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Maitotoxin Induces Acrosome Reaction and Histone Degradation of Starfish *Asterina pectinifera* Sperm

Tosikazu Amano, Yishihito Okita[1], Takeshi Yasumoto2[2] and Motonori Hoshi[3]

Department of Life Science, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuda, Midori-ku, Yokohama 227, Japan [2]*Department of Biological Chemistry, Faculty of Agriculture, Tohoku University, Tsutsumidori-Amemiyamachi, Aoba-ku, Sendai 981, Japan*

ABSTRACT—Maitotoxin (MTX) is a marine toxin which presumably activates Ca^{2+}-channels and stimulates phosphoinositide breakdown in various mammalian cells. We report here that MTX induces the acrosome reaction of starfish spermatozoa by a cooperative action with a diffusible fraction of the egg jelly. MTX alone does induce the degradation of sperm histones. Both reactions induced by MTX depend on extracellular Ca^{2+}. Verapamil, a Ca^{2+}-channel antagonist, inhibits MTX-induced acrosome reaction but not affects MTX-induced histone degradation. MTX bypasses the blockage by ARIS of the jelly-induced acrosome reaction and histone degradation.

INTRODUCTION

Changes in ion permeability of plasma membrane regulate sperm reactions to extracellular signals [6, 11, 27–29]. Egg jelly, with which spermatozoa interact first in the echinoderms, triggers Na^+- and Ca^{2+}-influx, and K^+- and H^+-efflux in spermatozoa, and eventually induces the acrosome reaction [19, 27, 28]. We have reported that the egg jelly also induces the degradation of sperm histones in the starfish, *Asterina pectinifera* [1]. Both reactions are induced by a cooperative action of homologous ARIS, a fucose sulfate rich glycoprotein having an extremely large molecular weight, and a diffusible fraction of the egg jelly (M8) containing Co-ARIS and sperm-activating peptides (SAP) [2, 14, 15, 17, 20, 22, 24, 26]. The acrosome reaction and the histone degradation induced by the egg jelly depend upon extracellular Ca^{2+} and are inhibited by Ca^{2+}-channel antagonists, suggesting that Ca^{2+}-influx via Ca^{2+}-channels plays important roles in these reactions [1]. However, A23187, a Ca^{2+} ionophore which induces the acrosome reaction of sea urchin sperm is not effective to induce the acrosome reaction and the histone degradation of *Asterina pectinifera* sperm [1]. It induces these reactions only in combinations with monensin or M8 (1; unpublished results). These results suggest that an increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) is essential but not sufficient to induce the acrosome reaction and histone degradation. Although these reactions of *Asterina pectinifera* sperm are induced by a cooperative action of ARIS and Co-ARIS [3], several lines of evidence indicate that two reactions are regulated by distinct pathways at least partly [1, 2].

Maitotoxin (MTX) has been isolated from a poisonous marine dinoflagellate, *Gambierdiscus toxicus* [23]. MTX stimulates the uptake of Ca^{2+} in various cells [4, 10, 16, 18, 30, 32] and elicits phosphoinositide breakdown in several cell lines [5, 12, 13]. Effects of MTX absolutely depend upon extracellular Ca^{2+}. In most cells, the stimulation of Ca^{2+}-uptake by MTX is blocked by Ca^{2+}-channel antagonists [16, 32], and therefore it

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[1] Present address: Suntory Pharmatech Center, Chiyodamachi, Gunma 370-05, Japan

[3] To whom all correspondence should be addressed.

is proposed that MTX is a direct activator of Ca^{2+}-channels [32]. In contrast to this, the stimulation of phosphoinositide breakdown by MTX is not affected by a variety of organic and inorganic Ca^{2+}-channel blockers [5, 12, 13].

In the starfish, *Asterias amurensis,* it is reported that, in alkaline seawater, MTX triggers only a part of acrosome reaction: it induces acrosomal exocytosis but not the formation of perfect acrosomal process [25].

In this paper, we show that MTX is effective to induce normal acrosome reaction in *Asterina pectinifera* and the effect of MTX on inducing the acrosome reaction is greatly enhanced by M8 and is susceptible to verapamil. MTX also initiates the degradation of sperm histones in a verapamil-insensitive manner.

MATERIALS AND METHODS

Artificial seawater, jelly components and drugs

Normal artificial seawater (ASW) consisted of 450 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, 30 mM $MgCl_2$, 20 mM $MgSO_4$, 15 mM N-2-hydroxyethylpiperazine-N′-propanesulfonic acid-NaOH, pH 8.2. Ca^{2+}-free seawater (CFSW) was prepared as above except for omitting $CaCl_2$.

Egg jelly, ARIS and M8 were prepared as previously described [1, 3]. Verapamil was dissolved in DMSO to make a 200 mM stock solution. For the control, an equal concentration of DMSO (1%) was run. MTX was dissolved in distilled water at a concentration of 300 μg/ml (87.6 μM), and diluted in ASW or CFSW.

Assays of the acrosome reaction and the histone degradation

Spermatozoa of the starfish, *Asterina pectinifera* were collected "dry" and kept on ice until use. Assays of the acrosome reaction and the histone degradation were performed as previously described [1, 3]. Spermatozoa with an acrosomal process were scored as "reacted". Each experiment was repeated at least two different batches of sperm.

RESULTS

Effects of MTX on starfish spermatozoa: It has been reported that MTX (0.06–0.73 μM) does not induce the acrosome reaction of *Asterias amurensis* spermatozoa in normal seawater, but that it induces only the acrosomal exocytosis in alkaline seawater (pH 9.5) [25]. We examined first the effects of MTX on the acrosome reaction and the histone degradation in *Asterina pectinifera.* MTX (0.6 μM) induced the acrosome reaction considerably, although at the concentration of 0.3 μM its effect was not appreciable (Table 1). Induction of the acrosome reaction by MTX was greatly enhanced by M8 but not by ARIS (Table 1). MTX-induced acrosome reaction was quite normal in the morphology (Data not shown). It required external Ca^{2+} and was completely blocked by 200 μM verapamil (Table 1).

TABLE 1. Induction of the acrosome reaction by MTX

Treatments	Acrosome Reaction (% Control)*
Jelly	100
ARIS+M8	82
ARIS	9
M8	0
MTX (0.6 μM)	78
+verapamil**	3
MTX (0.3 μM)	12
+ARIS	19
+M8	56
+M8 in CFSW	0
+M8+verapamil**	4

Sperm were treated with egg jelly (100 μg fucose/ml), ARIS (50 μg/ml), M8 (62.5 μg/ml) or combinations of them for 3 min and fixed for microscopic observation. *The percentage of jelly-induced acrosome reaction was referred as 100%. Values are means for two experiments. **Sperm were pretreated with verapamil (200 μM) for 3 min and then MTX plus M8, or MTX, was added to the sperm suspension.

In contrast to the acrosome reaction, the histone degradation was much sensitive to MTX: 0.3 μM of MTX induced the histone degradation as well as

TABLE 2. Induction of the histone degradation by MTX

Treatments	Histone H1 Degradation (%)
Jelly	52
ARIS	9
M8	2
MTX (0.6 μM)	64
+M8	73
+verapamil*	61
MTX (0.3 μM)	42
+ARIS	61
+M8	56

Sperm were treated with egg jelly (50 μg fucose/ml), ARIS (50 μg/ml) or M8 (25 μg/ml) and incubated for 60 min. Reactions were stopped by the addition of an equal volume of sample buffer for SDS-polyacrylamide gel electrophoresis.
* Sperm were pretreated with verapamil (200 μM) for 3 min and then MTX was added to the sperm suspension.

the egg jelly did (Table 2). Neither ARIS nor M8 significantly enhanced the effect of MTX on the histone degradation. MTX-induced histone degradation also required external Ca^{2+}, but it was not blocked by 200 μM verapamil (Table 2).

Sequential treatments of MTX and egg jelly components: It is known that spermatozoa treated with either ARIS or M8 become unresponsive to the egg jelly [2, 14, 21]. We examined whether MTX also showed a similar "pretreatment effect". As shown in table 3, when sperm were pretreated for 3 min with an insufficient concentration of MTX (0.3 μM) to induce the acrosome reaction, they did not undergo the acrosome reaction by the addition of M8. In response to the egg jelly they underwent the acrosome reaction, but much less than intact spermatozoa did (Table 3). ARIS-pretreated spermatozoa, which were unresponsive to the egg jelly, underwent the acrosome reaction in response to a mixture of MTX and M8 (Table 3).

Similarly, ARIS-pretreated spermatozoa did not undergo the histone degradation in response to the egg jelly [2] but they did so in response to MTX (Table 4). Previous study has shown that concanavalin A (Con A) specifically inhibits the jelly-induced histone degradation [1]. Thus, we examined the effects of Con A on the MTX-induced histone degradation. As shown in table 4, Con A (0.2 mg/ml) blocked the jelly-induced histone degradation but did not affect the MTX-induced one.

TABLE 3. Effects of sequential treatments of MTX and jelly components on the acrosome reaction

Treatments 1st	Treatments 2nd	Acrosome Reaction (% Control)*
—	Jelly	100
—	MTX+M8	56
ARIS	Jelly	23
ARIS	MTX+M8	70
MTX	Jelly	38
MTX	M8	6

Sperm were preincubated with ARIS (50 μg/ml) or MTX (0.3 μM) for 3 min and then egg jelly (100 μg fucose/ml), a mixture of MTX (0.3 μM) and M8 (62.5 μg/ml), or M8 (62.5 μg/ml) was added to the sperm suspension. The mixtures were incubated for another 3 min and fixed.
* The percentage of jelly-induced acrosome reaction was referred as 100%. Values are means for two experiments.

TABLE 4. Effects of sequential treatments of MTX and jelly components on the histone degradation

Treatments 1st	Treatments 2nd	Histone H1 Degradation (%)
—	Jelly	52
ARIS	Jelly	11
ARIS	MTX	70
Con A	Jelly	18
Con A	MTX	83

Sperm were treated with ARIS (50 μg/ml) or Con A (0.2 mg/ml) for 3 min, and then egg jelly (50 μg fucose/ml) or MTX (0.3 μM) was added to the sperm suspension. The mixtures were incubated for another 60 min and incubations were stopped by addition of sample buffer for SDS-polyacrylamide gel electrophoresis.

DISCUSSION

External Ca^{2+} is essential for inducing the acrosome reaction and histone degradation by the egg jelly in starfish spermatozoa [1, 21]. Pharmacolo-

gical studies suggest the involvement of voltage-dependent Ca^{2+}-channels in these reactions [1]. We have reported here that MTX (0.6 μM) induces morphologically normal acrosome reaction in *Asterina pectinifera*. This concentration is much higher than effective doses for mammalian cells, but it is comparable to that for the acrosomal exocytosis in *Asterias amurensis* [25]. Echinoderm sperm are generally much less sensitive to various drugs than mammalian cells. The effect of MTX on the acrosome reaction was greatly enhanced by simultaneous addition of M8 but not by ARIS. Similar to the stimulation of Ca^{2+}-uptake by MTX in various cells [16, 32], the acrosome reaction induced by MTX with or without M8 required extracellular Ca^{2+} and was completely blocked by verapamil. This suggests that MTX induces the acrosome reaction by activating Ca^{2+}-channels.

From the present data, we do not know exact roles of M8 in stimulation of MTX-induced acrosome reaction. It seems reasonable to assume that M8 facilitates the opening of Ca^{2+}-channels and/or other intracellular changes that are essential for complete acrosome reaction. The acrosome reaction consists of the acrosomal exocytosis and process formation [7, 8, 33]. Like many other exocytotic events, acrosomal exocytosis probably requires an increase in $[Ca^{2+}]i$. The process formation is thought to be regulated by an increase in intracellular pH (pHi) [31, 34]. Actually, in sea urchin sperm, both Ca^{2+}-uptake and a pHi increase can not be dissociated from the acrosome reaction. It is reported that MTX induces the acrosomal exocytosis significantly, but the process formation slightly, in mussel sperm at normal pH (8.0) and in *Asterias amurensis* sperm at pH 9.5 [25]. A Ca^{2+}-ionophore A23187 did not initiate the acrosome reaction in *Asterina pectinifera* [1]. Thus we think that an increase in $[Ca^{2+}]i$ is essential but not sufficient to induce the acrosome reaction of starfish spermatozoa. It is known in *Asterias amurensis* that ARIS and Co-ARIS induce the acrosome reaction without any detectable pHi increase, and SAP stimulates the reaction by increasing the pHi [14, 20, 22]. Stimulation by M8 of MTX-induced acrosome reaction in *Asterina pectinifera* suggests that M8 induces some other intracellular changes also required for the acrosome reaction, such as a pHi increase.

Pretreatment of sperm with MTX remarkably decreased the egg jelly-induced acrosome reaction suggesting that MTX induces irreversible changes in sperm, which may participate in the jelly-induced acrosome reaction. MTX plus M8 bypassed the blockage of the jelly-induced acrosome reaction in ARIS-pretreated spermatozoa suggesting that ARIS-pretreatment did not irreversibly inactivate MTX-sensitive Ca^{2+}-channels. Two mutually non-exclusive working hypotheses are being considered: (I) ARIS directly makes Ca^{2+}-channels unresponsive to the jelly, but MTX can activate ARIS-modified Ca^{2+}-channels. (II) ARIS acts on a site upstream to the MTX-site(s), which indirectly regulates Ca^{2+}-channels.

MTX was also effective to induce the histone degradation. This effect of MTX was not enhanced by M8 nor affected by verapamil. These results suggest that the action site of MTX for the histone degradation is different from that for the acrosome reaction. This is consistent with the hypothesis that two reactions are controlled by distinct pathways at least partly [1, 2]. It has been proposed that MTX is a potent activator of phosphoinositide breakdown and that Ca^{2+}-channel blockers hardly inhibit this effect [5, 12, 13]. It has been also reported that egg jelly promotes the formation of inositol 1,4,5-triphosphate in sea urchin spermatozoa [9]. Taking these into account, there is a possibility that MTX-induced phosphoinositide-breakdown in sperm cells triggers the histone degradation. Therefore, important questions to be answered are whether phosphoinositide breakdown is induced by the egg jelly also in starfish spermatozoa and, if so, whether it is involved in the induction of histone degradation.

Preincubation of sperm with Con A inhibits jelly-induced histone degradation [1] but it did not affect the MTX-induced one. MTX bypassed the blockage of the jelly-induced histone degradation in ARIS-pretreated sperm. These results suggest that the MTX-sensitive and verapamil-insensitive step in the reaction pathway leading to the histone degradation is later than the action sites of ARIS and Con A, or that MTX induces the histone degradation by a quite different way from the egg jelly does.

It is to be answered how and at which step, the two sperm reactions are linked together.

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