metry; fig., E). All neurones stained (locust: 11; cricket: 6) had, in principle, the same morphology. In one exceptionally good fill-preparation of a locust neurone, an axon collateral could be traced as far as its terminals on one of the antennal muscles.

A striking feature common to both locust and cricket antennal DUM neurones is that they send axon collaterals not only into each antennal motor nerve, but also into the 'Nervus corporis cardiaci III' (NCC III). So far these collaterals could only be followed into the main trunk of this nerve, where staining faded. Work is in progress to investigate the complex peripheral branching pattern of the NCC III in the locust, and to define the targets of the DUM neurone collaterals in this nerve. In other insect species, the nerve has been shown to innervate the retrocerebral glandular complex, the pharyngeal dilator muscles, and the antennal heart [15, 16].

Another conspicuous feature of the antennal DUM neurones is that most of their dendritic ramifications are found in the suboesophageal ganglion, and only a few within the brain (fig., E). This suggests that their activity is mostly determined by integrative processes within the suboesophageal ganglion. There is increasing evidence that this ganglion participates in the coordination of locomotory activity during flight [17] and walking [18]. The antennae participate actively in both behaviours: during flight they act as wind gauges [19], during walking they probe the substrate by movements correlated with the walking rhythm. It is possible that suboesophageal neurones influence the antennal motor system via the activation of the antennal DUM neurones, provided that these neurones also modulate neuromuscular transmission as do other DUM neurones.

Acknowledgments. We wish to thank G. A. Manley for kindly correcting the English text. Supported by the Deutsche Forschungsgemeinschaft (Br 882/1-2, Ho 463/16-1).

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0014-4754/90/030259-03$1.50 + 0.20/0

Role of endopeptidase in motility induction in apyrene silkworm spermatozoa; micropore formation in the flagellar membrane

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Received 25 August 1989; accepted 19 September 1989

Summary. Immotile apyrene spermatozoa of the silkworm have a thick electron-dense flagellar membrane. On activation of apyrene spermatozoa by initiatorin, a prostatic endopeptidase of the silkworm, or by trypsin, the flagellar membrane becomes thinner, with the formation of many micropores. It then resembles that of motile apyrene spermatozoa in vivo.
Key words. Apyrene spermatozoa; sperm motility; micropore formation; endopeptidase; initiatorin; *Bombyx mori.*

The flagellar membrane of insect spermatozoa is not a classical unit membrane, but has a complicated ultrastructure [1]. Lepidoptera produce both anucleated apyrene spermatozoa, and nucleated eupyrene spermatozoa that fertilize eggs [2]. In the silkworm, *Bombyx mori*, a specific energy-yielding system for spermatozoa, an arginine degradation cascade coupled with protein degradation [3–6] and extracellular glycolysis [3, 7] is present in the spermatophore. At the time of ejaculation, initiatorin, an endopeptidase of the serine protease type secreted from the glandula (g.) prostatica, triggers these reactions [8, 9] and the induction of motility of apyrene spermatozoa [7, 8, 10]. Thus the spermatophore formed in the female bursa copulatrix [11] is the site of sperm maturation as well as being a reactor, and the apyrene sperm stir its viscous contents to promote the dissociation of eupyrene bundles [3, 8].

Materials and methods

The ductus (d.) deferens near the testis, the ampulla d. deferentis and the vesicula (v.) seminalis were each excised from unmated male moths one day after emergence of the silkworm, *Bombyx mori*. The bursa copulatrix containing spermatophore was excised from freshly emerged female moths 30 min after the beginning of copulation. The tissues were prefixed with periodate-lysine-paraformaldehyde fixative at 4 °C for 2 h [12]. The materials were washed with 0.1 M phosphate buffer, pH 7.2 at 4 °C for 24 h, and refixed with 1 % osmium tetraoxide solution in acetate-veronal buffer, pH 7.4 at 4 °C for 1 h. The fixed materials were dehydrated in a graded series of ethanols, and embedded in Quetol 812 (Nissin EM Co., Ltd, Tokyo, Japan). Ultrathin sections stained with uranyl acetate and lead citrate were examined with a JEM-100S electron microscope (Japan Electron Optic Laboratory, Akishima, Japan).

Results

Apyrene spermatozoa that escape from the testis through the basal membrane move vigorously. They lose motility in the ampulla d. deferentis and vesicula seminalis, but become motile again in the spermatophore (fig. 1). The flagellar membranes of the motile spermatozoa in the d. deferens near the testis and in the spermatophore are similar in ultrastructure, but different from those of the immotile apyrene spermatozoa in the d. deferens near the ampulla d. deferentis and in the v. seminalis.

Immotile apyrene spermatozoa have a flagellar membrane (about 480 Å) consisting of five layers (fig. 1). Thin longitudinal sections of the flagellar membrane of immotile spermatozoa show that the surface layer, which is about 140 Å thick, consists of an orderly, linear arrangement of two types of granular material; hexagonal, electron-dense particles separated by materials of low electron density. Below this there is a second layer of about 80 Å thickness consisting of trapezoid, highly electron-dense granules also with material of low electron density between them. The thin third layer (about 40 Å) is uniformly electron dense. The fourth layer of about 100 Å thickness consists of linearly arranged, square electron-dense granules of almost uniform size separated by regions of such low electron density that they appear to contain no material. The fifth layer of 120 Å thickness likewise consists of rectangular structures of alternating high and low electron densities. The materials of low electron density in the flagellar membrane of immotile spermatozoa gradually become thicker as the spermatozoa migrate from the d. deferens to the v. seminalis through the ampulla d. deferentis.

The flagellar membrane of motile spermatozoa under natural conditions does not contain the materials of low electron density observed in the immotile spermatozoa, but has many slit-like microspaces (fig. 1). The membrane is also much thinner than that of immotile spermatozoa because layers 4 and 5 and sometimes also layer 3

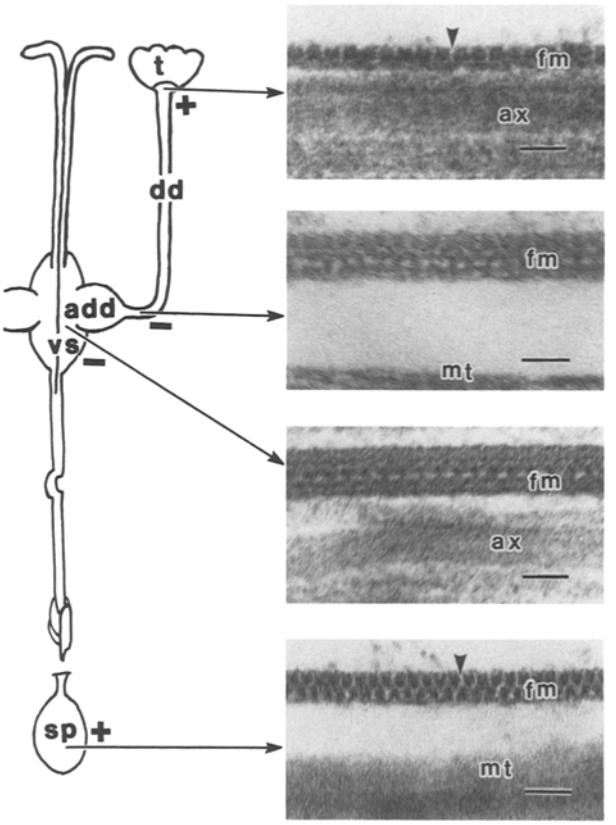

Figure 1. Migration path of silkworm spermatozoa from the testis to the spermatophore and longitudinal sections of the flagellar membranes of apyrene spermatozoa in the various regions. Left: The male reproductive system is drawn schematically. t: testis, dd: ductus deferens, add: ampulla ductus deferentis, vs: vesicula seminalis, sp: spermatophore. Right: The top of each ultrathin section shows the surface of the flagellar membrane. + and − indicate motility and immotility of sperm. fm: flagellar membrane, mt: mitochondrion, ax: axoneme. An arrowhead in the thin section of the ductus deferens near the testis or the spermatophore shows a slit-like microstructure at the flagellar membrane of motile apyrene spermatozoon. Scale bars: 500 Å.

have disappeared. Addition of porcine trypsin (20 µg/ml final concentration) or an extract of the g. prostatica containing initiatorin to the immotile apyrene spermatozoa obtained from the v. seminalis induces their motility [10, 13]. The ultrastructures of the flagellar membranes of spermatozoa activated by these two treatments are very similar, and are also similar to those of motile spermatozoa in the d. deferens near the testis and in the spermatophore. The membrane is thin and has numerous pore-like slits (fig. 2). The similarity in appearance of the flagellar membrane of motile apyrene spermatozoa after these treatments and under natural conditions strongly suggests that the structural changes induced in vivo and also the acquisition of motility are caused by the proteolytic action of an endopeptidase [8, 10, 13]. This possibility is supported by the fact that the spermatophore contains initiatorin derived from the g. prostatica [11, 13].

Discussion

There have been few reports on the ultrastructure of the flagellar membrane of animal spermatozoa [14], and no

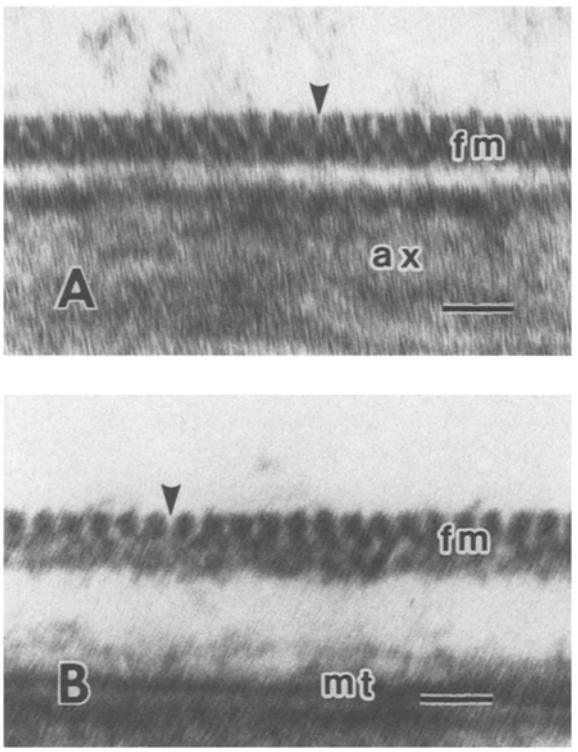

Figure 2. Ultrastructures of membrane of apyrene spermatozoa treated with prostatic secretions containing initiatorin (*A*) and porcine trypsin (*B*). The top of each ultrathin section is the surface of the membrane. fm: flagellar membrane, ax: axoneme. An arrowhead in each thin section shows a slit-like microstructure at the flagellar membrane of the motile apyrene spermatozoon induced by initiatorin or trypsin. Scale bars: 500 Å.

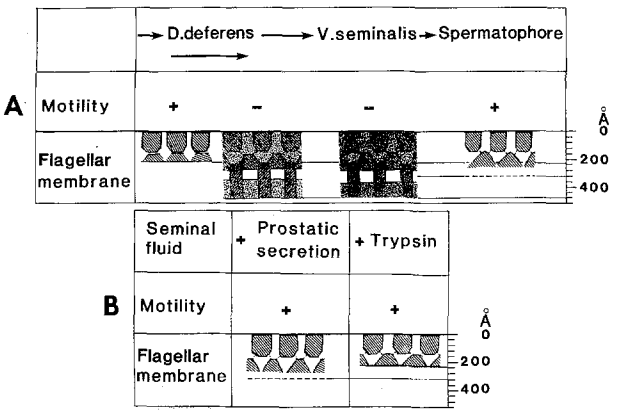

Figure 3. *A* Diagrams of ultrastructures of flagellar membranes of silkworm spermatozoa in various male reproductive organs. Arrows show the direction of migration of the apyrene spermatozoa from the testis to the spermatophore. *B* Comparison of ultrastructures of the flagellar membranes of silkworm spermatozoa treated with an extract of the glandula prostatica or porcine trypsin.

reports on its relation to motility of spermatozoa. Our findings show that the structural changes of the flagellar membrane of the silkworm spermatozoa caused by an endopeptidase are closely related to induction of motility, as shown in figure 3. The thickness of the flagellar membrane of the spermatozoa of some insects and of rats depends on the amount of exogenous glycoprotein deposited on it[1,15]. Initiatorin or trypsin may act on this component of the flagellar membrane. In this connection, it is interesting to note that cell division of cultured fibroblasts initiated by proteolysis with trypsin is due to unmasking of an agglutination site containing N-acetylglucosamine[16].

The slits seen in sections of the flagellar membrane are similar to those found among circumferential ribs of the fibrous sheath of the principal piece of mammalian spermatozoa[17]. They may also be assumed to be cross-sections of micropores of about 60 Å diameter running through the membrane. Namely, the direct action of initiatorin or trypsin on sperm is to make numerous micropores in the flagellar membrane by solubilizing susceptible proteins distributed at regular intervals in the membrane. It may be assumed that the function of these micropores is to transport substances necessary for sperm motility from the seminal plasma into the spermatozoa. The diameter of these pores is slightly less than that of the diffusion channels of the nuclear membrane of cells (about 90 Å in diameter)[18,19], which allow high-molecular-weight proteins of over 200 Å in diameter to be imported into the nucleus[20,21]. Thus the micropores in the flagellar membrane are sufficiently large to let at least peptides from the seminal plasma pass into the spermatozoa. The pores formed by the endopeptidase in the flagellar membrane of spermatozoa in the d. deferens, near the testis and the spermatophore, may even allow the endopeptidase itself to enter the spermatozoa. The possibility is consistent with the observation that apyrene spermatozoa treated with concentrated trypsin show marked degradation of axis with morphological changes of the flagellar membrane.

Initiatorin or trypsin activates apyrene spermatozoa in the seminal fluid from the v. seminalis[10,13], but not apyrene spermatozoa isolated from seminal plasma, unless adenosine 3':5'-cyclic monophosphate (cAMP) is added to the medium[22]. One v. seminalis (about 6.7 mg) inclusive of seminal fluid contains about 14 pmol of cAMP[23]. Thus cAMP from the seminal fluid may enter the apyrenes through the micropores formed by the action of an endopeptidase, and act as a direct inducer of apyrene motility. This possibility is consistent with reports that in mammalian and fish spermatozoa, cAMP acts as an essential component of a mechanism for control of sperm motility[24-26]. Phosphorylations catalyzed by cAMP-dependent protein kinase are required and are sufficient for flagellar motility of the human sperm[27]. Moreover, the motility of demembranated reactivated spermatozoa of some mammals is inhibited by protease inhibitors[28], suggesting that in mammals also an endopeptidase plays a role in the control of sperm motility. Besides cAMP, the substance necessary for apyrene motility is a respiratory substrate(s)[22] such as 2-oxoglutarate produced by the arginine degradation cascade[4]. This respiratory substrate(s) presumably also passes

through the micropores of the membrane to reach the mitochondria of the sperm. Direct evidence for the suggested functions must await future experiments.

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0014-4754/90/030261-04$1.50 + 0.20/0

Type VI collagen in experimental atherosclerosis

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Received 9 June 1989; accepted 3 August 1989

Summary. Diffuse intercellular immunofluorescence staining of type VI collagen was found in the experimentally thickened vascular wall and in control blood vessel tissues as well, superimposed by more intense staining around basement membranes. While the basement membrane staining disappeared in advanced mural thickenings, the diffusely distributed network of type VI collagen remained.
Key words. Aneurysm; atherosclerosis; haemodynamics; immunofluorescence; type VI collagen.

Type VI collagen was originally isolated from human aortic intima and named intima collagen [1]. Subsequently it has been localized in all three layers of the vascular wall and in a large number of other tissues (for recent review see Timpl and Engel [2]). The ubiquitous occurrence of this collagen variant indicates an important biological role for this protein. Despite its original detection in vascular tissue, virtually no data on its involvement in atherosclerosis are available. In this study, experimental saccular aneurysms have been examined by immunofluorescence histochemistry in thirteen sheep of post-operative ages, ranging from 11 to 98 months. It has been shown that histological changes in these vessels resemble human atherosclerosis [3, 4]. Comparison of the distribution of type VI collagen in haemodynamically stressed vessels with that in sham-operated control vessels, is reported here.

Materials and methods

The antiserum to type VI collagen was provided by Dr Mark Gibson (Univ. Adelaide, Adelaide, Australia). Monospecific antiserum to collagen type IV was obtained from Bioscience Products AG, Emmenbrucke, Switzerland. Saccular aneurysms were produced by anastomosing the right jugular vein to the right common carotid artery and then ligating the vein, proximally and distally to the anastomotic site, in sheep less than 12 months of age [3]. Control arteriotomies and phlebotomies were performed on the left common carotid artery and external jugular vein. Tissue sample preparation and immunofluorescence staining for type IV and type VI collagen were performed as described elsewhere [5].