The Effect of ATP Depletion upon the Acrosome Reaction in Guinea Pig Sperm

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The suspension of guinea pig spermatozoa in substrate-free medium results in the cessation of sperm motility and a 94% reduction in adenosine triphosphate (ATP) levels within minutes. ATP depletion was not deleterious to spermatozoa since motility was fully restored by the addition of pyruvate. In addition, sperm were able to synthesize ATP by metabolizing pyruvate. Following incubation for 0, 30, and 60 minutes in medium without substrate, acrosome reactions were measured before and after motility restoration. ATP depletion induced a large percentage of acrosome reactions relative to controls (32.29 ± 1.22% compared with 0.63 ± 0.02% at 60 minutes). Ca^{2+} was required for the effect. It is suggested that ATP depletion inactivates a Ca^{2+}-dependent ATPase expulsion mechanism for Ca^{2+}, thereby permitting Ca^{2+} influx and the acrosome reaction.

Key words: sperm motility, ATP depletion, acrosome.

Calcium was originally implicated in fertilization mechanisms in invertebrates (Dan, 1956). The demonstration of a Ca^{2+}-dependent ATPase on the outer acrosomal membrane of rabbits, humans (Gordon, 1973), guinea pigs (Gordon et al, 1974), mice, and rats (Gordon, unpublished observations) implicated Ca^{2+} as a potent factor in the mammalian acrosome reaction. Calcium was invoked both as a prerequisite for membrane fusion (Gordon, 1973) and as an activator of acrosomal enzymes which are necessary for sperm passage through egg investments (Williams et al, 1974). Subsequently, it was established that the mammalian acrosome reaction is dependent upon the presence of external Ca^{2+} (Yanagimachi and Usui, 1974) and that Ca^{2+} influx precedes or parallels its occurrence (Singh et al, 1978).

Mechanisms of Ca^{2+} entry into the sperm head are still not definitely known. However, based on observations of the activity of plasmalemmal and acrosomal membrane transport enzymes, Gordon (1973) considered that a Ca^{2+}-dependent ATPase on the outer acrosome membrane is inactive until the plasmalemma is modified during capacitation (Gordon et al, 1974), when Ca^{2+} levels rise in the periacrosomal fluid. At this point, Ca^{2+} is transported into the acrosome.

Most cells maintain low Ca^{2+} levels by active extrusion and mitochondrial sequestration (Kretsinger, 1979). It is clear that sperm Ca^{2+} must be kept low to prevent premature acrosome reactions. Since intracellular Ca^{2+} levels are dependent upon passive membrane permeability and an active Ca^{2+}-dependent ATPase, an alteration in either processes will result in altered Ca^{2+} flux. Oleate, lysolecithin, and Cd^{2+}, agents that increase Ca^{2+} membrane permeability (Hasselbach, 1972; Hurwitz et al, 1977), can induce the acrosome reaction in the presence of external Ca^{2+} (Gordon, unpublished observation). In the present study, the authors have examined the effect of depletion of ATP, and thus decreased activity of the Ca^{2+}-dependent ATPase expulsion mechanism, upon the guinea pig acrosome reaction.

Materials and Methods

Guinea pig spermatozoa were extruded from the cauda epididymidis into 0.9% NaCl in H_2O. After

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washing three times by centrifugation (1000 g; 4 minutes each) spermatozoa were suspended in substrate-free BWW medium (NaCl 94.6 mM, KCl 4.7 mM, $CaCl_2$ 1.71 mM, KH_2PO_4 1.19 mM, $MgSO_4$ 1.19 mM, $NaHCO_3$ 25.07 mM, and bovine serum albumin 1 g/l), *p*H 7.55, at a final concentration of 1×10^7 sperm/ml. Pyruvate-containing medium (10 mM) and Ca^{2+}-free medium were also employed. Spermatozoa were incubated at 37 C under air.

After suspension of spermatozoa in the substrate-free BWW, ATP was assayed at 0, 15, 30, 45, and 60 minutes. At 60 minutes, either glucose (8 mM) or pyruvate (8 mM) was added and ATP was assayed 15 minutes later. Assays were performed on two occasions. The caudae of three guinea pigs were pooled to perform each assay. According to the method of Cole et al (1967), ATP was extracted from aliquots of spermatozoa with perchloric acid and was measured by the luciferase assay as described by Stanley and Williams (1969).

Acrosome reactions were assessed by use of a hemocytometer (four chambers, 0.1 mm^3 each) after 0-, 30-, and 60-minute incubations (Santos-Sacchi and Gordon, 1980). For spermatozoa incubated in the absence of energy substrates, acrosome reactions were counted before and after motility restoration by 10 mM pyruvate. Acrosome reaction indices for spermatozoa incubated in medium with pyruvate from 0 time were based upon motile spermatozoa only. Spermatozoa were examined by light and phase contrast microscopy. Motile sperm are sperm with an active flagellar beat, including those freely swimming and those whose heads have attached to the slide during the counting procedure. The following formulae were used to calculate acrosome reaction (AR) responses: %AR = (AR spermatozoa/total spermatozoa) × 100; $\%AR^{tot}$ = [AR spermatozoa/(total spermatozoa − immotile spermatozoa)] × 100 (Santos-Sacchi and Gordon, 1980).

Results

Within 15 minutes after suspension in substrate-free BWW, over 95% of the spermatozoa become immotile. Figure 1 demonstrates the concomitant rapid decrease in sperm ATP levels, which dropped to about 6% of initial values (2.14 $\mu g/10^8$ cells) within 15 minutes. Pyruvate restores motility to a large proportion of spermatozoa (Fig. 2, insert), but glucose does not. A difference in the ability of ATP-depleted spermatozoa to utilize these substrates was also observed (Fig. 1). Whereas pyruvate increases sperm ATP levels to nearly half their initial levels within 15 minutes, glucose is essentially ineffective. The inability of pyruvate to restore motility to all spermatozoa was not the result of damage to spermatozoa by the absence of substrate, since approximately the same percentage of motile spermatozoa were present in controls where pyruvate had been present from 0 time (Fig. 2, insert).

Figures 2 and 3 show the effect of incubation

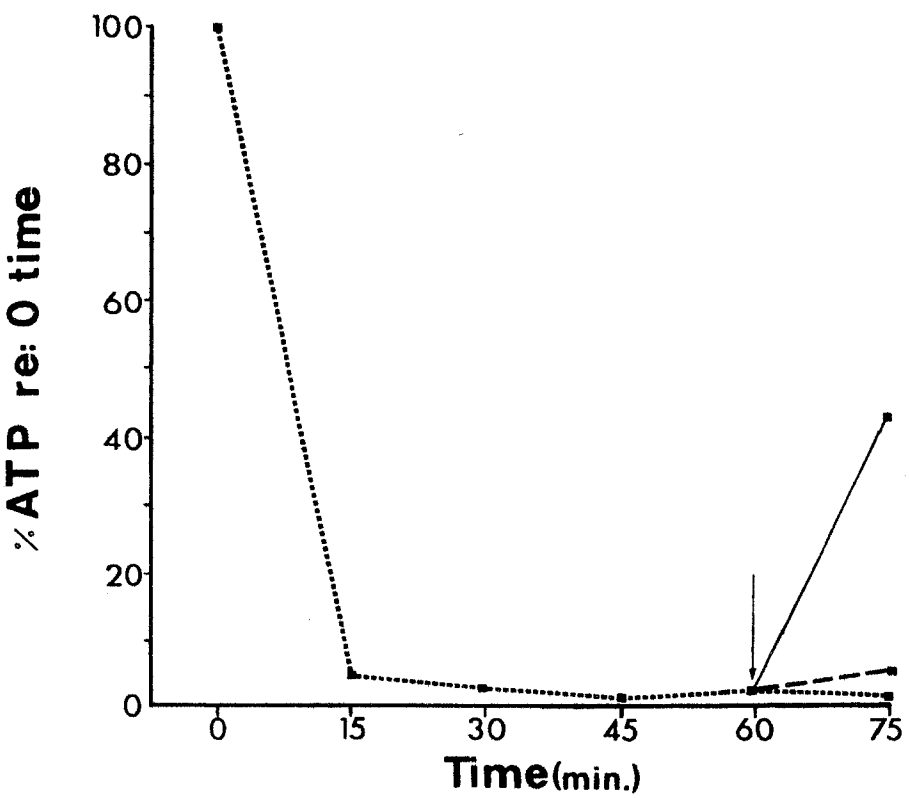

Fig. 1. Sperm samples were assayed for ATP at 15-minute intervals after suspension in substrate-free BWW (-----). At 60 minutes pyruvate or glucose (8 mM) was added (arrow) to samples, and ATP was assayed 15 minutes later (pyruvate ——; glucose – – –). The ATP levels determined at 0 time for the first and second assay were 2.22 uM/10^8 sperm and 2.14 uM/10^8 sperm, respectively. The 15-minute levels were reduced to 2.95% and 6.4% of initial values, respectively. The average of the two assays is reported here as a percentage of the 0 time value.

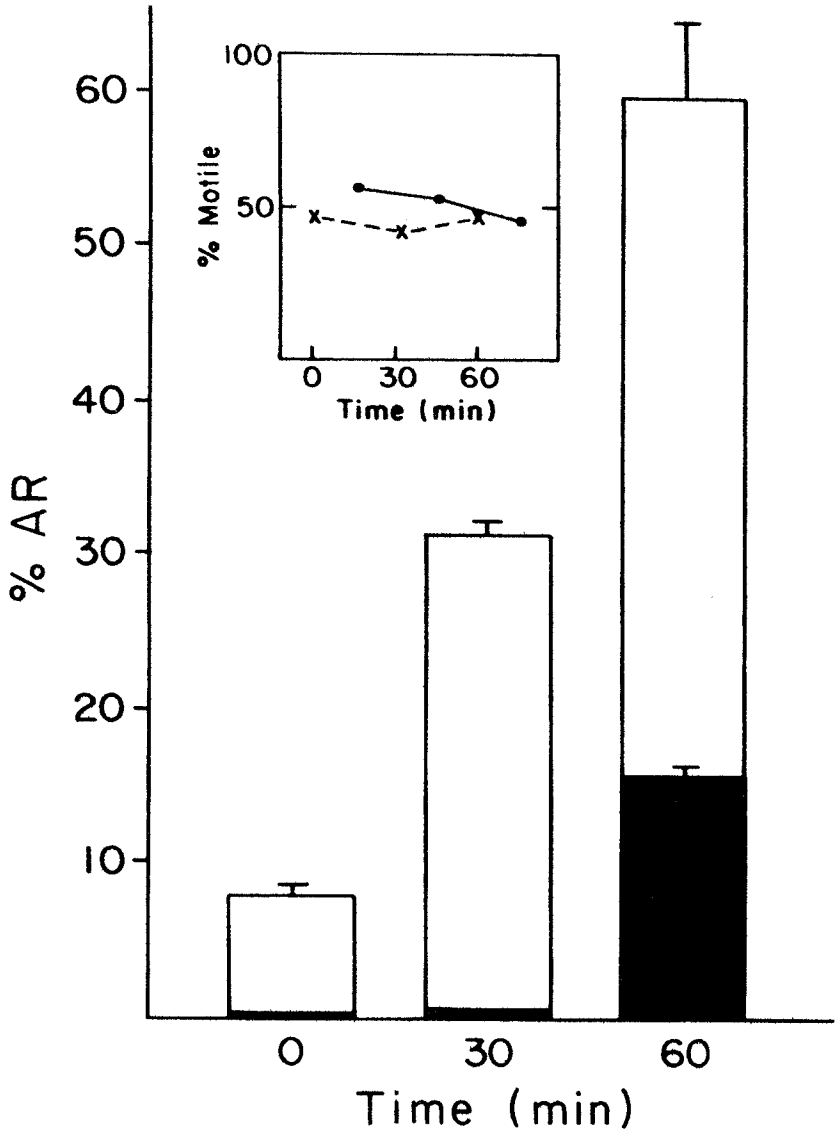

Fig. 2. Sperm were incubated in substrate-free BWW medium, and the percentages of acrosome reactions were determined prior to restoration of motility (light bars). The dark bars show the percentages of acrosome-reacted sperm whose motility was restored by pyruvate addition (10 mM). Insert: the percentages of sperm that remained motile during incubation in the presence of pyruvate from 0 time (——) and the percentages of sperm incubated in substrate-free BWW whose motility was restored by the addition of pyruvate (10 mM) at 0, 30, and 60 minutes (– – –).

time upon the induction of acrosome reactions. In the presence of Ca^{2+}, a time-dependent increase in the $\%AR^{tot}$ score is observed for spermatozoa incubated with or without an energy substrate. However, the $\%AR^{tot}$ scores for those spermatozoa incubated without substrate are dramatically higher than for those incubated in the presence of pyruvate (1 hour: 32.29 ± 1.22% vs. 0.63 ± 0.02%) (Fig. 3). The number of immotile acrosome-reacted spermatozoa that became motile upon the addition of pyruvate also increases over time (Fig. 2). Because pyruvate was added to restore sperm motility prior to determining the $\%AR^{tot}$ scores, it was necessary to establish whether pyruvate induced the acrosome reaction. The pyruvate effect on motility is not related to the acrosome reaction, because an increase in the number of *immotile acrosome-reacted spermatozoa* is found prior to pyruvate addition (Fig. 2). Furthermore, microscopic examination showed that acrosome-reacted and non-acrosome-reacted spermatozoa regain motility immediately (within 2 minutes) after pyruvate addition, with the acrosome-reacted spermatozoa exhibiting an "activated" pattern of motility (Yanagimachi and Usui, 1974).

In the absence of Ca^{2+}, the %AR score is very low relative to that in the presence of Ca^{2+}. After a 2-hour incubation in Ca^{2+}-free medium, only 27 ± 4% immotile acrosome-reacted spermatozoa are found. Pyruvate *did not* restore motility of

acrosome-reacted spermatozoa but did restore the motility of non-acrosome-reacted spermatozoa, indicating that acrosome degeneration occurred after the death of these sperm. The 2-hour $\%AR^{tot}$ score for spermatozoa incubated from 0 time with pyruvate in Ca^{2+}-free medium was also very low, equivalent to that obtained after 30 minutes in the Ca^{2+}-containing control.

Osmolality differences between substrate and substrate-free conditions were not responsible for the observed results, since glucose (5 mM) could replace pyruvate if added at the initiation of incubation. Under such circumstances, osmolality differences between substrate and substrate-free conditions were insignificant (5 mOsmol).

Discussion

Gordon (1973) originally suggested that an outer acrosome membrane Ca^{2+}-dependent ATPase initiates the acrosome reaction by actively increasing acrosomal Ca^{2+} concentration. Green (1978), however, suggested that influx of calcium during the acrosome reaction is a result of passive diffusion. The induction of the acrosome reaction after ATP depletion suggests passive Ca^{2+} influx. Thus, rather than activity of the ATPase, the entry of Ca^{2+} for initiation of membrane fusion and exocytosis may be due to inhibition of an active Ca^{2+}-dependent ATPase expulsion mechanism for calcium. The consistent demonstration of an active Ca^{2+}-dependent ATPase on the outer acrosome membrane in epididymal and seminal spermatozoa (Gordon, 1973) lends support to this idea. The demonstration that plasmalemmal glycoproteins (Gordon et al, 1974) and enzymes (Gordon and Dandekar, 1977) are modified during capacitation of rabbit sperm may now be interpreted as a prerequisite to inactivation of the acrosomal membrane Ca^{2+}-dependent ATPase. Thus loss of plasmalemmal integrity may affect the functional properties of the outer acrosomal membrane. In fact, Gordon et al (1978) demonstrated that the loss of Ca^{2+}-dependent ATPase on the acrosome surface was correlated with destabilization of the plasma membrane of guinea pig sperm.

It is interesting to note that ATP levels decrease during capacitation (Rogers and Morton, 1973). A decrease in acrosomal membrane ATPase activity could be due to a decrease in substrate availability as well as to a decline in enzyme activity. Stores of ATP could be depleted within cellular compartments located near the acrosome independently of axonemal ATP. The sperm head is effectively isolated from the flagellum by a tight membrane junction (Fawcett, 1975). Therefore, the acrosome reaction may occur as a result of localized ATP depletion, despite intense flagellar "activation." In this study, ATP depletion produced immotility. Since pyruvate fully restored motility, ATP depletion does not damage the flagellar-mitochondrial system. The inability of glucose to restore motility is probably due to the ATP requirement for glucose utilization, since glucose alone can maintain motility if present at onset of incubation, ie, when ATP levels are high.

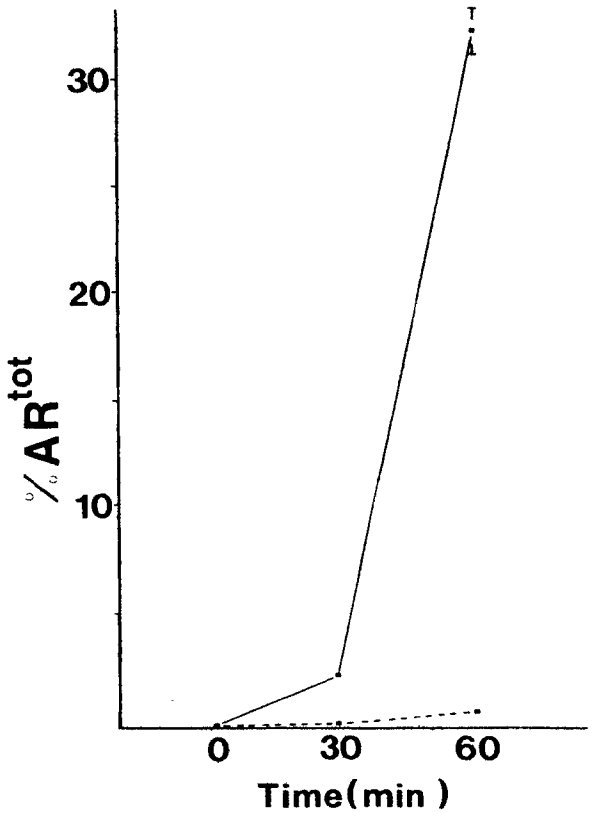

Fig. 3. The percentages of motile acrosome-reacted sperm as a function of incubation time. The motility of sperm incubated in substrate-free BWW (——) was restored by pyruvate addition (10 mM) at 0, 30, and 60 minutes, whereupon acrosome reactions were assessed. Sperm incubated in the presence of pyruvate from 0 time were assessed as well (– – –).

The consequences of ATP depletion on cAMP production may be significant. If cAMP generation is reduced or abolished, this may affect Ca^{2+} flux and the acrosome reaction. It has been shown that exogenous cAMP inhibits (Garcia and Rogers, 1978; Rogers and Garcia, 1979) and exogenous cGMP in the presence of Ca^{2+} induces the guinea

pig acrosome reaction (Santos-Sacchi and Gordon, 1980; Santos-Sacchi et al, 1980).

The rate of induction of acrosome reactions by ATP depletion is slower than that induced by changes in membrane Ca^{2+} permeability. For example, after a 1-hour incubation in glucose-free BWW, the addition of 4.5 μg oleate/10^7 spermatozoa induces a high percent acrosome reaction within a few minutes (Gordon, unpublished observations). This difference in induction time can be explained by the intrinsically poor Ca^{2+} permeability of plasma membrane (Lee and Shin, 1979). It appears that inhibition of Ca^{2+}-dependent ATPase in an otherwise intact membrane will not increase the rate of passive Ca^{2+} permeation but will permit a slow equilibration due to the inability to extrude Ca^{2+}. Agents that increase the passive Ca^{2+} permeability, on the other hand, result in a rapid influx of Ca^{2+}, despite the presence of an active Ca^{2+}-dependent ATPase (Hasselbach, 1972). Hence the rapidity of onset of Ca^{2+}-dependent events is related to the particular mechanism disrupted, whether passive or active.

The spermatozoon is a terminal cell. Irreversible changes in the sperm surface during capacitation prepare the sperm for the acrosome reaction. Thus the breakdown of a Ca^{2+} expulsion mechanism associated with the external acrosome membrane would follow the breakdown of the sperm surface membrane barrier. The fluid compartment between the plasma membrane and acrosome would facilitate such a sequence of membrane changes.

Although the exact mechanism of acrosome reaction induction needs further study, the present findings suggest that Ca^{2+} concentration can increase in the acrosome as a result of ATP depletion. Decreased activity of a Ca^{2+} expulsion mechanism may therefore be the cellular mechanism that initiates the acrosome reaction and prepares the sperm for fertilization.

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