile cells. Higher concentrations of caffeine (>3 mM) appeared to decrease the percentage of motile spermatozoa after 2 h incubation but overall the effect of the drug on this parameter was not significant in this experiment. However, the percentage of motile spermatozoa declined with time even in the control incubation and this effect was significant (Table 2). The percentage of progressively motile spermatozoa showing the yawing mode of progression

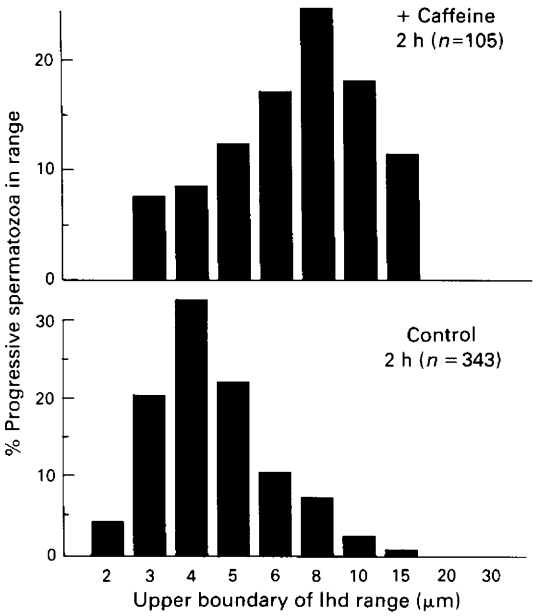

Fig. 2. The frequency of different lateral head displacements in spermatozoa incubated for 2 h with and without 6 mM-caffeine. Only the upper boundary of the ranges is shown under each bar; the lower boundary is the figure under the preceding bar.

Table 2. The effect of different concentrations of caffeine on the motility of human spermatozoa incubated at 37°C

	Incubation time (h)	Caffeine (mM)						
		0	0·38	0·75	1·5	3	6	12
No. of motile spermatozoa	0	77	52	48	35	24	26	27
	1	69	32	41	19	48	37	15
	2	48	9	38	21	29	25	—
% Progressively motile spermatozoa	0	89	81	100	94	92	100	100
	1	88	88	78	47	65	76	93
	2	68	67	68	57	52	12	—
% Progressive spermatozoa yawing	0	20	13	27	33	50	25	26
	1	26	27	55	77	71	84	100
	2	9	67	74	63	77	100	—
Mean ± s.e.m. lateral head displacement (μm)	0	4·1 ± 0·29	4·4 ± 0·35	6·6 ± 0·50	6·3 ± 0·39	6·7 ± 0·64	5·0 ± 0·55	6·5 ± 0·63
	1	4·6 ± 0·34	5·7 ± 0·53	6·3 ± 0·36	6·0 ± 0·65	6·3 ± 0·41	6·4 ± 0·68	5·9 ± 0·34
	2	4·9 ± 0·38	—*	5·3 ± 0·36	5·5 ± 0·48	6·2 ± 0·4	—*	—*

Significance of treatment effects (P): analysis of variance.

	Caffeine	*Time*	*Caffeine × time*
% Progressive	>0·1	<0·005	—
% Yawing	<0·05	<0·01	—
lhd	0·0001	0·0001	0·0001

Individual differences in lhd between caffeine concentrations significant at $P < 0·05$ (Tukey test): 0, 0·38 < 0·75, 1.5, 3, 6, 12

* < 10 spermatozoa analysed.

Table 3. The effect of different concentrations of pentoxifylline on the motility of human spermatozoa incubated at 37°C (pooled data from 2 experiments)

	Incubation time (h)	Pentoxifylline (mM)				
		0	0·15	0·30	0·60	1·2
% Progressively motile (no. of spermatozoa)	0	66 (484)	56 (403)	66 (417)	63 (564)	75 (555)
	4	66 (412)	63 (406)	69 (432)	64 (408)	61 (444)
% Motile spermatozoa yawing	0	11·9	12·3	9·9	11·2	9·6
	4	24·2	18·0	20·1	24·1	21·9
Mean ± s.e.m. velocity (μm/sec)	0	65 ± 1·4	69 ± 1·59	74 ± 1·2	74 ± 1·0	73 ± 1·0
	4	68 ± 1·4	73 ± 1·4	78 ± 1·2	75 ± 1·4	67 ± 1·3
Mean ± s.e.m. lateral head displacement (μm)	0	3·7 ± 0·11	3·6 ± 0·10	3·8 ± 0·10	3·9 ± 0·10	4·0 ± 0·09
	4	5·2 ± 0·14	5·7 ± 0·21	5·2 ± 0·14	5·5 ± 0·17	5·6 ± 0·15

Significance of treatment effects (P): analysis of variance

	Replicates	*Pentoxifylline*	*Time*	*Pentoxifylline × time*
% Progressively motile*	—†	>0·10	>0·10	—
% Yawing*	—†	>0·10	<0·005	—
Velocity	0·0001	0·0001	0·008	0·0001
lhd	0·0001	>0·10	0·0001	0·0043

*One observation/cell.
†Not tested.

was significantly increased by all concentrations of caffeine and the increase was greater after longer incubation times (Table 2). Sperm velocities are not shown in full but concentrations of caffeine ≥ 3 mM decreased sperm velocity to a significant extent ($P < 0·0001$, Analysis of Variance), the velocity after 1 h incubation being 54, 54, 59, 69, 49, 45 and 38 μm/sec in the presence of 0, 0·38, 0·75, 1·5, 3, 6 and 12 mM-caffeine respectively.

The effect of the other phosphodiesterase inhibitor, pentoxifylline, on motility was different. It had no significant effect on the % progressively motile spermatozoa or the % of yawing spermatozoa and the effect on lateral head displacement was slight and barely significant. However, all concentrations increased sperm velocity ($P < 0·0001$, Analysis of Variance). Velocity did not change significantly with respect to time but there was a significant interaction between concentration and time, with 0·15 and 0·3 mM-pentoxifylline producing a greater enhancement after 4 h than at zero time and 1·2 mM causing a decrease in velocity after 4 h. In this experiment the % of yawing spermatozoa and lhd increased with time whether pentoxifylline was present or not, but there was a significant interaction between time and pentoxifylline concentration (Table 3).

Metabolic measurements

The rate of metabolism of [U-^{14}C]lactate to $^{14}CO_2$ was linear for 60 min but declined slightly during the 2nd hour of the incubation in the controls (Fig. 3a). It was not significantly affected by 6 mM-caffeine ($P > 0·15$, Analysis of Variance). The rate of glycolysis estimated by the release of tritiated water from [3-^{3}H]D-glucose was linear for 2 h in the control incubations and in the presence of 6 mM-caffeine: 6 mM-caffeine produced a 40% increase in the rate of glygolysis ($P < 0·005$, Analysis of Variance) (Fig. 3b).

Caffeine had no effect on the concentration of ATP in the spermatozoa but this changed significantly with time, increasing during the first 30 min incubation and then declining again

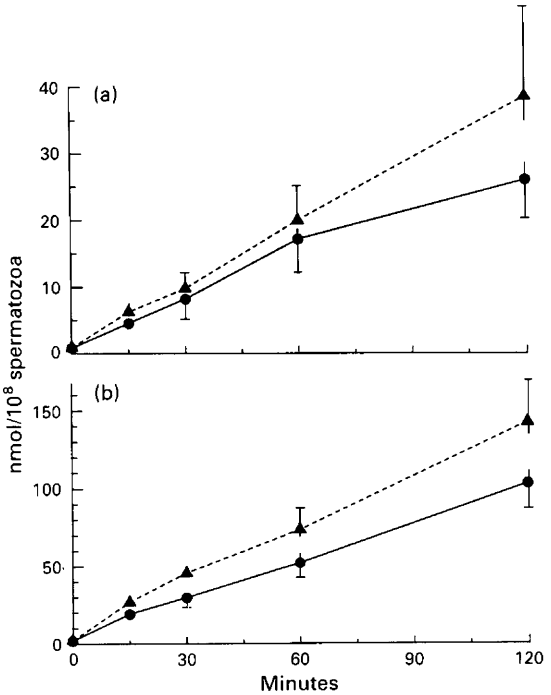

Fig. 3. The rate of conversion of (a) 1 mM-[U-^{14}C]L-lactate to $^{14}CO_2$ and (b) 1 mM-[3-^{3}H]D-glucose to tritiated water by human spermatozoa incubated at 37°C in the presence of 6 mM-caffeine (– –▲– –) or controls (—●—). Values are means ± s.e.m. for 4–6 observations.

(Table 4) ($P < 0.0001$, Analysis of Variance). The concentration of ADP in control spermatozoa decreased after 30 min incubation but remained the same in the presence of 6 mM-caffeine (Table 4: $P < 0.01$, Analysis of Variance).

Table 4. The effect of 6 mM-caffeine on the concentrations (nmol/10^8 spermatozoa) of ATP and ADP in human spermatozoa incubated at 37°C

	Caffeine (mM)	Incubation time (min)				
		0	15	30	60	120
ATP	0	7·3 ± 0·97 (5)	8·7 ± 0·91 (3)	9·1 ± 2·15 (4)	5·4 ± 1·30 (4)	7·9 ± 1·88 (5)
	6	7·7 ± 1·58 (5)	8·2 ± 1·55 (4)	9·1 ± 2·65 (4)	6·9 ± 1·49 (5)	7·8 ± 1·12 (6)
ADP	0	3·0 ± 0·26 (5)	3·2 ± 0·88 (4)	2·2 ± 0·25 (4)	2·6 ± 0·31 (4)	2·0 ± 0·16 (5)
	6	3·0 ± 0·19 (5)	3·0 ± 0·53 (4)	3·0 ± 0·14 (4)	3·2 ± 0·30 (5)	2·8 ± 0·15 (6)

Values are mean ± s.e.m. for the no of observations in parentheses

Significance of treatment effects (P): analysis of variance

	Replicates	*Caffeine*	*Time*	*Caffeine × time*
ATP	0·001	0·34	0·0001	0·16
ADP	0·15	0·07	0·17	0·39

Pentoxifylline, at 0–0·6 mM, had no effect on the metabolism of pyruvate but at 1·2 mM it increased the production of $^{14}CO_2$ by about 50%. The rate of glycolysis was increased by all concentrations of pentoxifylline tested and the size of the increase was related to the pentoxifylline concentration, being about 40% with 0·15 and 80% with 1·2 mM-pentoxifylline. Pentoxifylline had no significant effect on the concentration of ATP (Table 5).

Table 5. The effect of different concentrations of pentoxifylline on the metabolism of 1 mM-[2-^{14}C]-pyruvate and 1 mM-[3-^{3}H]D-glucose by human spermatozoa incubated at 37°C for 4 h (nmol/10^8 spermatozoa/h) and the concentrations of ATP and ADP (nmol/10^8 spermatozoa)

	Pentoxifylline (mM)				
	0 (Control)	0·15	0·3	0·6	1·2
[2-^{14}C]pyruvate to $^{14}CO_2$	19 ± 2·4	20 ± 3·5	22 ± 4·5	22 ± 3·2	28 ± 3·9*
[3-^{3}H]glucose to ^{3}HOH	52 ± 6·0	73 ± 11·5*	76 ± 9·5*	89 ± 16·5*	93 ± 17·1*
ATP	9·3 ± 1·03	8·8 ± 0·49	9·6 ± 0·67	9·6 ± 10·73	9·2 ± 0·48
ADP	1·9 ± 0·34	2·0 ± 0·34	1·4 ± 0·39	2·2 ± 0·08	1·7 ± 0·20

Values are mean ± s.e.m. for 5 or 6 observations.
*Significantly different from control ($P < 0·05$), analysis of variance and Tukey test.

Discussion

The effect of caffeine on the lateral head displacement of human spermatozoa has not been reported previously, possibly because this parameter was not measured in previous studies (Schoenfeld *et al.*, 1973; Schill, 1975; Traub *et al.*, 1982; Aitken *et al.*, 1983; Moussa, 1983; Prins & Ross, 1985). It could be related to the increase in lateral head displacement observed during the capacitation of human spermatozoa (Morales *et al.*, 1988; Ginsburg *et al.*, 1989). The change in the frequency distribution of sperm lateral head displacement observed after 6 h capacitation by Morales *et al.* (1988) was qualitatively similar to that produced by caffeine in our experiments (Fig. 2). Capacitation is regulated by a number of factors including cAMP (see Fraser & Ahuja, 1988; Garbers, 1989) and caffeine can enhance the penetrating power of poor quality sperm suspensions in the hamster egg test (Aitken *et al.*, 1983; Prins & Ross 1985; Cai & Marik, 1989). However, the motility changes observed here are quite different from the type of hyperactivated or 'whiplash' motility seen in capacitating mouse or hamster spermatozoa (see Fraser, 1984) and the complex 'star-spin' and related trajectories observed in human spermatozoa (Robertson *et al.*, 1988). The possibility that the observed effect is an artefact of the measuring system must also be considered: the transition from rolling to yawing progression is very similar to the change in the motility pattern between bovine sperm swimming in the centre and by the walls of a flattened capillary tube filled with cervical mucus (Katz *et al.*, 1981). The increase in yawing produced by caffeine might result from more spermatozoa swimming close to the surface of the slide or coverslip due to more spermatozoa colliding with the surface because of their more vigorous motility.

Pentoxifylline had no effect on the proportion of yawing spermatozoa or on the lateral head displacement but did increase velocity. Since the two methyl xanthines had different effects on sperm function it is unlikely that they both act as pure phosphodiesterase inhibitors. Possibly they contribute to intracellular alkalinization or to other processes to different extents.

Both methyl xanthines had similar effects on sperm metabolism, producing a very significant increase in the rate of glycolysis but a smaller less consistent increase in the oxidation of lactate or pyruvate to CO_2. In spite of the smaller increase in flux, respiration could still produce a greater increment of ATP production because of the much higher yield from oxidative compared with

substrate level phosphorylation. However, it is unlikely that the release of $^{14}CO_2$ reflects the complete oxidation of lactate or pyruvate. Substantial amounts of [U-^{14}C]lactate will be converted to pyruvate and decarboxylated to acetate without further metabolism (see Ford & Rees, 1990) and although [2-^{14}C]pyruvate should be a better probe because the labelled carbon atom is not released until it has been incorporated into the pool of tricarboxylic acid cycle intermediates, some $^{14}CO_2$ could still be released by the action of malic enzyme rather than complete oxidation. It is probable, therefore, that the increased flux through the glycolytic pathway provides a substantial proportion of the additonal ATP required to sustain increased motility.

In the caffeine experiments the rate of metabolism remained linear despite a decrease in the percentage of motile spermatozoa. Either the immotile spermatozoa continued to metabolize or the motile ones underwent a larger increase in rate than the overall data show.

The increased glycolytic flux produced by caffeine could have been mediated in part by the relatively greater ADP concentration, but the change in ADP did not occur until 30 min of incubation whereas the change in glycolytic rate was immediate. Pentoxifylline produced no changes in adenine nucleotide concentrations. Thus, in human spermatozoa the metabolic rate can be increased to meet a greater demand for ATP in such a way that the concentrations of ATP or ADP are not perturbed to any marked degree. This is in contrast to bull (Garbers *et al.*, 1973a; Halangk *et al.*, 1985) or boar (Garbers *et al.*, 1973b) spermatozoa in which caffeine caused a decrease in ATP and corresponding increases in ADP and AMP. However, the conditions and the time scale were different in these experiments and we cannot rule out the possibility that transient changes in ATP concentration may have occurred here.

Whatever the mechanism, it is clear that human spermatozoa can adjust their metabolic performance effectively to meet changes in energy demand but that the glycolytic pathway is more responsive than mitochondrial respiration, at least in terms of the relative change in flux. Consequently, the presence of a glycolytic substrate is likely to be advantageous when human spermatozoa are manipulated *in vitro*.

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