Stimulation of an exocytotic event, the hamster sperm acrosome reaction, by *cis*-unsaturated fatty acids

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The *cis*-unsaturated fatty acids oleic, arachidonic and *cis*-vaccenic stimulated the hamster sperm acrosome reaction in vitro (an exocytotic event which occurs in the sperm head and which is essential for fertilization). The *trans*-isomers of oleic and vaccenic acids did not stimulate the acrosome reaction, nor did the *cis*-unsaturated fatty acids petroselenic and docosahexaenoic or the saturated fatty acids lauric, myristic or stearic. This is the first report of a stimulatory effect of *cis*-unsaturated fatty acids on an exocytic event in an intact viable cell.

Exocytosis *Fatty acid* *Acrosome reaction* *(Hamster sperm)*

1. INTRODUCTION

As a requirement for mammalian fertilization, the acrosome, an enzyme-containing organelle in the sperm head, undergoes a unique form of exocytosis involving the fusion of the outer acrosomal membrane with the overlying sperm plasma membrane, followed by vesiculation and eventual loss of the vesiculated membranes [1–3]. This process is called the acrosome reaction (AR) and occurs in sperm which have undergone a series of partially defined cellular changes known as capacitation [1–3].

Sperm phospholipase A_2 and its lysophospholipid products may have a role in the mammalian AR [3–8]. Mammalian sperm phospholipids, potential substrates of phospholipase A_2 contain several *cis*-unsaturated fatty acids [9], and it has been demonstrated that some *cis*-unsaturated, but not *trans*-unsaturated, or saturated fatty acids stimulate breakage of membrane barriers between aggregated, isolated, adrenal chromaffin granules [10]. Those results led to the suggestion that the release of such *cis*-unsaturated fatty acids by phospholipase activity could stimulate exocytosis [10].

The present report describes studies of the effect of *cis*-unsaturated, *trans*-unsaturated and saturated fatty acids on the hamster sperm AR. Part of this work was presented elsewhere in abstract form [11].

2. MATERIALS AND METHODS

Golden Syrian hamsters were purchased from Harlan Sprague-Dawley. Fatty acids were from Sigma Chemical Co. and all other chemicals and materials were from sources described in [5].

2.1. *Preparation and incubation of sperm*

Unless otherwise noted, the preparation and incubation of a 90% motile washed cauda epididymal sperm suspension were done as in [5]. In brief, initial sperm incubation suspensions of 90 μl consisted of: 20 μl bovine serum albumin, 30 mg/ml (purified as in [3]) in a phosphate-buffered saline (pH 7.3); 5 μl taurine, 10 mM in the phosphate-

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buffered saline; 5 μl epinephrine, 1.4 mM in modified Tyrode's solution (25 mM $NaHCO_3$); 60–Y μl of the modified Tyrode's solution containing 5 mM glucose, 12.5 mM lactate, 0.25 mM pyruvate and 1.5 mg/ml purified bovine serum albumin; and Y μl sperm suspension in the modified Tyrode's solution plus metabolites and albumin (Y chosen so as to give a final concentration of 2.5×10^6 sperm/ml in 100 μl). Penicillin was not present in any solutions, and the final concentration of serum albumin was 6.2–6.3 mg/ml in various experiments (but identical in all suspensions within a particular experiment). These sperm suspensions were incubated at pH 7.4–7.6 under capacitating conditions at 37°C in a humidified 5% CO_2–95% air atmosphere. After 4.5 h incubation, 5 μl fatty acid + 5 μl PBS or 5 μl solvent control + 5 μl PBS were added, and sperm were incubated under capacitating conditions for an additional 15–30 min and then assayed.

2.2. *Assays*

Motility and hyperactivation (whiplash-like flagellar movements characteristic of capacitated hamster sperm) were estimated by dark-field microscopy, and the percentage of AR was determined by phase contrast microscopic counts of the number of AR in 100 motile sperm, all as in [5]. Statistical analysis was carried out utilizing Student's t-test for matched samples with the Bonferroni correction for multiple comparisons [12].

2.3. *Preparation of fatty acid stock solutions*

Fatty acids were dissolved in a chloroform:methanol solution (2:1, v/v), except *trans*-vaccenic acid which was dissolved in DMSO. Each was diluted to a stock concentration of 10 mg/ml, purged with nitrogen, and stored at −80°C in light-safe screw-cap glass test tubes with teflon cap liners.

Aliquots (100 μl) of 10 mg/ml stock fatty acid solutions were transferred to 13 mm × 75 mm disposable culture tubes. The chloroform:methanol or DMSO was evaporated using a gentle stream of nitrogen. The solvent free fatty acids, except *trans*- and *cis*-vaccenic acids, were emulsified in 1 ml degassed ice-cold PBS using a Sonifier Cell Disruptor (model W185D) equipped with a micro probe, at a power setting of 30 W for 15 s. During sonication the samples were maintained at 4°C and continuously purged with nitrogen.

trans-Vaccenic acid was dissolved in 200 μl DMSO, 1.8 ml degassed PBS was then added to

Table 1

The effect of addition of *cis*- and *trans*-unsaturated fatty acids on the acrosome reaction (AR)

Fatty acid or solvent control ($n = 5$–6)[a]	Conc. (μg/ml)	min after addition	% AR (mean ± SD)
Oleic acid [*cis*-9-octadecenoic acid (18:1)]	50	15	58.0 ± 19.3[b]
Control	–	15	16.5 ± 9.79
Elaidic acid [*trans*-9-octadecenoic acid (18:1)]	50	30	15.8 ± 8.32[b]
Control	–	30	17.0 ± 9.06
cis-Vaccenic acid [*cis*-11-octadecenoic acid (18:1)]	25	15	51.6 ± 15.4[b]
Control	–	15	13.2 ± 9.16
trans-Vaccenic acid [*trans*-11-octadecenoic acid (18:1)]	25	15	14.8 ± 10.3
Arachidonic acid [all *cis*-5,8,11,14 eicosatetraenoic acid (20:4)]	50	15	60.2 ± 20.8[b]
	50		
Control	–	15	16.5 ± 9.79
Petroselinic acid [*cis*-6-octadecenoic acid (18:1)]	50	30	31.4 ± 15.2
Control	–	30	32.8 ± 17.9
Docosahexaenoic acid [all *cis*-4,7,10, 13,16,19 (22:6)]	50	30	40.0 ± 14.9
Control	–	30	36.2 ± 19.3

[a]Hamster sperm were incubated under capacitating conditions for 4.5 h (section 2) and fatty acids or solvent controls added. Assays were by light microscopy methods. Motility (70–80%) and flagellar hyperactivation (50–60%) were the same in control and experimental samples within each experiment

[b]Fatty acid-stimulated AR over control, $p < 0.02$

prepare a 500 μg/ml suspension, and sonication was carried out as described for the other fatty acids except at 15–20°C to prevent supercooling and solidification of the DMSO. *cis*-Vaccenic acid was dissolved in 1.8 ml PBS, 200 μl DMSO were added, and sonication was carried out as for *trans*-vaccenic acid.

All fatty acid stock solutions were then diluted to the desired concentration with degassed PBS, purged with nitrogen, sealed to the atmosphere, stored at 4°C in a cryoblock, maintained in the dark and used within 10 min.

3. RESULTS

At 4.5 h, motility was 70–80% and hyperactivation 60–70% in all experiments. The *cis*-isomers, but not the *trans*-isomers of 9-octadecenoic and 11-octadecenoic acids stimulated the AR when added at 4.5 h (table 1). Three other *cis*-unsaturated fatty acids, docosahexaenoic, petroselinic and arachidonic acids were tested. Arachidonic acid stimulated the AR when added at 4.5 h, but the other two did not (table 1).

Three saturated fatty acids, lauric (12:0), myristic (14:0) and stearic (18:0) did not stimulate the AR when tested at 50 μg/ml. In 5 such experiments the following percentage AR results (mean ± SD) were obtained (30 min after addition of fatty acid): lauric 28.4 ± 16.1, control 32.8 ± 16.3; myristic 32.6 ± 12.2, control 36.2 ± 17.7; stearic 16.0 ± 8.83, control 17.0 ± 8.27.

4. DISCUSSION

Creutz [10] reported that oleic acid, *cis*-vaccenic acid, and arachidonic acid (as well as several other *cis*-unsaturated fatty acids) stimulated membrane breakage between aggregated isolated adrenal chromaffin granules, but that elaidic acid (the *trans*-isomer of oleic) or saturated fatty acids did not. The present results are the first to demonstrate such differential effects of fatty acids on an exocytotic event in an intact viable cell.

Karnovsky et al. [13] have suggested that *cis*-unsaturated fatty acids may cause local perturbations in membranes which lead to local increases in the disorder of gel-like regions of membrane. Since increased membrane fluidity appears to be important for the AR [14], perhaps the effective *cis*-unsaturated fatty acids stimulate the AR, at least in part, through such local increases in membrane disorder. Arachidonic acid can also be metabolized via the cyclooxygenase and lipoxygenase pathways [15] to products which have important effects on cells. Preliminary evidence [16] suggests that prostaglandin E_2 and 5- and 12-hydroxyeicosatetraenoic acids, arachidonic acid metabolites of those pathways, can stimulate the hamster AR. Whatever the effect of the stimulatory fatty acids, a more complete capacitation is required for its manifestation because addition of oleic or arachidonic acids at only 2.5 h incubation did not increase AR over control values (not shown).

The lack of stimulation by petroselenic acid may have been due in some way to the closer location of the single *cis*-unsaturated bond to the fatty acid head group (as suggested in [10] to explain why petroselenic acid did not stimulate breakage between aggregated chromaffin granules). However, it is not yet clear why docosahexaenoic acid failed to stimulate.

Oleic, arachidonic and docosahexaenoic acids are present in mammalian sperm phospholipids [9], and sperm phospholipase A_2 activity has been implicated in the AR [3–8]. We suggest the sperm phospholipase A_2 activity stimulates the AR in previously capacitated sperm, at least in part, by its release of oleic acid and arachidonic acid from sperm phospholipids.

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NOTE ADDED IN PROOF

Corey, E.L., Shih, C. and Cashman, J.R. [Proc. Natl. Acad. Sci. USA (1983) 80, 3581], have reported that docosahexaenoic acid is an inhibitor of the synthesis of prostaglandins from arachidonic acid. We have now found that docosahexaenoic acid will inhibit the stimulation of the acrosome reaction by arachidonic acid but not that by oleic acid (in preparation). Perhaps, the *cis*-unsaturated fatty acid docosahexaenoic acid failed to stimulate the hamster acrosome reaction in the

above work because it inhibited sperm prostaglandin synthesis.

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