THE ACTIVATION OF MOTILITY IN QUIESCENT HAMSTER SPERM FROM THE EPIDIDYMIS BY CALCIUM AND CYCLIC NUCLEOTIDES

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SUMMARY: Control is exerted on the movement of mammalian spermatozoa at ejaculation and at capacitation. Here the activation of motility in motionless pre-ejaculated sperm was investigated. This was done by isolating quiescent caudal epididymal sperm from the hamster and observing that the addition of either calcium cAMP, cGMP, or cUMP conferred full motility upon them. Other salts, nucleotides, caffeine, sugars, or oxygen did not. Epididymal fluid which contains phosphodiesterase had too little calcium to activate the sperm while seminal plasma had more than enough. The cAMP content of quiescent sperm was low, but ATP levels were high. At the activation of motility, sperm cAMP synthesis became very rapid. It thus appears that sperm are quiescent on the male because they lack cAMP, and that calcium, supplied at ejaculation, initiates rapid cAMP synthesis to produce motility.

Control of the movement of mammalian spermatozoa is exerted at two critical stages in their existence. One of these occurs when the sperm reaches the upper reproductuve tract of the female. There, through the process of capacitation, sperm motility is stimulated significantly above basal levels (1). This apparently enables the sperm to penetrate the zona pellucida surrounding the egg (1). We have recently reported that this stimulus of motility (2) results from elevated cyclic AMP synthesis by sperm adenyl cyclase under capacitating conditions (3,4). The possible involvement of cAMP in sperm capacitation was first suggested by Garbers et al. (5) and later by Hicks et al. (6).

The other major control point in the movement of mammalian spermatozoa occurs at ejaculation when motility is activated for the first time in the life cycle of this cell. Here we report that this activation of motility occurs by a calcium triggered initiation of sperm cAMP synthesis.

METHODS

Epididymides excised from adult hamsters were submerged under mineral

oil in a petri dish and the caudal area punctured with a #24 syringe needle. About 0.3 ml fluid, containing about 10^8 sperm by hemocytometer count, was extruded from the cauda epididymis with forceps in such a way that it adhered to the bottom of the container. 5 µl aliquots of this sperm suspension were transferred when needed to 45 µl of 0.25 M sucrose diluent under oil in small watch glasses. Motility observations were made with a phase contrast microscope at 100 diameters. Movement was evaluated in terms of rate (0-10) and percent moving.

Calcium was measured using a calcein (G.F. Smith Chemical Company, Columbus, Ohio) fluorescence assay by G.K. Turner Associates, Palo Alto, California. Sperm-free epididymal fluid was obtained by centrifuging the undiluted caudal contents of four epididymides at 30,000 g for 10 minutes and separating the supernatant fraction for assay.

The ATP assay was based upon our luciferin-luciferase adaptation (2) while cAMP measurements employed the protein binding assay of Gilman (7). In these measurements the sample for each time point consisted of the entire caudal contents of a hamster epididymis (0.3 ml) diluted to 1 ml with the indicated activating medium and extracted at the appropriate time point by the addition of 3 ml ice cold acetone while vortexing. The samples in preweighed 12 ml corex tubes were then placed on ice for 15 minutes, centrifuged for 5 minutes at 20,000 g and the supernatant fractions evaluated to remove the acetone. Capillary bubble sources were used to avoid bumping. The required aliquots were then removed for nucleotide analysis.

RESULTS

We recently have found that hamster caudal epididymal fluid contains an active cAMP-phosphodiesterase which is sensitive to methyl xanthines (B. Morton and J. Harrigan-Lum, unpublished). This suggested the hypothesis that sperm are quiescent in the male because they lack cAMP. If this were true, it would be important to surround the sperm with extracellular phosphodiesterase. This would prevent accidental activation of sperm

motility in the male which could occur if cAMP from other sources leaked into the lumen of the epididymal tubules.

In order to evaluate this hypothesis, it was necessary to isolate epididymal spermatozoa without activating flagellation. This was accomplished by extruding hamster caudal epididymal contents without diluents directly under mineral oil. Such an immotile sperm suspension was viable at room temperature for several days.

The bottom two curves of Figure 1 indicate that some motility was

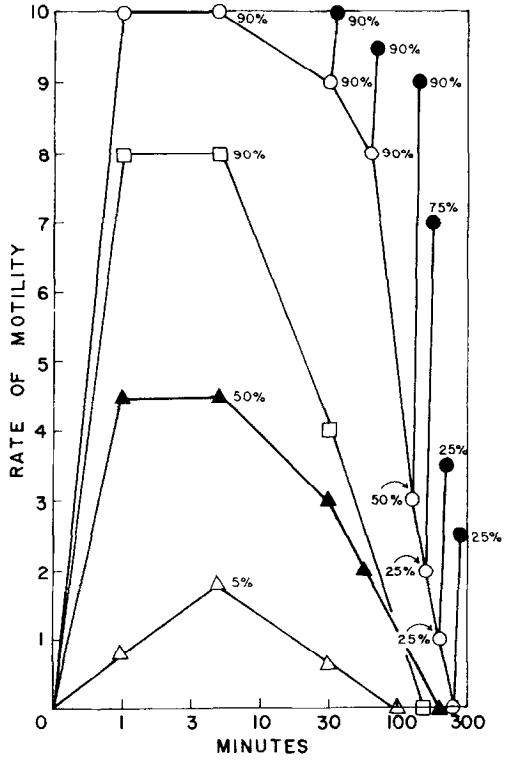


Figure 1: The activation of motility in quiescent hamster sperm by calcium. Sperm were prepared and diluted as described in METHODS. Open triangles = 24 hour sperm diluted in sucrose. Closed triangles = fresh sperm diluted in sucrose. Squares = 24 hour sperm diluted in 1 mM $CaCl_2$-sucrose. Open circles = fresh sperm diluted in 1 mM $CaCl_2$-sucrose. Closed circles = the effect of adding 10 mM caffeine at the time point indicated. Numbers by the data points refer to percent of sperm moving.

conferred upon sperm from this suspension by tenfold dilution into 0.25 M sucrose. If the sperm suspension was stored 24 hours under oil, negligible movement was elicited by this dilution.

If 1 mM $CaCl_2$ was also present in the sucrose diluent (upper curves, Figure 1), a very strong activation of sperm motility occurred. All viable cells became violently motile whether or not they had previously been stored 24 hours under oil. This motility decayed in a manner that could substantially be reversed by the addition of 10 mM caffeine, an agent known to inhibit sperm cAMP degradation by phosphodiesterase (5).

Glucose, fructose, NaCl, KCl, $MgSO_4$, $NaHCO_3$, K_2HPO_4 or caffeine did not activate quiescent hamster sperm above the sucrose background. Neither did the presence of air in experiments where mineral oil was omitted over the sucrose diluent.

Figure 2 indicates 1 mM or greater calcium was required for strong

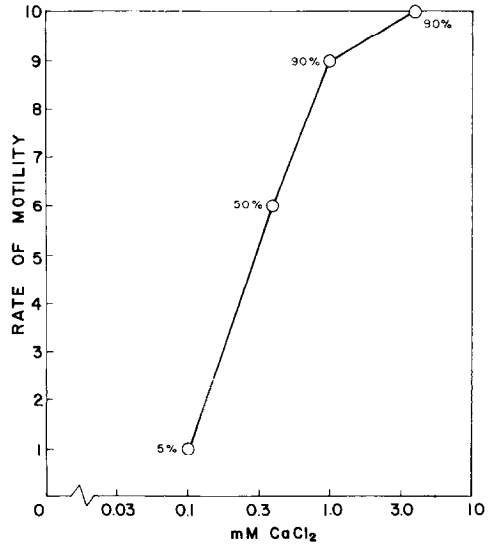
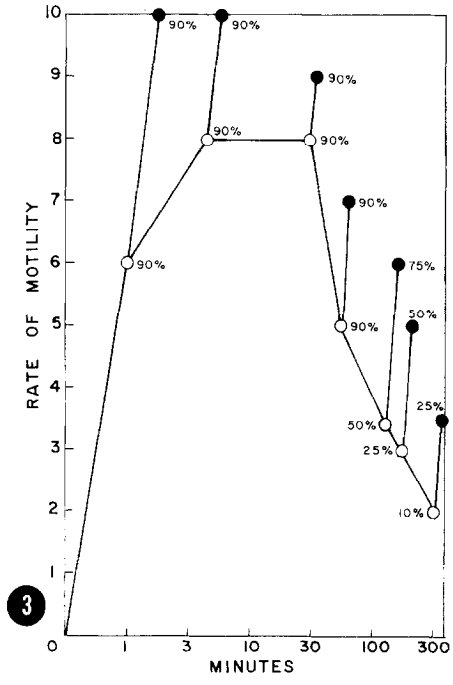


Figure 2: The effect of calcium concentration upon the activation of quiescent hamster sperm. Conditions as in METHODS. The indicated concentrations refer to that in the sperm suspension after dilution in sucrose. Numbers by data points refer to percent of sperm moving.



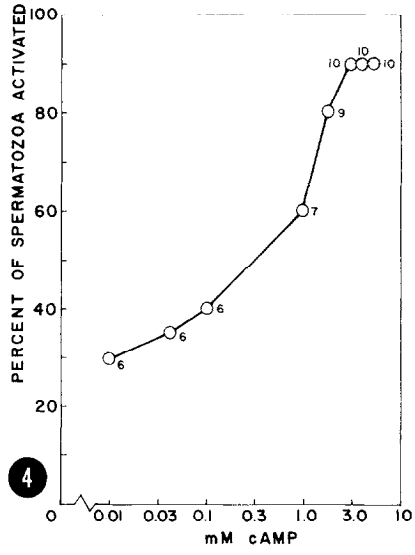


Figure 3: The activation of quiescent hamster sperm by cAMP. Conditions as in Figure 1. Open circles = sperm diluted in 1 mM cAMP-sucrose. Closed circles = the addition of 10 mM caffeine at the time point indicated. Numbers by the data points refer to percent of sperm moving.

Figure 4: The effect of cAMP concentration upon the activation of motility in quiescent hamster sperm. The indicated concentrations refer to that in the sperm suspension after dilution in sucrose. Numbers by the data points refer to the rate of sperm movement (0-10).

motility activation. The average calcium content of human seminal plasma is 8 mM (8). We found that sperm-free undiluted epididymal fluid from hamster contained 0.025 $\pm$ 0.03 mM calcium. This amount, when present in the sucrose diluent, did not activate quiescent hamster sperm.

To investigate how calcium might activate motility, we first replaced calcium in the sucrose diluent with calcium-free, 1 mM cAMP. The results, shown in Figure 2, indicate that cAMP also activated quiescent hamster sperm motility and that the presence of 10 mM caffeine magnified this effect. The

concentration requirements for cAMP activation of sperm motility are shown in Figure 4 where it may be seen that as little as 0.01 mM cAMP caused significant activation.

Cyclic GMP and cyclic UMP were similar to cAMP in their ability to activate. Cyclic GMP, ATP, or AMP were inactive. Epididymal sperm from mice were similar to those from hamster in all regards inspite of an unusual requirement by the latter for calcium (9).

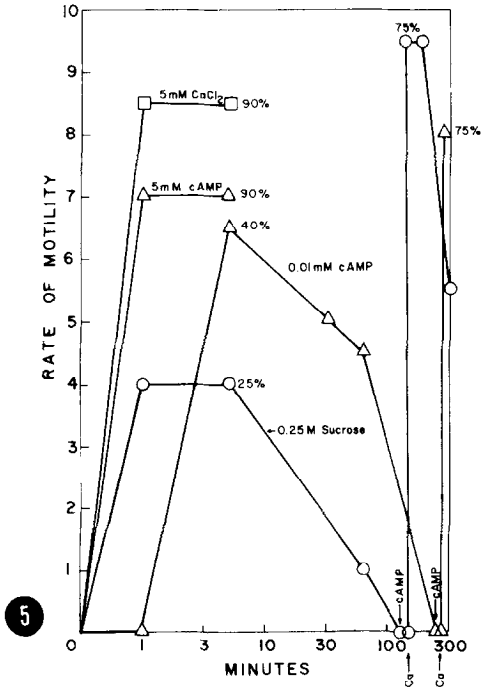
That calcium was superior to cAMP in reactivating motility in hamster sperm samples whose movement had briefly ceased is indicated in Figure 5. Note, however, that in Figure 1 caffeine also reactivated motility. If more than a few minutes elapsed after sperm lost motility, they could not be reactivated.

An important hypothesis (10) states that sperm in the epididymis are quiescent because they lack oxygen and anaerobic substrate (and thus ATP). The ATP contents of quiescent and activated epididymal hamster sperm, shown in Figure 6, indicates that quiescent sperm have more than enough ATP to support motility.

We were thus in the position to test our original hypothesis that sperm are quiescent in the epididymis because they lack cAMP. Cyclic AMP levels within activating epididymal hamster sperm are shown in Figure 6. It may be seen that the cAMP content of quiescent sperm indeed approaches zero. As activation begins, a spike in the cAMP level occurs, followed by stabilization at amounts normally seen in motile hamster sperm (B. Morton and J. Harrigan-Lum, unpublished).

DISCUSSION

To perform these experiments properly, it was necessary to avoid calcium contamination and the dilution effect (11). Only a few seconds delay in the preparation of the zero time sample for cAMP analysis resulted in very misleading results (12) due to the extreme rapidity of cAMP synthesis at



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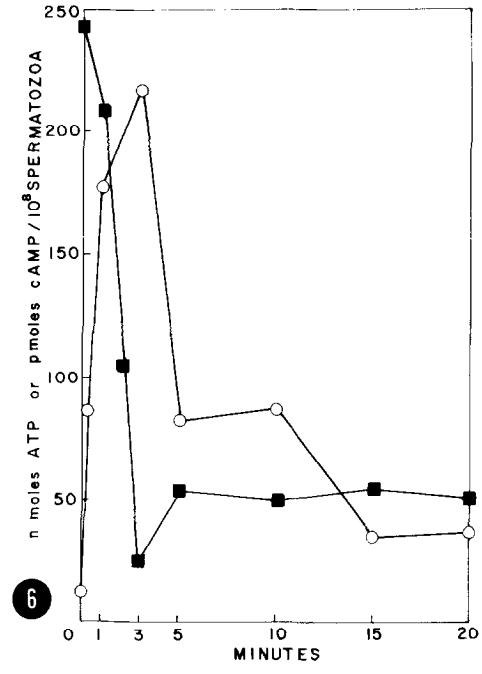

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Figure 5: Reactivation of motility by calcium. Conditions as in Figures 1 and 3. Arrows at lower right refer to additions of 1 mM cAMP followed by 1 mM $CaCl_2$ to immotile diluted hamster sperm. Numbers by data points refer to percent of sperm moving.

Figure 6: ATP and cAMP content of hamster sperm during motility activation. Conditions as in METHODS. Activation diluent contained 1 mM $CaCl_2$. Squares = ATP. Circles = cAMP.

activation. Acetone was substituted for acid extraction techniques because the latter produced very high cAMP backgrounds.

This work while answering some interesting questions has raised several new ones. At present it is unknown how calcium and cAMP interrelate to control sperm motility, or to control many other biological processes (13). One thing is clear. The actual contractile apparatus of hamster sperm does not require calcium (14), as opposed to that in muscle. It is possible that in sperm calcium may activate sperm motility because it is required by an enzyme in the cascade beyond cAMP-dependent protein kinase, as in glycogen breakdown. However, if this is so, the activation of sperm motility by

minute quantities of cAMP in the absence of calcium implies the existence of an internal calcium compartment whose contents are released via cAMP. Conversely calcium, and possibly cAMP could be direct effectors of sperm adenyl cyclase, inducing the unusual burst of cAMP synthesis observed, with no further cellular calcium requirements involved. Certainly quiescent sperm are much more sensitive to external cAMP than are motile sperm. We continue to investigate this interesting and possibly prototypic system.

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REFERENCES

1. Yanagimachi, R., J. Reprod. Fert. 23, 193 (1970).
2. Rogers, B.J. and Morton, B., Biol. Reprod. (to appear in 1973).
3. Morton, B., Rogers, B.J. and Chang, T.S. K., Biol. Reprod. (to appear in 1973).
4. Morton, B., and Albagli, L., Biochem. Biophys. Res. Commun. 50, 697 (1973).
5. Garbers, D.L., Lust, W.D., First, N.L. and Lardy, H.A., Biochem. 10, 1825 (1971).
6. Hicks, J., Martinez-Manautou, J., Pedron, N. and Rosado, A. Fert. Steril. 23, 172, 886 (1972).
7. Gilman, A.G., Proc. Nat. Acad. Sci. 67, 305 (1970).
8. Lundquist, F., Acta Physiol. Scand. 19, Suppl. 66 (1949).
9. Morita, Z. and Chang, M.C., Biol. Reprod. 3, 169 (1970).
10. Mann, T., The Biochemistry of Semen and the Male Reproductive Tract, p. 54, Methuen, London, 1964.
11. Morton, B. and Chang, T.S.K., J. Reprod. Fert. (to appear in 1973).
12. Morton, B., Harrigan-Lum, J. and Jooss, T., Biol. Reprod. 9, 71 (abs)
13. Rasmussen, H., Science 170, 404 (1970).
14. Morton, B., Exptl. Cell Res. 79, 106 (1973).