Human and Ram Seminal Plasma Both Contain a Calcium-Dependent Regulator Protein

Calsemin

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Biochemical aspects of post-testicular sperm maturation have been studied. Partial purification of either human or ram seminal plasma yields a heat-stable, acidic protein which is a Ca^{2+}-dependent regulator of 3′:5′-cyclic nucleotide phosphodiesterase. This protein fraction has been provisionally named calsemin. Human calsemin activates the flagellar plasma membrane Ca^{2+}-ATPase of ram epididymal and ejaculated spermatozoa by 290% and 20%, respectively. Activation is abolished by trifluoperazine. The addition of calsemin plus Ca^{2+} to isolated ram caudal spermatozoa results in a three-fold stimulation of flagellar beat activity. These results lead us to propose that only after interaction between calsemin and the sperm flagellar plasma membrane can the Ca^{2+}-ATPase obtain full expression to maximise Ca^{2+} efflux from the flagellum and thereby cause a stimulation and coordination of sperm flagellar beat activity. This finding may provide a partial biochemical explanation for the development of mammalian sperm motility.

Key words: Spermatozoa, initiation of motility, Ca^{2+}-dependent regulator, Ca^{2+}-ATPase, epididymis, seminal plasma.

The biochemical events associated with the epididymal and ejaculated stages of sperm maturation and, in particular, the initiation of motility have not been completely resolved. It is known, however, that the plasma membrane undergoes considerable changes in composition during post-testicular maturation (Nicolson and Yanagimachi, 1979). Of particular interest are glycoproteins that coat the plasma membrane (Olson and Hamilton, 1978). It is unlikely that these proteins are synthesized *de novo* by spermatozoa since these cells lack a cytoplasmic protein synthesizing system (Clermont, 1977). Recent studies have shown that synthesis of certain epididymal proteins and glycoproteins is androgen-dependent (Jones et al, 1980a; Brooks & Higgins, 1980) and probably occurs in the epididymal epithelia (Moore, 1980). Some of these epididymal proteins attach to the sperm plasma membrane during epididymal transit (Kohane et al, 1980). To date, no biochemical function has been directly assigned to these sperm proteins; however, they could be involved in the acquisition of coordinated flagellar movement. Hoskins et al (1979) have proposed that a multimeric, heat-stable, acidic glycoprotein found in seminal plasma (referred to as forward motility protein) is largely responsible for inducing changes in sperm motility (Acott and Hoskins, 1978). The metabolic basis for these changes is unknown. In this paper we report the partial purification from both human and ram seminal plasma of a heat-stable, acidic protein fraction which acts as a Ca^{2+}-dependent regulator of two enzymes: Ca^{2+}-dependent Mg^{2+}-ATPase and 3′:5′-cyclic nucleotide phosphodiesterase. This protein fraction, which has been provisionally named calsemin, may function as a critical regulator of sperm processes, and in particular may be involved in the initiation of sperm motility.

This work was supported by grants from the Ford Foundation (790-0658) and the Medical Research Council of New Zealand.

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Submitted for publication August 12, 1981; revised version received November 23, 1981; accepted for publication December 3, 1981.

0196-3635/82/0900/0289/$01.20 © American Society of Andrology
(J Androl 1982; 3:289–296)

Materials and Methods

Sperm-free semen from vasectomized human donors was centrifuged twice at 40,000 × g for 30 minutes and the supernatant fraction heated for 5 minutes at 90 C. The suspension was then centrifuged at 40,000 × g for 1 hour, the pellet was discarded, and the supernatant adjusted to *p*H 4.0. The acidified fraction was centrifuged at 100,000 × g for 1 hour to recover precipitated protein which was redissolved with gentle stirring in buffer 1 (10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid [HEPES], 0.5 mM $CaCl_2$, *p*H 7.0). Partial purification of calsemin from the heat-treated, acid-precipitated seminal plasma was achieved using fluphenazine-affinity chromatography (Charbonneau and Cormier, 1979). The seminal plasma extract was loaded onto the fluphenazine affinity column and the column was washed with buffer 1 until protein elution ceased. Non-specifically bound protein was eluted using buffer 2, (10 mM HEPES, 0.15 mM $CaCl_2$, 0.5 m NaCl, *p*H 7.0). Finally buffer 3, (10 mM Tris HCl, 10 mM ethyleneglycol-bis-(β-aminoethyl ether) N,N'-tetraacetic acid [EGTA], *p*H 8.0) was applied to the column to remove protein bound specifically to the immobilized fluphenazine. At each stage of the purification, 20 μl samples were removed from the 8 ml column fractions and tested for their ability to activate 3':5'-cAMP phosphodiesterase (PDE). The PDE assay, carried out as previously described (Loten et al, 1978), contained in a final volume of 0.15 ml: 50 mM Tris HCl, *p*H 7.5, 1.0 mM EGTA, 2.30 mM $CaCl_2$, [^{3}H] cAMP (37.7 Ci/mmol), 0.25 mM cAMP (unlabeled), and 25 mM $MgCl_2$. The assay was initiated by the addition of 10 μl of PDE (2 mg/ml). The activator-deficient bovine heart PDE used in the assays was obtained from Sigma Chemical Company. The fractions (80 to 95) eluted with buffer 3 were pooled, dialyzed against 10 mM HEPES-Tris, *p*H 7.0, and a sample (40 μg protein) was analyzed by electrophoresis on 10 to 15% SDS-polyacrylamide gels using the method of Owens and Haley (1976). Calsemin was purified from ram seminal plasma by methods identical to those used for human calsemin.

Flagellar plasma membranes of ram ejaculated spermatozoa were prepared as previously described (Bradley and Forrester, 1980a). Washed ram spermatozoa were diluted tenfold with isotonic buffer (30 mM Tris HCl, 103 mM NaCl, 12.5 mM KH_2PO_4, 2.5 mM K_2HPO_4, 30 mM $MgCl_2$, 0.4 mM EDTA, *p*H 7.4), and 5 ml portions were sonicated using an ultrasonic disintegrator (MSE Scientific Instruments, England, model 100 W) (20% of full amplitude for 20 seconds) to dissociate sperm heads from flagella. The sonicates were pooled, diluted with isotonic buffer and repeatedly centrifuged at 600 × g for 15 minutes to remove the sperm heads. The flagellar fraction, recovered by centrifuging the supernatant at 1500 × g for 10 minutes, was resuspended in two volumes of double-distilled water and forced several times through a pasteur pipette. The resulting homogenate was fractionated by centrifugation (100,000 × g for 2 hours in a Beckman L3-50 ultracentrifuge) through a discontinuous density gradient of 20.0% (10 ml), 37.6% (15 ml), and 45.3% (15 ml) sucrose w/v, respectively. The membrane fraction was recovered from the central region of the 37.6% sucrose band, diluted in 18 mM histidine-18 mM imidazole buffer, *p*H 7.2, containing 5 mM EGTA, centrifuged again at 100,000 × g for 1 hour and finally resuspended in a volume of 18 mM histidine-18 mM imidazole buffer, *p*H 7.2. The yield of membrane vesicles was 50 to 100 μg protein/10^9 sperm.

The total Ca^{2+}-ATPase activity was determined as previously described (Bradley and Forrester, 1980b) using a reaction mixture (1 ml) containing: 18 mM histidine-18 mM imidazole buffer, *p*H 7.2, 3 mM ATP, 15 mM KCl, 5 mM EGTA, 0.1 mM ouabain, 4.85 mM $CaCl_2$, and approximately 50 μg of plasma membrane protein. After incubation for 1 hour at 37 C, the extent of ATP hydrolysis was determined using an enzyme-linked assay to measure ADP produced (Jawonek et al, 1974). The ATPase activity obtained in the absence of $CaCl_2$ is defined as the Mg^{2+}-ATPase. The increase in Mg^{2+}-ATPase activity in the presence of free Ca^{2+} (4.85 μM) represents the Ca^{2+}-ATPase activity. Free Ca^{2+} levels were estimated using the computer program of Perrin and Sayce (1967). Protein determinations were performed according to the method of Bradford (1976).

Caudal spermatozoa were isolated and from fresh ram testes as previously described (van Eerten and Forrester, 1980), except that the isolation buffer contained 30 mM Tris HCl, 103 mM NaCl, 5.0 mM KCl, 30 mM $MgCl_2$, 0.4 mM EDTA, *p*H 7.4. Briefly, the method involved removal of the tunica, rinsing the testes in isolation buffer, and dissection of the cauda region of the epididymis. The cauda epididymides were then finely minced into isolation buffer and the tissue gently stirred for 30 minutes to allow release of spermatozoa. The material was then strained through muslin and the filtrate centrifuged at 1000 × g for 10 minutes. The spermatozoa were identified as a discrete cream-colored layer over a firm pellet of epididymal tissue and were carefully removed with a pasteur pipette and repeatedly washed by centrifugation (1000 × g for 10 minutes) using isolation buffer. This procedure yielded a highly purified preparation of ram caudal spermatozoa. Flagellar plasma membranes of ram caudal spermatozoa were prepared as described above for ejaculated sperm samples. Motility assays were performed by incubating approximately 1 × 10^8 ram caudal spermatozoa (100 μl) for 10 minutes at 37 C in a final reaction mixture (1 ml) which contained one of the following: isolation buffer, isolation buffer + 2.5 μg calsemin, isolation buffer + 0.6 mM Ca^{2+}, isolation buffer + 0.6 mM Ca^{2+} + 2.5 μg calsemin. Motility assessments were made using a Leitz Orthoplan phase-contrast microscope with heating stage at 37 C (Bradley and Forrester 1981). Motility was assessed by a double-blind procedure in which five separate observers were required to score the degree of flagellar beat activity of the various samples on an arbitrary scale of one to five units (five being the most vigorous). Flagellar beat activity is simply defined as the intensity of flagellum movement.

Results

The isolation of the Ca^{2+}-dependent regulatory protein from human seminal plasma was attempt-

ed using a phenothiazine affinity column. This method of purification was chosen because of our earlier demonstration that the Ca^{2+}-dependent activating potential of human seminal plasma extracts is readily inhibited by phenothiazines such as trifluoperazine and chlorpromazine (Forrester and Bradley, 1980a). A typical protein elution profile from the affinity column is shown in Fig. 1. A total of three peaks eluted from the column during the elution sequence employing buffers 1, 2 and 3. However, a Ca^{2+}-dependent stimulation of bovine heart phosphodiesterase (PDE) was detected only in the protein fraction eluted with buffer 3 (fractions 80 to 90). We have named this fraction calsemin. Examination of the PDE activating peak by SDS-polyacrylamide gel electrophoresis revealed four separate protein species with molecular weights of approximately 18,500, 33,000, 45,000, and 60,000 (Fig. 1). Of these four species, the 18,500 and the 33,000 weight components gave a positive reaction with periodate-Schiffs reagent, according to the method of Zacharius et al (1969). Further purification of the calsemin fraction using an ACA 54 Ultragel column resulted in the elution of only one protein peak. Analysis of this fraction by the phenolsulfuric acid method (Dubois et al, 1956) clearly indicated the presence of carbohydrate moieties. However SDS-polyacrylamide gel electrophoresis of this column fraction revealed that it still contained the same four protein species that previously eluted from the fluphenazine column. Various combinations of molecular exclusion and ion exchange column chromatography has to date failed to separate these four protein species. When ram seminal plasma was fractionated by the same procedure employed for human seminal plasma, an identical protein fraction (calsemin) was obtained.

Table 1 demonstrates that the stimulation of PDE by human calsemin was strictly Ca^{2+}-dependent. Neither the seminal plasma protein fraction nor Ca^{2+} alone altered PDE activity, whereas calsemin plus Ca^{2+} produced a large (3.5-fold) stimulation of PDE activity when compared with the PDE and Ca^{2+} value. No PDE activity could be detected in the calsemin fraction alone (data not shown). It appears that calsemin is a very potent Ca^{2+}-dependent activator of PDE, since maximal stimulation (3.5-fold) was obtained with calsemin present at 10 μg/ml in the assay (Fig. 2). At higher levels of calsemin (40 μg/ml), PDE activation was slightly reduced. In all cases, the Ca^{2+}-dependent activation of PDE by calsemin was abolished by trifluoperazine (60 μM). Trifluoperazine alone at this concentration does not inhibit PDE activity. Table 1 also demonstrates the effect of calsemin from ram seminal plasma on PDE. The PDE activity is stimulated twofold by

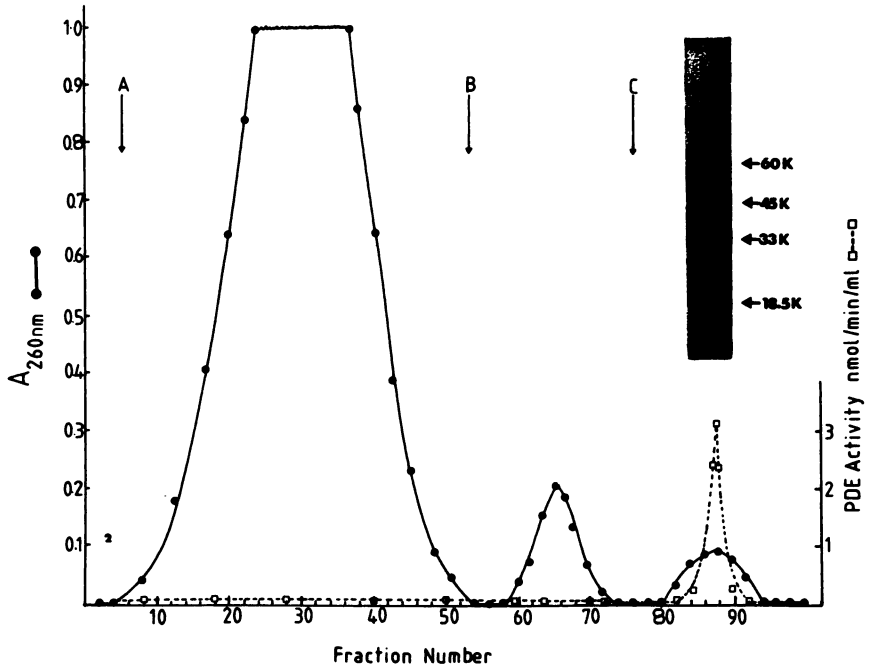

Fig. 1. Purification of human seminal plasma extract by chromatography on a fluphenazine-Sepharose-4B affinity column. The sequential addition of eluting buffers (1, 2, 3) is indicated (A, B, C). (Buffer composition described in Materials and Methods). Elution of the protein fraction containing calsemin was monitored by activation of phosphodiesterase. PDE assay results are expressed as nmol of cAMP hydrolysed/min/ml of added PDE. The assay conditions for PDE are as defined in Materials and Methods. SDS-polyacrylamide gel electrophoresis (10 to 15%) of the activating fraction is shown (inset) along with approximate molecular weights (K = 1×10^{-3}).

TABLE 1. Stimulation of Activator-Requiring Phosphodiesterase by Calsemin from Human and Ram Seminal Plasma*

Assay Components	Phosphodiesterase Activity nmol/min/ml.	Fold Activation
Human Calsemin		
PDE Basal	3.78 ± 0.7	—
PDE + Ca^{2+}	3.28 ± 0.7	—
PDE + calsemin	3.30 ± 0.6	—
PDE + Ca^{2+} + calsemin	11.44 ± 1.9	3.49
PDE + Ca^{2+} + calsemin + trifluoperazine	2.72 ± 1.3	—
Ram Calsemin		
PDE Basal	3.22 ± 0.50	—
PDE + Ca^{2+}	3.18 ± 0.60	—
PDE + calsemin	3.20 ± 0.80	—
PDE + calsemin + Ca^{2+}	6.76 ± 0.60	2.13
PDE + Ca^{2+} + calsemin + trifluoperazine	2.86 ± 0.60	—

* The components and conditions of the basal PDE assay are as defined in Materials and Methods. Where indicated, additional components were present at a final concentration of: free Ca^{2+} (1.3 mM); calsemin (10 μg/ml); trifluoperazine (60 μM). Results are expressed as nmol of cAMP hydrolysed/min/ml of added PDE. The results presented are the mean (± SD) of four separate determinations.

ram calsemin at the same concentration (10 μg/ml) used for the human calsemin activation studies. The activation is strictly Ca^{2+}-dependent and is inhibited in the presence of 60 μM trifluoperazine. Control samples of ram calsemin were also found to contain no PDE activity.

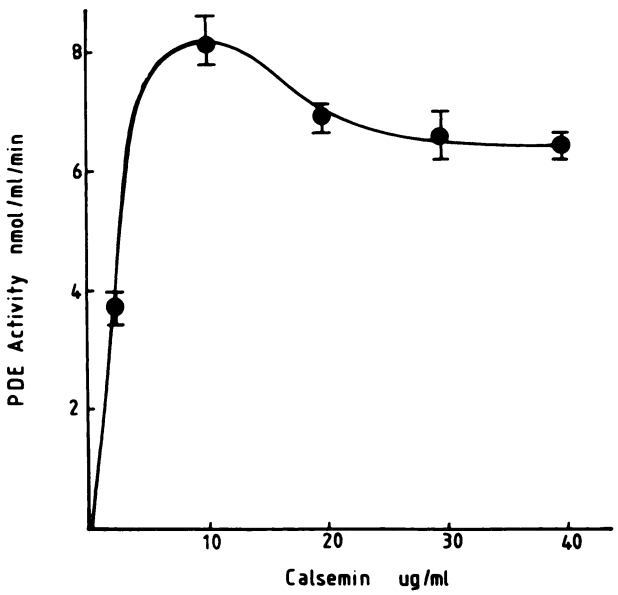

Fig. 2. Calcium-dependent activation of activator-requiring phosphodiesterase by human calsemin. The conditions for PDE assay were as described for Table 1. Final calsemin concentrations in the assay are expressed as μg/ml. PDE activity is expressed as nmol of cAMP hydrolysed/min/ml. Results are the mean ± SD of three separate experiments.

The human calsemin fraction was found to be an effective activator of the Ca^{2+}-ATPase present in flagellar plasma membranes of ram epididymal and ejaculated spermatozoa (Table 2). The plasma membranes from these two phases of sperm maturation are characterized by differences in both the level of Ca^{2+}-ATPase activity and the degree of activation induced by calsemin. The Ca^{2+}-ATPase activity in the flagellar plasma membranes of ram caudal epididymal spermatozoa is approximately 40% of that found in ram ejaculated sperm membranes. Upon the addition of human calsemin, the epididymal Ca^{2+}-ATPase experiences a much greater increase in activation (297%) when compared with the Ca^{2+}-ATPase located in the ejaculated membranes (20% activation). Similarly, Table 3 shows that the Ca^{2+}-ATPase in the plasma membranes of ram caudal spermatozoa is greatly increased (198%) in the presence of ram calsemin (5 μg/ml). Little change (8.5%) in the flagellar plasma membrane Ca^{2+}-ATPase of ram ejaculated spermatozoa is produced by the addition of ram calsemin.

The levels of human calsemin required to maximally activate the flagellar plasma membrane Ca^{2+}-ATPase of ram caudal spermatozoa are critically defined, as demonstrated in Fig. 3. Maximal Ca^{2+}-ATPase activation was obtained at 3.5 μg of calsemin, with a sharp reduction in Ca^{2+}-ATPase activation for calsemin concentrations that were both lower and higher than the stated optimum. It is of interest that a similar "roll over" phenome-

TABLE 2. The Effect of Human Calsemin on the Ca^{2+}-ATPase Activity in Ram Sperm Flagellar Plasma Membranes*

Source of Flagellar Membranes	Ca^{2+}-ATPase Activity	Percent Activation
Ejaculated Spermatozoa		
+ Ca^{2+}	3.33 ± 0.90	—
+ Ca^{2+} + calsemin	3.98 ± 0.10	19.5
+ Ca^{2+} + calsemin + trifluoperazine	3.00 ± 0.25	—
Epididymal Spermatozoa		
+ Ca^{2+}	1.38 ± 0.78	—
+ Ca^{2+} + calsemin	5.48 ± 0.80	297
+ Ca^{2+} + calsemin + trifluoperazine	1.12 ± 0.70	—

* Where indicated, 3 μg/ml of human calsemin was present, as well as trifluoperazine at a final concentration of 10 μM. The results presented are the mean (± SD) of at least three separate determinations. The Ca^{2+}-ATPase assays were performed as described in Materials and Methods. Activity is expressed as μmol of ADP produced/mg of membrane protein/hour. Trifluoperazine at this concentration does not inhibit basal Ca^{2+} ATPase activity.

TABLE 3. The Effect of Ram Calsemin on the Ca^{2+}-ATPase Activity in Ram Sperm Flagellar Plasma Membranes*

Source of Flagellar Membranes	Ca^{2+}-ATPase Activity	Percent Activation
Ejaculated Spermatozoa		
+ Ca^{2+}	2.95 ± 0.35	—
+ Ca^{2+} + calsemin	3.20 ± 0.30	8.5
+ Ca^{2+} + calsemin + trifluoperazine	2.75 ± 0.20	—
Epididymal Spermatozoa		
+ Ca^{2+}	0.97 ± 0.30	—
+ Ca^{2+} + calsemin	2.89 ± 0.10	198
+ Ca^{2+} + calsemin + trifluoperazine	1.15 ± 0.20	—

* Where indicated, 5 μg/ml of ram calsemin was present, as well as trifluoperazine at a final concentration of 10 μM. The results presented are the mean (± SD) of at least three separate determinations. The Ca^{2+}-ATPase assays were performed as described in Materials and Methods. Activity is expressed as μmol of ADP produced/mg of membrane protein/hour. Trifluoperazine at this concentration does not inhibit basal Ca^{2+} ATPase activity.

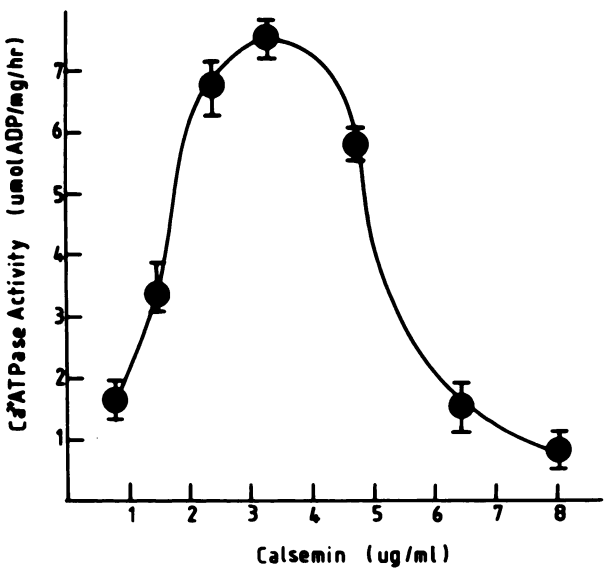

Fig. 3. Calcium-dependent activation of the flagellar plasma membrane Ca^{2+}-ATPase of ram caudal spermatozoa by human calsemin. The assay was carried out as described in Materials and Methods. Free Ca^{2+} was present at a concentration of 4.85 μM. ATPase activity is expressed as μmol ADP produced/mg membrane protein/hour. Final calsemin concentrations are expressed as μg/ml. Results are the mean ± SD of two separate determinations.

non is observed during the interaction of calmodulin and Ca^{2+} with the red blood cell [Ca^{2+} + Mg^{2+}]-ATPase (Vincenzi et al, 1980). This effect may be due to the preferential binding of Ca^{2+} to the activator protein, resulting in a lowering of free Ca^{2+} and diminished ATPase activity.

The effect of human calsemin on ram caudal epididymal sperm motility was examined. The results are presented in Fig. 4. Caudal spermatozoa that had been preincubated in isolation buffer (treatment A) were characterised by slow, uncoordinated flagellar movement. For these spermatozoa, a motility score of 1.2 ± 0.45 (mean ± SD) was obtained. This flagellar activity was unaltered by the addition of calsemin (treatment B). In the presence of buffer and added Ca^{2+} (treatment C), the motility score (1.6 ± 0.55) was not significantly different ($P < 0.3$) from that of treatments A and B. The Ca^{2+}-dependent action of calsemin was demonstrated by the incubation of caudal spermatozoa in the presence of Ca^{2+} and calsemin (treatment D). This treatment resulted in a threefold increase in flagellar activity (motility score = 3.6 ± 0.5, $P <$ 0.0001) over the control systems (treatments A and B). In assessing sperm motility no attempt was made to distinguish between forward movement and other types of motility. The assessments were an index of flagellar beat activity only.

The difference in Ca^{2+}-ATPase activity between the flagellar plasma membranes of ram caudal and ejaculated spermatozoa and the greater sensitivity of the caudal Ca^{2+}-ATPase to activation by calsemin prompted us to examine the flagellar plasma membranes from both sources by SDS polyacrylamide gel electrophoresis (Fig. 5). Both types of membrane show a large number of protein bands. However, the plasma membranes from ram ejaculated spermatozoa are distinct in containing a protein species with an approximate molecular weight of 18,500. This protein cannot be detected in the flagellar plasma membranes of ram epididymal spermatozoa.

Discussion

The results we have presented demonstrate that both human and ram seminal plasma contain a Ca^{2+}-dependent regulatory protein fraction, which we have named calsemin. The calsemin fractions did not contain endogenous PDE or Ca^{2+}-ATPase activity. In some respects, calsemin is similar to calmodulin, the ubiquitous intracellular Ca^{2+}-dependent regulator: for example, they both stimulate activator-requiring phosphodiesterase and Ca^{2+}-ATPase activity; enzyme activation is Ca^{2+}-dependent; activation is abolished by phenothiazines; and both calmodulin and calsemin are acidic, heat-stable, low molecular weight proteins (Means and Dedman, 1980). Al-

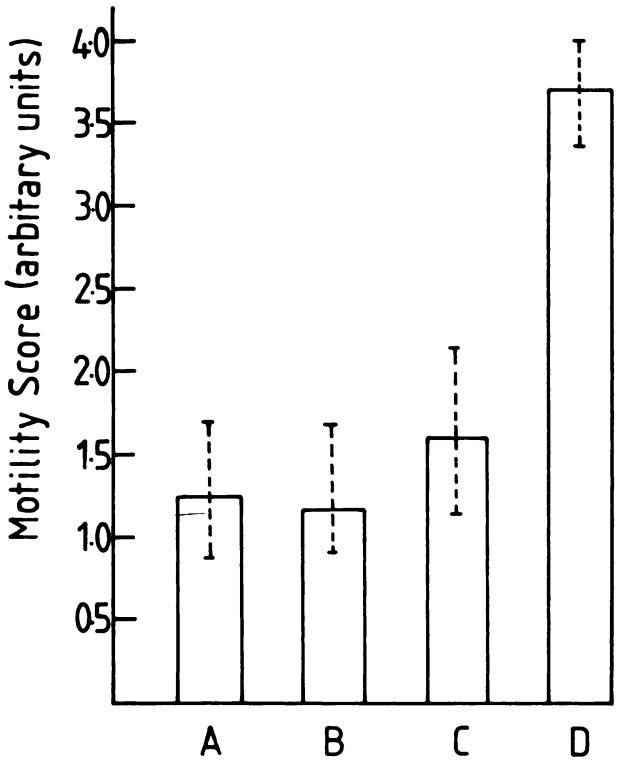

Fig. 4. The effect of human calsemin and Ca^{2+} on ram caudal sperm motility. Motility assays were performed as described in Materials and Methods. A-spermatozoa + isolation buffer; B-spermatozoa + isolation buffer + 2.5 μg/ml calsemin; C-spermatozoa + isolation buffer + 0.6 mM Ca^{2+}; D-spermatozoa + isolation buffer + 0.6 mM Ca^{2+} + 2.5 μg/ml calsemin. Results are the mean ± SD of two separate experiments.

though it is known that mammalian spermatozoa contain calmodulin (Jones et al, 1980b), we believe that contamination of the calsemin fractions with sperm calmodulin did not occur, as the human calsemin is prepared using seminal plasma from vasectomized humans. Furthermore, a major difference between the two proteins is that calsemin is largely extracellular in nature, with synthesis apparently taking place in a tissue external to its site of action. This concept is linked to the fact that mammalian spermatozoa are so highly differentiated that ejaculated spermatozoa carry out no cytoplasmic protein synthesis, and even if the mitochondrial protein synthesizing system were functioning, it would be an unlikely source of either new cytoplasmic or plasma membrane proteins. A more likely source of calsemin would appear to be the accessory glands (eg seminal vesicles, prostate gland) of the male reproductive tract. To date we have been unable to detect calsemin in ram caudal fluids.

Fig. 5. SDS-polyacrylamide gel electrophoresis of plasma membrane isolated from the flagella of (A) ram ejaculated spermatozoa and (B) ram epididymal spermatozoa. Membrane protein (70 μg) was loaded per 10 to 13% gel. Electrophoresis was performed as previously described (Forrester and Bradley, 1980a) and proteins identified by Coomassie blue staining. Indicated molecular weights ($K = 1 \times 10^{-3}$) were determined using BioRad Standard marker proteins.

Our results provide some tentative evidence to suggest that calsemin may be glycoprotein in nature. Preliminary support for this concept is shown by the positive reaction of calsemin with periodate-Schiffs reagent and the determination of carbohydrate using the phenolsulfuric acid procedure. Verification of this preliminary result clearly needs more rigorous analysis.

The plasma membranes of ejaculated ram spermatozoa appear to contain a low molecular weight protein similar in molecular size to the 18,000 mo-

lecular weight protein band of both ram and human calsemin fractions. Furthermore, this protein is absent from the flagellar plasma membrane of ram epididymal spermatozoa. This finding would be consistent with our earlier proposal that the 18,000 molecular weight protein of calsemin and plasma membranes of ejaculated spermatozoa could be identical (Forrester and Bradley, 1980b), and that the increased impermeability of ejaculated spermatozoa to Ca^{2+} may be a consequence of seminal plasma factors interacting with epididymal spermatozoa to produce an activation of the plasma membrane Ca^{2+}-ATPase. Further research is now required to fully elucidate the physical characteristics of calsemin.

Although Ca^{2+} is recognized as a key regulator of sperm motility and the acrosome reaction, the mechanisms involved in the control of Ca^{2+} homeostasis in mammalian spermatozoa are still unresolved (Bradley and Forrester, 1980b; Babcock et al, 1976). Calsemin, as a Ca^{2+}-dependent regulator, may be directly associated with these sperm functions. It is now known that in ram spermatozoa the flagellar plasma membrane contains two Ca^{2+}-transporting systems; an energy-dependent Ca^{2+}-pump (Bradley and Forrester, 1980b) and a Na^{+}/Ca^{2+} exchange antiporter (Bradley and Forrester, 1980d). Since these systems are probably responsible for the bulk of Ca^{2+} efflux from the spermatozoa, any modification in their action would be expected to alter intrasperm free Ca^{2+} and subsequently to influence Ca^{2+}-dependent sperm functions. Experimental evidence to support this concept is provided by the finding that various pharmacological agents that inhibit the flagellar plasma membrane Ca^{2+}-ATPase of ram ejaculated spermatozoa cause immotility or change the pattern of sperm motility *in vitro* (Bradley and Forrester, 1980e). A natural counterpart of this situation, with activation of the flagellar plasma membrane Ca^{2+}-ATPase of epididymal spermatozoa by calsemin, may occur *in vivo*. This idea is further enhanced by the finding that trifluoperazine (25μM) reduces the flagellar plasma membrane Ca^{2+}-ATPase of ram ejaculated spermatozoa by 50%. Using the same concentration of trifluoperazine, the motility of ram ejaculated spermatozoa *in vitro* is reduced by 30% (unpublished results).

During the post-testicular maturation of mammalian spermatozoa, the pattern of sperm motility changes from a circular, twitching swimming motion to one of coordinated forward motility (Hoskins et al, 1979). This is a complex phenomenon involving several biochemical factors such as Ca^{2+}, cyclic nucleotides, and the phosphorylation of flagellar proteins (Hoskins, 1973; Garbers and Kopf, 1980). Also, as shown by Morton et al, (1974), spermatozoa could be transformed from a quiescent state to one with vigorous flagellation by the addition of Ca^{2+}, cyclic nucleotides, or by simple dilution, but this was species specific (Morton et al, 1978). Another factor in the development of sperm motility that needs to be considered is forward motility protein (Acott and Hoskins, 1978). Hoskins and his coworkers have proposed that a heat-stable, acidic glycoprotein fraction (molecular weight 37,000), found in bovine seminal plasma, initiates motility in bovine caput spermatozoa (provided high concentrations of theophylline are present). Our studies would suggest that calsemin and forward motility protein have many common properties. They are both heat-stable, acidic, and appear to exist in multiple aggregates. However, as yet, the specific metabolic function of forward motility protein has not been defined. Whether calsemin and forward motility protein are identical is still unresolved.

From our earlier studies of Ca^{2+} transport in mammalian spermatozoa, we postulated that changes in sperm motility may be associated with a concommitant alteration in the activity of the flagellar plasma membrane Ca^{2+}-ATPase (Forrester and Bradley, 1980b, Bradley and Forrester, 1980c). The results presented in this paper now extend the argument to include a Ca^{2+}-dependent stimulation of the flagellar plasma membrane Ca^{2+}-ATPase by calsemin. As demonstrated in this paper, ram calsemin can activate the flagellar plasma membrane Ca^{2+}-ATPase of ram epididymal spermatozoa to a level approximating that in the plasma membranes of ejaculated ram spermatozoa. By comparison, the activation of the flagellar plasma membrane Ca^{2+}-ATPase of ram epididymal spermatozoa by human calsemin produces a level of activity higher than that found in flagellar plasma membranes of ram ejaculated spermatozoa. As yet we are unable to explain this result. Furthermore, a Ca^{2+}-dependent stimulation of ram caudal sperm motility is achieved by the addition of calsemin. We have not been able to demonstrate that calsemin promotes forward motility, but rather have shown that a Ca^{2+}-dependent stimulation of flagellar beat activity oc-

curs in the presence of calsemin. We recognize that the interaction of calsemin with the plasma membrane Ca^{2+}-ATPase may be only one of a series of metabolic reactions that occur at ejaculation; however, our findings do provide a partial biochemical explanation of the transitional change in sperm motility associated with post-testicular maturation.

Acknowledgments

We are grateful to the New Zealand Ministry of Agriculture and Fisheries, Invermay Agricultural Research Centre for the supply of ram semen, Waitaki New Zealand-Refrigerating Company for the supply of ram testes, E. R. Squibb & Sons Pty. Ltd. (Australia) for their generous gift of fluphenazine-2HCl, and Dr. J. Singh for his help in establishing the phosphodiesterase assays.

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