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Enzyme activity in energy supply of spermatozoon motility in two taxonomically distant fish species (sterlet *Acipenser ruthenus*, Acipenseriformes and common carp *Cyprinus carpio*, Cypriniformes)

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ABSTRACT

As spermatozoon motility duration differs significantly among fish species, the mechanism of ATP generation-regeneration and its distribution along the flagellum may be species-dependent. The present study compared the role of creatine kinase (CK) with that of adenylate kinase (AK) in ATP regeneration during motility of demembranated spermatozoa of taxonomically distant fish species, sterlet, and common carp, allowing investigation for the presence of the creatine-phosphocreatine (PCr) shuttle in sterlet spermatozoa. The flagellar beat frequency of demembranated spermatozoa was measured in reactivating media in the presence or absence of ATP, ADP, PCr, and CK and AK inhibitors. After demembranation, AK, CK, and total ATPase activity was measured in spermatozoon extracts. Beat frequency of demembranated spermatozoa was found to be positively correlated with ATP levels in reactivating medium and to reach a plateau at 0.8 mM and 0.6 mM ATP for carp and sterlet, respectively. It was shown for the first time that sterlet axonemal dynein ATPases have a higher affinity for ATP than do those of carp. Supplementation of reactivating medium with ADP and PCr without ATP resulted in beat frequencies comparable to that measured with 0.3 to 0.5-mM ATP for both studied species. The presence of the PCr-CK phosphagen system and its essential role in ATP regeneration were first confirmed for sturgeon spermatozoa. The inhibition of CK exerted a high impact on spermatozoon energy supply in both species, whereas the inhibition of AK was more pronounced in sterlet than in carp. This was confirmed by the quantification of enzyme activity in spermatozoon extracts. We concluded that spermatozoa of these taxonomically distant species use similar systems to supply energy for flagella motility, but with different efficacy.

1. Introduction

Spermatozoon motility must be activated to initiate the processes that form the basis of successful fertilization: spermatozoa must reach, bind to, and penetrate the egg. In external fertilization, spermatozoa cannot obtain energy from the substrate of the milieu into which they are shed

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and therefore must possess considerable endogenous energy, mostly to sustain motility. Glycolysis, phospholipid catabolism and triglyceride metabolism, the Krebs cycle, and oxidative phosphorylation are the main energy-supply pathways for fish spermatozoa [1–3]. Although it is generally accepted that the source of energy for flagella activity is the hydrolysis of ATP [4], the metabolic pathways involved in its generation-regeneration and distribution along the length of the flagellum may be species-specific and are not fully understood. Possible existence of differing metabolic strategies for storage and generation of ATP was suggested by Ingermann [2], on the basis of differences in spermatozoon motility duration among fish species [5].

As a result of ATP hydrolysis during spermatozoon movement, ADP is generated and may be used as a source of ATP regeneration via adenylate kinase (AK) or, in the presence of phosphocreatine (PCr), by the action of creatine kinase (CK). The contribution of AK and CK to ATP regeneration was studied in demembranated rainbow trout spermatozoa by Saudrais et al. [6] who showed the presence of a creatine (Cr)-PCr shuttle and found that AK was less effective in ATP regeneration than CK.

The present study was carried out to investigate the presence of the Cr-PCr shuttle (which is known to be present in spermatozoa of salmonids and cyprinids [2]) in sturgeon sperm and to evaluate the relative contribution of CK and AK in ATP regeneration in the motile phase of demembranated spermatozoa of sterlet and carp, taxonomically distant fish species. The species were selected on the basis of their differing motility duration, 30 to 40 seconds in carp [7,8] and approximately 4 minutes in sterlet [9], as well as their modes of spermatozoon motility activation, osmotic in carp [8], and ionic in sterlet [10]. Spermatozoa of carp and sterlet also show differences in structure, including the presence of an acrosome in sterlet, which is absent in carp. Carp and sterlet spermatozoa differ in head shape (basically spherical in carp, elongated in sterlet) and size (estimated average head length 5.14 μm in sterlet, including mid-piece and acrosome, and 2.45 μm in carp), as well as in flagellum length (30.7 μm in carp and 42.3 μm in sterlet) [11,12].

2. Materials and methods

2.1. Ethics

All experiments were performed in accordance with National and Institutional guidelines on animal experimentation and care, and were approved by the Ethics Committee for the Protection of Animals in Research of the University of South Bohemia in Ceske Budejovice, Research Institute of Fish Culture and Hydrobiology, Vodnany based on the European Union–harmonized Animal Welfare Act of the Czech Republic.

2.2. Fish rearing conditions

Experiments were conducted using common carp *Cyprinus carpio* and sterlet *Acipenser ruthenus*. The experimental fish groups were reared at the hatchery of South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Vodnany, Czech Republic.

Five mature common carp males (2.5–3 kg) were maintained in 4000-L aquaculture tanks at a temperature of 18 °C for 14 days before hormone injection.

Six mature sterlet males (0.6–1.0 kg) were transferred from aquaculture ponds (water temperature 8 °C–10 °C) into a 0.8-m^3 closed water recirculation system. Within 24 hours, water temperature was increased to 15 °C, and fish were held 4 days before beginning experimentation.

2.3. Sperm collection

Spermiation in carp and sterlet was stimulated by intramuscular injection of carp pituitary powder dissolved in 0.9% NaCl solution at 1 and 4 mg kg^{-1} body weight, respectively. Carp sperm was collected into 10-mL plastic syringes 24 hours after injection using abdominal massage. Sterlet sperm was collected at the urogenital sinus by aspiration using a 4-mm plastic catheter connected to a 20-mL syringe 24 hours after hormone injection. Sperm samples showing 80% to 100% motility after dilution with water from the recirculation system were used for experiments. Spermatozoon motility parameters were assessed using standard video-microscopy techniques. Sperm was stored at 4 °C before and during experiments. In all samples, spermatozoon concentration was determined using a Burker cell hemocytometer (Meopta, Czech Republic) and Olympus BX 50 phase contrast microscope (200 × magnification; Olympus, Japan).

2.4. Spermatozoon demembranation and reactivation

For carp, 2 μL of sperm was mixed at room temperature (18 °C–20 °C), with 198-μL demembranating medium (DM; 0.15-M KCH_3COO, 20-mM Tris, pH 8.2, 0.5-mM EDTA, 0.1-mM ethyleneglycoltetraacetic acid [EGTA], 1-mM dithiothreitol [DTT], 0.04% Triton X-100). After 40 seconds, 6 to 9 μL of this mixture was transferred into 191 to 194-μL reactivating medium (RM) to obtain a spermatozoon concentration of 0.6×10^7 cells/mL. For sterlet, 5 μL of sperm was mixed at room temperature with 45 μL DM (20-mM NaCl, 20-mM Tris, pH 8.2, 0.5-mM EDTA, 1-mM DTT, 0.04% Triton X-100). After 60 seconds, 11 to 50 μL of this mixture was transferred into 150 to 189 μL RM to obtain a spermatozoon concentration of 0.6×10^7 cells/mL. Beat frequencies (BFs) were measured immediately after the RM addition. Composition of DM/RM and procedures for demembranation/reactivation were selected and adapted on the basis of experimentation and previous studies [6,8,13–16].

The RM for carp was composed of 0.15-M KCH_3COO, 20-mM Tris, pH 8.2, 0.5-mM EGTA, 1-mM DTT, 0.5% pluronic acid, and 1-mM $MgCl_2$; and for sterlet of 20-mM NaCl, 20-mM Tris, pH 8.2, 2-mM EGTA, 1-mM DTT, 1-mM $MgCl_2$, 200-μM cyclic adenosine monophosphate, and 0.5% pluronic acid. Depending on experimental conditions, the RM may also have contained ATP (vanadate free) or ADP in varying concentrations (ATP: 0.05–1.2 mM; ADP: 0.1 or 0.5 mM); 15 mM PCr with or without 20-μM fluorodinitrobenzene (FDNB, inhibitor of CK), or