Original article

Adenylate cyclase activity increases concomitantly with the onset of capacitation in heparin-treated bovine spermatozoa

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Summary — This study examined the effect of metal ions Ca^{2+}, Mg^{2+}, and Mn^{2+} and sonication on ejaculated frozen-thawed bovine sperm adenylate cyclase activity, and whether cAMP levels in sperm changed during capacitation with heparin. The sperm adenylate cyclase was almost insensitive to Ca^{2+} at concentrations up to 10 mM when cold-shocked homogenized spermatozoa were used. Adenylate cyclase activity, as observed by cAMP formation (pmol/mg protein/min), did not increase significantly in the presence of 5 mM Mg^{2+}. However, a 40-fold stimulation (cAMP 400–800 pmol/mg protein/min) occurred in the presence of 5 mM Mn^{2+}. Although Ca^{2+} per se had no effect, it acted synergistically with Mg^{2+} and Mn^{2+} in stimulating sperm adenylate cyclase. Adenylate cyclase activity was highest in cold-shocked, homogenized spermatozoa. Sonication of cold-shocked spermatozoa resulted in loss of adenylate cyclase activity, and a logarithmic decrease in cAMP production (1 155.5 to 109.7 pmol cAMP) occurred when sonication was increased from 2 x 5 to 2 x 25 s on ice. Cyclic AMP levels in spermatozoa incubated under non-capacitating conditions, both untreated and treated with glucose or heparin plus glucose, remained higher ($P < 0.01$) compared with those incubated with heparin for the first 4 h of incubation. When spermatozoa were incubated under non-capacitating conditions, cAMP levels increased ($P < 0.01$), especially during the first hour of incubation, and then declined gradually throughout the incubation. In contrast, cAMP levels of heparin-treated spermatozoa declined gradually for 3 h, at which time they began to rise, peaked at 4 h and then remained fairly stable until 6 h. Glucose antagonized the effect of heparin on adenylate cyclase activity but not for more than 4 h. We conclude that: i) Ca^{2+} stimulates adenylate cyclase in the presence of Mg^{2+}; ii) homogenization by sonication reduces cyclase activity in frozen-thawed, cold-shocked spermatozoa; iii) adenylate cyclase activity is inhibited by heparin but rises concomitantly with the onset of capacitation (after 4 h) in spermatozoa incubated under capacitating conditions; and iv) glucose, which prevents capacitation by heparin, antagonizes heparin action on adenylate cyclase.

cation / sonication / adenylate cyclase / heparin / glucose

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Résumé — La capacitation de spermatozoïde bovins induite par l'héparine s'accompagne d'une élévation de l'activité adényl cyclase. *Cette étude a évalué l'effet des ions métalliques calcium, magnésium et manganèse sur l'activité de l'adénylate cyclase des spermatozoïdes de bovin en présence ou en absence d'un agent capacitant, l'héparine. Les résultats obtenus démontrent une insensibilité de la cyclase au calcium à des concentrations atteignant 10 mM avec des préparations de spermatozoïdes homogénéisés à froid. L'activité de l'adénylate cyclase telle que mesurée par l'accumulation d'AMP cyclique n'augmente pas avec les faibles doses de magnésium (5 mM). En revanche, avec le manganèse à cette même dose (5 mM) l'augmentation est de 40 fois la valeur contrôle. Bien que le calcium n'ait pas d'effet seul, il augmente la réponse au magnésium et au manganèse. Nos résultats ont aussi démontré que ces réponses demeuraient perceptibles lorsque les spermatozoïdes étaient homogénéisés à froid contrairement à une homogénéisation par ultrason qui entraîne une perte graduelle de l'activité (1 155,5 pmol et 109,7 pmol respectivement pour des temps de 2 x 5 et 2 x 25 secondes). Le niveau basal d'AMP cyclique observé avec les spermatozoïdes incubés dans des conditions non-capacitantes en présence ou en absence de glucose demeure plus élevé que dans des conditions capacitantes (p < 0,01). En absence d'héparine, les niveaux d'AMP cyclique ont augmenté durant la première heure d'incubation pour décliner par la suite. Dans le cas des spermatozoïdes incubés avec héparine, il y a eu diminution graduelle pour 3 heures suivi d'une augmentation à 4 heures pour ensuite rester stable jusqu'à 6 heures d'incubation. La présence du glucose a eu un effet inhibiteur de l'effet de l'héparine pour les premières 4 heures seulement. En conclusion, i) le calcium stimule l'adénylate cyclase seulement en présence de magnésium, ii) l'utilisation des ultrasons pour homogénéiser les cellules détruit l'activité de l'adénylate cyclase, iii) l'activité basale de l'adénylate cyclase est d'abord inhibée puis augmente de façon concomitante avec la capacitation, iv) ce qui n'est pas le cas en présence de glucose qui est reconnu pour inhiber la capacitation.*

cation / ultrasons / adénylate cyclase / héparine / glucose

INTRODUCTION

It has long been known that when mammalian spermatozoa leave the male reproductive tract, they are capable of independent motility but not of fertilizing oocytes. Chang (1951) reported that preincubation of spermatozoa in the female genital tract is a prerequisite for fertilization of the oocyte. Acquisition of the capacity to fertilize an oocyte is termed capacitation (Austin, 1952). In bovine animals, capacitation normally occurs in the female reproductive tract, however, it can be achieved in vitro to obtain normal progeny (Brackett et al, 1982; Lambert et al, 1986).

Capacitation requires a species-specific length of time for completion (Bedford, 1970), and successful protocols are available to induce capacitation. However, the biochemical reactions involved in the process are still poorly understood. At present, no obvious characteristics have been found that can be evaluated to measure the degree of transition toward the fully capacitated state. No gross morphological changes, which might be monitored easily, occur (Bedford, 1970; Yanagimachi, 1988), and no biochemical parameters have been defined that could give such information.

Over the past decade, evidence had been accumulating that the cyclic nucleotide cAMP plays a role in mammalian sperm motility, capacitation, and acrosome reaction (reviewed by Garbers and Kopf, 1980; Tash and Means, 1983; Fraser, 1983; Fraser and Ahuja, 1988). The observation that intracellular cAMP levels increase during capacitation suggests changes in activity of the enzymes involved in its metabolism. Evidence that adenylate cyclase activity increases during this time has been obtained for the guinea pig (Morton and Albagli, 1973), mouse (Stein and Fraser, 1984; Monks et al, 1986) and hamster (White and Aitken,

1989) using epididymal spermatozoa capacitated in vitro.

Epididymal spermatozoa may differ from ejaculated spermatozoa in terms of normal physiological functions. Indeed, the zona pellucida failed to induce acrosome reaction on epididymal spermatozoa (Florman and First, 1988b). Differences in adenylate cyclase activity have also been reported in spermatozoa from caput epididymis compared with those from caudal epididymis (Vijayaraghavan and Hoskin, 1985). In addition, the capacitation status of mouse sperm was evaluated by the acrosome reaction rate (Fraser, 1981; Stein and Fraser, 1984), so the increase in adenylate cyclase activity may relate to acrosome reaction rather than to capacitation in these studies, and the processes of capacitation and acrosome reaction could be confused. A rise in adenylate cyclase activity was reported for freshly ejaculated boar spermatozoa incubated for 2 h in vivo in the gilt uterus (Berger and Clegg, 1983), but whether these conditions support capacitation is not known. More recently, Uguz et al (1992) reported that cAMP is elevated during capacitation of bovine sperm by heparin or oviduct fluid.

Because heparin, a gluycosaminoglycan, induces bovine sperm capacitation in vitro (Parrish et al, 1988, 1989a,b), independent of acrosome reaction unless lysophosphatidylcholine (Parrish et al, 1988) or solubilized zone pellucida (Florman and First, 1988a) are added, this study was conducted to determine whether incubation of bovine sperm in vitro with heparin affects the cAMP-generating system during capacitation. The cAMP nucleotide has been associated with an increase in sperm motility, but its action has been characterized mainly within the tails of demembraned epididymal sperm. The influence of cAMP in the capacitation process suggests an effect in the head and possibly in the midpiece. Therefore, to evaluate the influence of cAMP during the capacitation process,

homogenized sperm must be used in cyclase assay to measure maximal basal adenylate cyclase activity during capacitation. We report that a procedure such as sonication to homogenize cellular components reduces adenylate cyclase activity in frozen-thawed, cold-shocked bovine spermatozoa, and that intracellular cAMP levels rise concomitantly with the onset of capacitation in spermatozoa incubated under capacitating conditions.

MATERIALS AND METHODS

Sperm culture

A single frozen pool of semen from five different bulls was prepared and donated by the Centre d'Insemination artificielle du Quebec Inc (CIAQ Inc, Sainte-Hyacinthe, Quebec). The straws were thawed in a 35 °C water bath for 1 min and washed twice in Sp-TALP (Parrish et al, 1988) supplemented with 6 mg/mL fatty-acid-free bovine serum albumin (BSA, Sigma, St-Louis, MO). The spermatozoa were finally resuspended at 10×10^6 cells/mL in Sp-TALP, which could contain 10 µg/mL heparin (166 IU/mg, Sigma), 5 mM glucose, or other substances to be evaluated as indicated in the text and were incubated (39 °C, 5% CO_2) for up to 6 h. Samples of these suspensions were collected at the indicated times for adenylate cyclase assay.

Sperm preparation for adenylate cyclase assay

At precise times during the incubation of spermatozoa, a thoroughly mixed aliquot (2.5 mL) of the suspension was centrifuged for 5 min, 600 g at room temperature. The supernatant was discarded and the pellet resuspended in Hepes buffered saline [10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (Sigma), 150 mM NaCl, pH 7.2] supplemented with 1 mM ethylenediaminetetraacetic acid (EDTA, Sigma) and centrifuged as above. The supernatant was discarded, and the sperm pellet was 'cold-shocked' by resuspension in ice-cold (0–4 °C) homogenization buffer

(1/10 of the initial volume of sperm suspension), which contained 50 mM Hepes, 1 mM ethylene-glycol-bis-(β-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA, Sigma) 10% dimethyl sulfoxide (DMSO, Sigma), pH 7.6. The spermatozoa were then homogenized or sonicated on ice. Homogenization was achieved by passing the sperm suspension through an 18-G needle ten times using a 5 mL syringe. Sonication comprised two bursts each of 5, 10, 15, 20 or 25 s applied to a 5 mL suspension of spermatozoa using a 3 mm probe on a Vibra Cell Ultrasonic tissue disintegrator (Sonics and Materials Inc, Danbury, CT). The microtip output control limit was set at 4.5, and bursts were given in a continuous pulse manner at maximum power.

Adenylate cyclase assay

Adenylate cyclase activity in spermatozoa was determined by measuring the rate of conversion of $[a-^{32}P]$ATP to $[^{32}P]$cAMP. The adenylate cyclase assay was performed as usual in our facilities for uterine cells from cows (Fortier et al, 1988) and rabbits (Fortier et al, 1989; Lambert et al, 1990) with some modifications. For the assay, 40 μL of the sperm suspension prepared as above was added to the assay tube containing (in the final concentration) 50 mM Hepes, 1 mM 3-isobutyl-1-methylxanthin (IBMX, Sigma), 10 mM KCL, 16 μg creatine kinase (800 IU/mg 37 °C, Boehringer Mannheim, Laval, Quebec), 3.2 mM creatine phosphate (Boehringer Mannheim), 0.25 mM adenosine triphosphate (ATP, Boehringer Mannheim), 4 mM DTT (1,4-dithiothreitol, Boehringer Mannheim), and 0.4 μCi $[a-^{32}P]$ATP (New England Nuclear Research Products, Boston, MA (NEN)) in a final volume of 150 μL, pH 7.6. Mn^{2+} at 5 or 20 mM was used as the standard divalent cation. In experiment 1 it was replaced by Mg^{2+} or Ca^{2+} as specified. The reaction was performed at 39 °C for 15 min. Then 100 μL of the stopping solution [36.4 mM ATP, 10 mM 3',5'-cyclic AMP (Sigma), 1% sodium dodecyl sulfate and 30 000 cmp $[^{3}H]$cAMP (NEN)] was added to the assay tubes to terminate the reaction, and the tubes were boiled for 5 min. The $[^{32}P]$cAMP formed was recovered following dual chromatography using Dowex and alumina columns (Salomon et al, 1974), and the tritiated cAMP was used as an internal standard for the evaluation of recovery.

Determination of sperm membranes and cyclase assay mixture stability

The stability of sperm membranes and cyclase assay was determined over a 6 h period. For this experiment, an intact sperm homogenate was prepared and divided into three aliquots: the cyclase activity of one aliquot was assessed immediately (0 h) using freshly prepared assay mixture; the second and third were kept on ice for 6 h. Cyclase activity in the second and third aliquots was then assessed using respectively the assay mixture that was prepared at 0 h (and then kept on ice) and a freshly prepared assay mixture. The assay was performed in the presence of 5 mM Mn^{2+}.

Protein determination

A 100 μL aliquot of sperm suspension was taken to measure the protein contents, which were assayed according to Lowry et al (1951) using BSA as a standard.

Statistical analysis

Frozen-thawed sperm were assayed for adenylate cyclase activity within 6 h after washing and incubation. The adenylate cyclase assays were conducted in triplicate, and experiments were repeated at least three times. Data were analysed by a multiple regression approach. Analysis of variance was performed to determine the differences in treatments. Means from analyses that were different at $P < 0.05$ were tested using the multiple F-test (Duncan, 1955). The results are expressed as means ± SD.

RESULTS

Effect of divalent cations Ca^{2+}, Mg^{2+} and Mn^{2+}

In the first set of experiments, cold-shocked homogenized spermatozoa were used to

standardize the adenylate cyclase assay for frozen-thawed spermatozoa. The effects of the metal ions Ca^{2+}, Mg^{2+} and Mn^{2+} and the length of the assay were evaluated first and the results using untreated spermatozoa are shown in figure 1. The sperm adenylate cyclase was almost insensitive to Ca^{2+} even up to 10 mM concentrations (fig 1a).
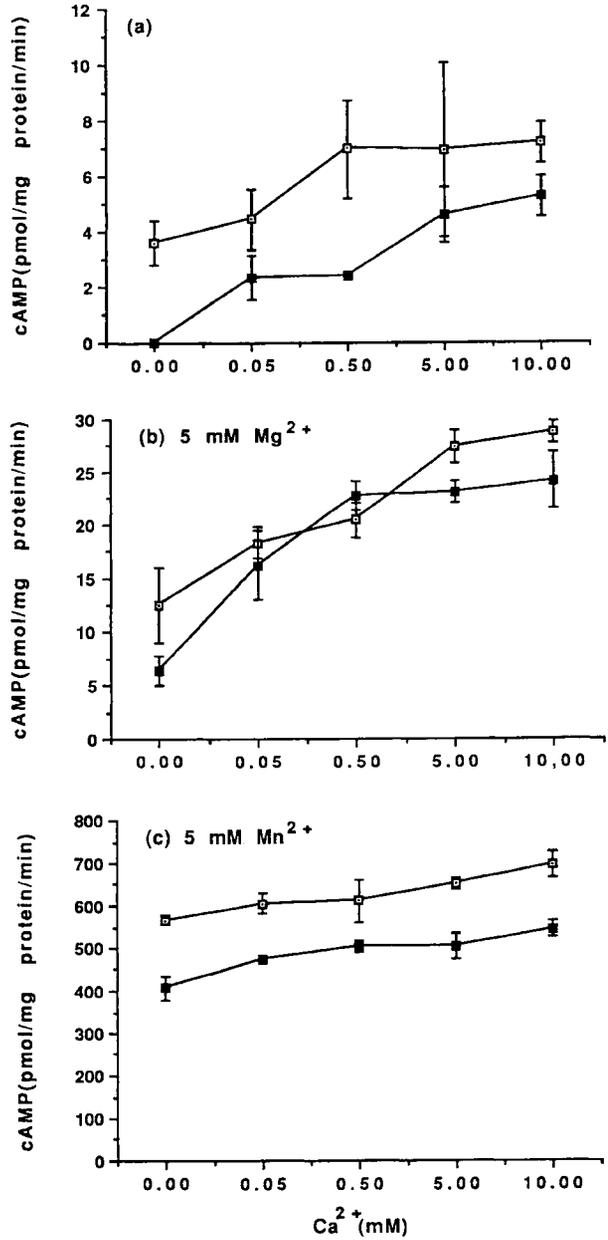
Fig 1. Effect of increasing concentrations of Ca^{2+} on adenylate cyclase activity in frozen-thawed spermatozoa. The dose-responses to Ca^{2+} (0–10 mM) were measured alone (**a**) or in the presence of 5 mM Mg^{2+} (**b**) or 5mM Mn^{2+} (**c**). Open symbols represent 0 h and closed symbols represent 6 h incubation. The responses at the two different times were significantly different ($P < 0.01$). Data are the means ± SD of three different experiments.

Although Mg^{2+} is recognized as the physiological cofactor in most adenylate cyclase systems, the sperm adenylate cyclase responded poorly to 5 mM Mg^{2+} (twice the concentration representing half-maximal stimulation; Brown and Casillas, 1986). However, the presence of Mg^{2+} conferred a dose-response sensitivity of sperm cyclase ($P < 0.05$) to increasing concentration of Ca^{2+} (fig 1b). When the assay was performed in the presence of 5 mM Mn^{2+} (twice the concentration representing half maximal concentration; Braun, 1975), the level of cAMP formation was remarkably higher (fig 1c), but Ca^{2+} sensitivity was reduced compared to Mg^{2+}. A decreased rate ($P < 0.05$) of cAMP formation was observed at 6 h compared with 0 h in these experiments.

Effect of sonication

Sonication appears to affect adenylate cyclase activity more than standard methods of homogenization (Towns and Luke, 1976). Therefore, the second set of experiments was performed to optimize the length of sonication to maximize adenylate cyclase activity. The effect of sonication was studied before and after a 6 h incubation period in the presence of 5 mM (fig 2a) or 20 mM (fig 2b) Mn^{2+}. Figure 2 shows that: (1) cAMP levels were higher ($P < 0.01$) in cold-shocked, homogenized spermatozoa (0 = no sonication); and (2) not only were cAMP levels lower in sonicated spermatozoa, but also there was a linear decrease ($P < 0.05$) in cAMP production (1 155.5 to 109.7 and 765.5 to 38.0 pmol cAMP at 0 and 6 h respectively; fig 2a) when the length of sonication was increased from 2 x 5 to 2 x 25 sec.

In the above experiments, cAMP levels were lower ($P < 0.05$) at 6 h than at 0 h. An experiment was therefore performed to evaluate whether this decrease in cAMP levels was due to the length of spermatozoal incu-

bation or to a degradation of the regeneration system (creatine kinase, creatine phosphate) of the cyclase assay mixture over time. Figure 3 demonstrates that sperm membranes were stable for at least 6 h when kept on ice in homogenization buffer. It also shows that the cyclase assay mixture was stable for the entire length of the protocol. Therefore, the reduction in cyclase activity following incubation of sperm for 6 h at 39 °C is due to metabolism within sperm cells.

Effect of heparin and glucose on adenylate cyclase activity

Adenylate cyclase activity measured in heparin-treated homogenized spermatozoa in the presence of 5 mM Mn^{2+} gradually declined until 3 h, a significant increase ($P < 0.01$) was observed at 4 h, and activity was fairly stable up to 6 h. Untreated spermatozoa, however, showed increased ($P < 0.01$) activity at 1 and 3 h of incubation compared with 0 h. Adenylate cyclase activity in untreated spermatozoa was higher ($P < 0.001$) than in heparin-treated spermatozoa up to 4 h of incubation (fig 4a).

The effect of 5 mM glucose, which retards capacitation induced by heparin (Parrish et al, 1989) was then evaluated in terms of adenylate cyclase activity. Unlike heparin-treated spermatozoa, the pattern of adenylate cyclase activity in glucose-treated spermatozoa was similar to that of untreated spermatozoa. However, throughout the incubation, cAMP levels in glucose-treated spermatozoa remained lower ($P < 0.05$) than in untreated spermatozoa except at 3 h (fig 4). No difference in adenylate cyclase activity was seen between the untreated cells and those treated with heparin plus glucose throughout incubation (fig 4a).

As illustrated in figure 4b, adenylate cyclase activity in glucose-treated sperma-

tozoa, when measured with 20 mM Mn^{2+}, was higher ($P < 0.05$) than those of untreated, heparin-treated and heparin plus glucose-treated spermatozoa at 0 h. This cyclase activity in heparin-treated spermatozoa gradually decreased until 3 h and steeply increased ($P < 0.001$) at 4 h. Although adenylate cyclase activity in glucose-treated cells was higher ($P < 0.001$) compared with heparin-treated cells through-

out the incubation, at 6 h the pattern of activity was similar in both treatments.

Unlike heparin and glucose-treated cells, cyclase activities of untreated cells and of those treated with heparin plus glucose increased ($P < 0.001$) from 0 to 1 h and then decreased gradually thoughout the incubation. No difference in adenylate cyclase activity was observed between untreated spermatozoa and those treated with hep-
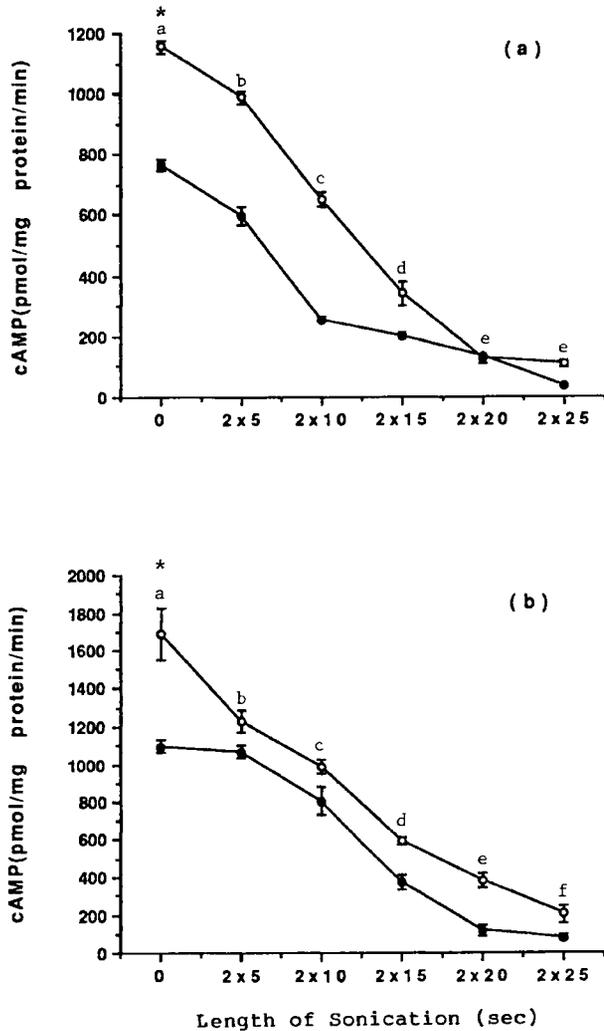
Fig 2. Effect of cold-shock and sonication on adenylate cyclase activity of untreated spermatozoa in the presence of (**a**) 5 mM Mn^{2+}, or (**b**) 20 mM Mn^{2+}. Open symbols represent 0 h and closed symbols represent 6 h incubation. Data shown are means ± SD ($n = 3$). * Cold-shocked spermatozoa. Data points with different superscripts were significantly different ($P < 0.05$).
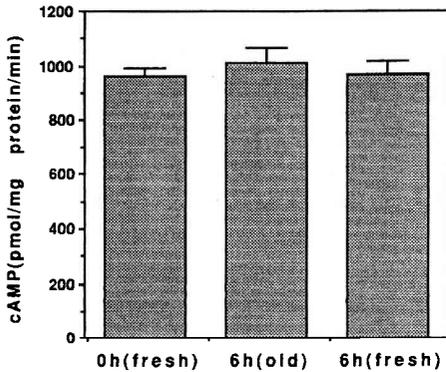
Fig 3. Effect of length of storage of regeneration system of the adenylate cyclase assay mixture. Untreated sperm homogenate was prepared, divided into three aliquots and kept on ice. 0 h (fresh) = assay mixture prepared at 0 h and half used immediately for one sperm aliquot; 6 h (old) = second half of the assay mixture kept on ice for 6 h and used for the second sperm aliquot; 6 h (fresh) = freshly prepared assay mixture used for third sperm aliquot.

arin plus glucose, but the activity was greater ($P < 0.001$) than for the heparin-treated spermatozoa throughout the incubation except at 4 h (fig 4b).

DISCUSSION

In the present study, the adenylate cyclase activity of bull spermatozoa was first evaluated in cold-shocked homogenized spermatozoa in the presence of metal ions Ca^{2+}, Mg^{2+} and Mn^{2+}. Bovine sperm adenylate cyclase, like the adenylate cyclase systems of other mammalian spermatozoa, uses metal-ATP as a substrate (Braun, 1975; Herman et al, 1976; reviewed by Fraser and Monks, 1990).

Although Ca^{2+} per se has no effect on sperm adenylate cyclase, it acts synergistically with Mg^{2+} and Mn^{2+} in stimulating sperm adenylate cyclase. Augmentation by

Ca^{2+} of the stimulatory effects of Mg^{2+} and Mn^{2+} on adenylate cyclase is not unique to bull sperm (Braun, 1975) but has been observed in other species as well (rat: Bianchi, 1963; guinea pig: Hyne and Garbers, 1979). Although 0.1–1 mM Ca^{2+} inhibits or completely abolishes adenylate cyclase activity in various somatic cell types (reviewed by Robison et al, 1971), 0.05–10 mM Ca^{2+} potentiated adenylate cyclase stimulation by Mg^{2+} and Mn^{2+} in our study. These concentrations are at the upper limit of the physiological range. However, this information might be significant with regard to sperm function in vivo. In mammals acquisition of motility by spermatozoa occurs in the corpus of the epididymis, however; spermatozoa become motile for the first time at ejaculation. A rapid flow of Ca^{2+} into the sperm might occur at ejaculation and result in divalent ion translocation to the catalytic and/or regulatory site(s) of the sperm adenylate cyclase, because bovine seminal plasma has a much higher Ca^{2+} content (8.5 mM; Mann, 1964) than spermatozoa (1 mM; Bianchi, 1963). Alternatively, Ca^{2+} influx through the sperm membrane coupled with ion translocation might displace one (or more) substance(s), for example, Cu^{2+} restricts enzyme activity (Braun, 1975).

Although Mg^{2+} and Mn^{2+} both stimulated adenylate cyclase activity of spermatozoa, the effect was remarkably greater in the presence of Mn^{2+} (fig 1). When Mn^{2+} alone or Mn^{2+} plus Mg^{2+} were used, the specific activity of adenylate cyclase was several times higher than with Mg^{2+} alone. Further, at concentrations of Mn^{2+} near saturation, Mg^{2+} does not produce an additional increase in activity, indicating that Mn^{2+} can completely satisfy the requirement for divalent cations (Herman et al, 1976); however, the mechanism of the stimulatory effect of Mn^{2+} is unknown.

Adenylate cyclase from mature mammalian spermatozoa is membrane-bound (Brown and Casillas, 1986; Peterson et al,

1980) and its activity is found primarily in the particulate fraction (Garbers and Kopf, 1980), although Herman et al (1976) recovered at least 30% of the total enzyme activity in bull spermatozoa in the soluble fraction. The cold-shock procedure has been shown to be a good treatment for evaluating adeny-

late cyclase activity in rams (Towns and Luke, 1976) and sonication, which disrupts the different parts of the cell, was added to enhance the activity. Adenylate cyclase activity was highest when the frozen-thawed sperm used in this study were cold-shocked and homogenized (without sonication), and
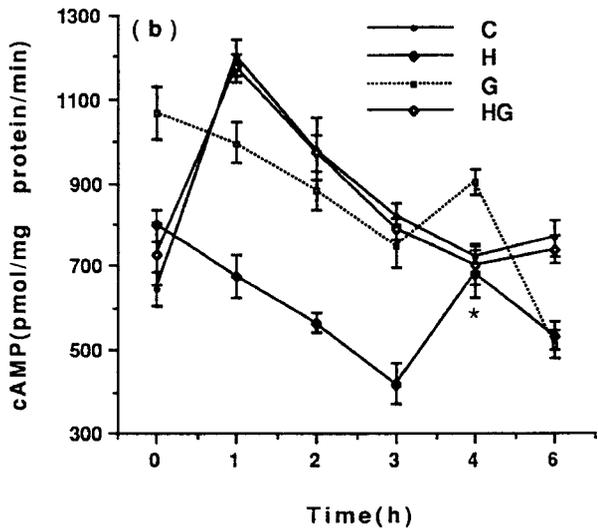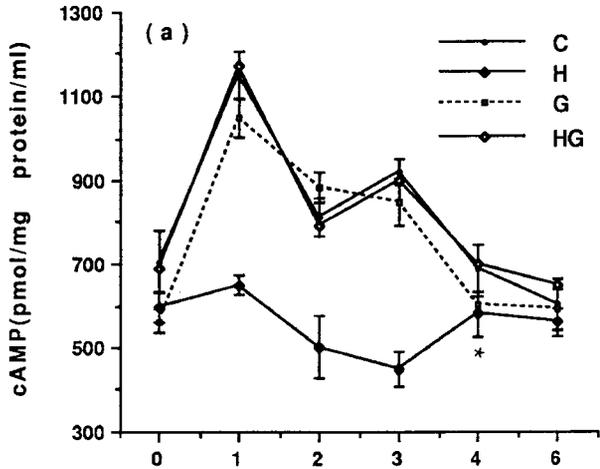
Fig 4. Effect of 10 µg/mL heparin (H), 5 mM glucose (G), and 10 µg/mL heparin plus 5 mM glucose (HG) on adenylate cyclase activity of spermatozoa. Untreated cold-shocked homogenized spermatozoa were used as controls (C) and assays were performed in the presence of (a) 5 mM Mn^{2+} and (b) 20 mM Mn^{2+}. Data are means ± SD (n = 5) * Shows a significant capacitation-related increase (P < 0.01) in cAMP levels in heparin-treated spermatozoa which began to rise at 3 h and peaked at 4 h.

decreased when the length of sonication increased (fig 2).

We could not find any report detailing the effect of sonication on adenylate cyclase activity of frozen-thawed, cold-shocked spermatozoa; however, Stephens et al (1979) reported that sonication periods longer than 30 s decrease the activity of both particulate and soluble fractions of cAMP phosphodiesterases in cold-shocked bovine spermatozoa. It has also been reported that longer sonication (5 min) results in significantly lower adenylate cyclase activity than shorter sonication (1, 2, 3 and 4 min) in freshly ejaculated, cold-shocked boar spermatozoa (Berger and Clegg, 1983). Towns and Luke (1976) reported higher adenylate cyclase activity in spermatozoa subjected to freeze-thawing, and lower adenylate cyclase activities, in order, in spermatozoa subjected to cold-shock and sonication. Because sonication destroys all cellular organization, including possible regulatory relationships based on structural integrity, it is not surprising that a decrease in enzyme activity is observed with an increase in duration of sonication. As a result, enzyme activities determined in suspensions frozen-thawed, cold-shocked and then sonicated may be poor indicators of the actual activities prevailing in situ.

Basal adenylate cyclase activity evaluated with Mn^{2+} at two concentrations was different in spermatozoa incubated under non-capacitating and capacitating conditions (fig 4). Since heparin capacitates bovine sperm in vitro (Parrish et al, 1985), and capacitation of sperm by heparin requires at least 4 h of exposure (Parrish et al, 1988), our results, showing no increase in basal adenylate cyclase activity in heparin-treated spermatozoa during the first 3 h and then an increase concomitant with the onset of capacitation (from 3 to 4 h), are similar to those observed in mouse spermatozoa (Stein and Fraser, 1984; Monks and Fraser, 1987). Stein and Fraser (1984)

and Monks et al (1986), using a well characterized mouse in vitro capacitation and fertilizing system, found a significant increase in adenylate cyclase activity when sperm suspensions were sampled, first early during capacitation and then at the end of capacitation, when cells are highly fertile (Fraser, 1983). It has also been shown that phosphodiesterase activity decreases significantly during capacitation (Monks and Fraser, 1987). Together, this potential for increasing the formation of cAMP and decreasing the breakdown of cAMP to 5'-AMP suggests an increasing availability of cAMP during capacitation. In a recent study Uguz et al (1992), using cAMP radioimmunoassay, observed an increase in the absolute amount of cAMP in freshly ejaculated bovine spermatozoa incubated with heparin or oviduct fluid compared with controls, thus supporting the hypothesis of reduced phosphodiesterase activity in capacitated spermatozoa.

We observed an increase in cAMP levels at 4 h which corresponds to the onset of capacitation in heparin-treated spermatozoa, but the levels remained low compared to untreated spermatozoa. In the literature cAMP levels have been reported to be higher in capacitated spermatozoa than non-capacitated controls (mouse: Stein and Fraser, 1984; Monks et al, 1986; hamster: White and Aitken, 1989). In addition to species differences, some differences between these data and ours may be explained by their use of another system in which demembraned epididymal spermatozoa were capacitated in the absence of heparin. Moreover, in these other studies the accumulation of cAMP over the capacitating process was measured, whereas we measured a functional response in membranes of capacitated spermatozoa.

Adenylate cyclase activity in spermatozoa treated with glucose or with heparin plus glucose was no different from the controls; however, it was significantly different from

the heparin-treated spermatozoa during the first 3 h of incubation. Because glucose counteracts the effect of heparin during the first 4 h of incubation (Parrish et al, 1989a), the aforementioned finding was not surprising but may indicate a pathway to explain the difference.

A significant increase in adenylate cyclase activity was also observed, especially during the first hour, comparing glucose treatment with heparin alone. Glucose regulation of sperm motility was reported by Mujica et al (1991). It has been shown that an increase in pH of the incubation medium results in an increase in sperm motility (Ijaz and Hunter, 1988). Similarly, an increase in incubation temperature modulates both adenylate cyclase activity and motility (Hammerstedt and Hay, 1980). Increasing evidence correlates the cAMP content of spermatozoa with motility (reviewed by Tash and Means, 1983; Fraser and Monks, 1990). Moreover, the increase in cAMP content of the control and glucose plus heparin-treated spermatozoa may be explained partly by an increase in the pH of the incubation medium from 7.4 to 7.8 during the first 3 h of incubation (unpublished data). It may also be due to an increase in the incubation temperature from approximately 22 °C during manipulations at the beginning of the treatment to 39 °C during incubation. However, no detrimental effect of a capacitating concentration of heparin on sperm motility has been reported during incubation at 39 °C for up to 6 h (Parrish et al, 1988; Florman and First, 1988a,b). Accordingly, the motility in heparin and other treatments remained similar in our experiments (data not shown).

In conclusion, our results indicate that Mn^{2+} completely satisfies the requirement for divalent cations for sperm adenylate cyclase. Heparin delays the increase in cAMP production by spermatozoa during incubation at 39 °C; the addition of glucose prevents the effect of heparin.

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