SMOOTH MUSCLE CONTRACTILE ACTION OF THE VENOM FROM THE CROWN-OF-THORNS STARFISH, *ACANTHASTER PLANCI*

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ABSTRACT—The fraction (venom B) of spine venom from the crown-of-thorns starfish (*Acanthaster planci*) caused contractions of the uterus of rats and enhanced vascular permeability in rabbits. The venom B-induced contraction of the smooth muscle was depressed by inhibitors of prostaglandin synthesis such as indomethacin or aspirin, but not by the anticholinergic agent, atropine. The fraction with the uterus contractile action was partially purified from venom B through column chromatography. This fraction contains phospholipase and proteinase activities and was different from the lethal factor in the venom. These results suggest that the uterine contractile action caused by venom B is mediated by prostaglandins and partly contributed by the activity of phospholipase in the venom.

KEY WORDS : Starfish venom, Muscle contraction, Prostaglandin.

INTRODUCTION

The crown-of-thorns starfish, *Acanthaster planci* is commonly found in coral reefs around Okinawa islands and their voracious eating of coral causes a serious problem in the biological environment in reefs. In addition, the starfish has numerous spines on the body surface and contacts with the spines cause harmful symptoms such as pain, swelling, vomiting and paralysis in human. Biological and toxicological properties of the venom of the starfish spines have been reported by Taira *et al.* (1975) and Shiomi *et al.* (1985 ; 1988 ; 1990). Thus it was shown that the spine venom involves lethal and hemolytic activities and the purified lethal factor is a potent hepatotoxin. The effect of the venom on car-

diovascular system has also been studied as seen in systemic hypotension in rats (Yara *et al.*, 1992) or thrombocytopenia and leukopenia in dogs (Shiroma *et al.*, 1994). However, other properties of the spine venom are not well understood. In the present study, uterine contractile action of the venom and the partial purification of the contractile factor were examined.

MATERIALS AND METHODS

Preparation of spine venom : Spines of a crown-of-thorns starfish (*Acanthaster planci*) collected from reefs around Onna township, Okinawa Island were removed and stored at -20°C until used. The spines were homogenized with three volumes of 0.005 M phosphate buffer (pH 7.0) by a Polytron homogenizer (Kinematica, Switzerland) on ice, and centrifuged at $4,000\times g$ for 30 min. The resultant supernatant was made with

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ammonium sulfate 50% saturated. The supernatant and precipitate thus obtained were called venom A and venom B, respectively. After precipitation of venom A with ammonium sulfate 100% saturated, each precipitate was dialyzed against water followed by lyophilization and used for biological assays.

Smooth muscle contractile action : The contractile action of the venom was examined by using the uterus isolated from Sprague-Dawley rats (200–350 g) which were treated with estradiol (0.13 mg/kg, intraperitoneally) 24 hr prior to the experiment. The uterus with endometrium was isolated from the rat followed by store in Jalon solution (NaCl 9.0 g, KCl 0.42 g, CaCl₂ 0.06 g, glucose 0.5 g, NaHCO₃ 5 g in 1,000 ml) at 4°C for 6 hr and then the contractile force was measured along longitudinal direction of the muscle by a Magnus apparatus (10 ml) at 30°C in Jalon solution. Acetylcholine (ACh, 10⁻⁷ g/ml) was used to produce standard contraction. Kinin releasing action of the venom was measured as described previously (Aniya and Matsusaki, 1979) : Briefly, human plasma (1 ml) heated at 56°C for 3 hr was incubated as a kininogen with a spine venom (0.1 ml) at 37°C for 10 min followed by ultrafiltration (Centricut 10, Grace Co. Tokyo). The filtrate was added to the Magnus apparatus and the contractile force of the uterus was measured. For the measurement of contractile force of fractions obtained from column chromatography, the lyophilized sample was used.

Measurement of biological and enzymatic activities : Vascular permeability and blood coagulation activity were measured as previously described (Aniya *et al.*, 1985). The enzyme activity of phospholipase or proteinase was measured according to the method described in previous report (Aniya and Matsusaki, 1982).

Determination of lethal dose : The lethal dose of the starfish venom was determined by using five ICR mice in each group. Venom B, dissolved in a 0.9% sodium chloride solution, was given intraperitoneally and its lethality at 24 hr after the injection was observed. The LD₅₀ value was calculated by the methods of Behrens (1929) and Kärber (1931). Protein concentration in the venom was measured by the method of Lowry *et al.* (1951).

Isolation of smooth muscle contracting factor :

Gel filtration of venom B through a Sephadex G-100 column was carried out using 0.005 M potassium phosphate buffer (pH 7.0). The fraction with uterus contracting activity was applied to a DEAE-cellulose column after dialysis against 0.01 M Tris-HCl buffer (pH 8.0) and eluted with a gradient of 0.3 M NaCl in the same buffer. The protein content of the eluate was measured by UV absorption at 280 nm.

Chemicals : Acetylcholine (ACh) and estradiol benzoate were purchased from Daiichi-seiyaku and Teikokuzoki, Tokyo, respectively. Atropine sulfate (Nichidokuyakuhin, Tokyo), aspirin (Toriiyakuhin, Tokyo), purified casein (Difco Laboratory, Detroit) and indomethacin (Sigma Chemicals, St. Louis) were used. Sephadex G-100 (particle size 40–120 nm) and DEAE-cellulose were provided by Pharmacia Fine Chemicals (Uppsala, Sweden) and Nacalai Tesque (Kyoto), respectively. Other reagents used were of analytical grade.

RESULTS

Lethality of Venom B : Lyophilized venom B showed 1.1% of the spine weight and the LD₅₀ of venom B was 7.4 mg/kg in mice.

Uterine contractile action of spine venom : As shown in Fig. 1, venom B caused uterine contractions with a dose dependent manner. The contractile force of the venom (168 µg/ml) was 92% of that of the standard ACh (10⁻⁷g/ml) and the onset of the contraction was delayed for 40 sec as compared with that of ACh. The time lag seen in the venom-induced contraction was increased with the decrease in the venom concentrations. Since the contractil activity was weakened once after the treatment of the uterus by the venom (Fig. 1B), freshly prepared uterus was used for each contraction. Fig. 2 depicts the effect of various inhibitors on the uterus contraction. Inhibitors in the prostaglandin synthesis, indomethacin and aspirin, inhibited the venom-induced contraction of the uterus, but no effect was observed in atropine.

The contractile action of venom B was decreased to 33% of the control after heated at 75°C for 5 min. Venom A did not cause the uterus contraction. When kinin releasing action

Fig. 1. Effect of venom B on smooth muscles of the uterus of rat. (A) : Each concentration of venom B was added to the uterus and the contractile force was measured as described in the Materials and Methods. The freshly prepared uterus was used for every concentration of the venom. (B) : Venom B (168 μ g/ml) was added to the uterus and after several washing of the uterus with Jalon solution the same concentration of the venom was again applied to the uterus. Ach ; acetylcholine, B ; venom B.

Fig. 2. Effect of various agents on venom B-induced contraction of the uterus. Indomethacin (Ind), aspirin (Asp) and atropine (Atr) were added prior to treatment of the uterus with venom B and the contractile force was measured. Ach ; acetylcholine, B ; venom B.

of venom B was examined by using human plasma as a source of kininogen, no contraction of the uterus was observed.

Vascular permeability: As shown in Fig. 3, a marked enhancement of the permeability was observed by the injection of the venom.

Isolation of uterus contracting factor from venom B: Fig. 4 indicates the gel filtration of venom B on Sephadex G-100. Protein was recovered in four fractions and the second fraction (G-100-2) caused the uterus contraction which was depressed by indomethacin. Thus the G-100-2 fraction was chromatographed by a DEAE-cellulose column. Three fractions were obtained from the column and the first fraction, designated as DE-1, contracted the uterus (Fig. 5). The contractile action of DE-1 fraction was lost after heating at 75°C for 5 min. Although the recovery of DE-1 fraction was 0.8% of venom B, the contractile force of DE-1 has increased 1.6-fold as shown in Table 1.

Enzyme activity in the venom: Phospholipase A and proteinase activities were detected in venom B and still remained in DE-1 fraction (Table 2). Both activities were almost lost by heating at 75°C for 5 min. Anticoagulant activity was observed in venom B but was not detected in DE-1 fraction.

DISCUSSION

It was demonstrated that venom B caused the uterus contraction with a time lag. The time-delayed contraction of the uterus muscle suggests that venom B itself does not act on the uterus but some mediator(s) that released from the uterus during the treatment of venom B may result in the contraction. Thus the possibility of prostaglandin or kinin as the mediator was examined. The venom B-induced contraction of the uterus was depressed by indomethacin and aspirin which are known to inhibit cyclooxygenase followed by inhibition of prostaglandin synthesis. On the other hand, when the filtrate obtained from the reaction of venom B with human plasma was added to the uterus, no contraction was observed. As reported previously (Aniya and Matsusaki, 1979), when kinin was formed from kininogen in the plasma by the venom, the contract of uterine muscle took place. Thus the

Fig. 3. Effect of spine venom B on vascular permeability.

Each dose of venom B (1; 1.95 mg, 2; 0.98 mg, 3; 0.65 mg, 4; 0.49 mg, 5; 0.33 mg) or vehicle (C and 11; 0.9% NaCl) was injected intracutaneously to the rabbit after intravenous injection of Evans blue and the spots were observed as described in Materials and Methods.

results presented here clearly showed that kinin was not related with the venom-induced contraction. Anticholinergic agent atropine could not inhibit the contraction of the uterus by venom B. Furthermore, venom B-induced contraction of the uterus was not inhibited by serotonin antagonist tryptamine and kinin antagonist homochlorcyclizine (data not shown). It was therefore clarified that venom B causes the uterus contraction by releasing prostaglandins. Shiroma *et al.* (1994) reported that the starfish venom induced severe systemic hypotension in dogs which was suppressed by indomethacin. Although the spine homogenate instead of venom B was used in their study, same component(s) as involved in venom B may cause hypotension through vasodilating prostaglandins. Furthermore, it is likely

Fig. 4. Column chromatography of venom B on Sephadex G-100.

Venom B (323 mg) was applied to a Sephadex G-100 column (2.5×50 cm) and eluted with 0.005 M potassium phosphate buffer (pH 7.0). Protein content of fractions was measured at 280 nm. The inserted figure shows a DEAE cellulose column chromatogram of Sephadex G-100 fraction. Sephadex G-100 fraction (72 mg) was applied to a DEAE-cellulose column (1.5×24 cm) and eluted with a gradient of 0.3 M NaCl in 0.01 M Tris-HCl (pH 8.0).

Fig. 5. Contractile action of DE-1 fraction. Dialyzed and lyophilized DE-1 fraction was dissolved in Jalon solution and the contractile force was measured.

Table 1. Purification of the uterus contractile factor from venom B.

Steps	Protein (g)	Recovery (%)	Contractile action ¹⁾ (%/μg)
Spines	250		
Venom B	2.775	100	0.46
G-100-2	0.209	7.5	1.92
DE-1	0.021	0.8	2.40

¹⁾ Contractile action : percent of the uterus contraction to that of the standard ACh (10^{-7} g/ml) per microgram protein of each fraction.

Table 2. Enzyme activity of each fraction.

Fraction	Phospholipase A (min/mg) ¹⁾	Proteinase (OD ₂₈₀ /mg) ²⁾
Venom B	20 (9)	0.441
G-100-2	>30 (13)	0.511
DE-1	>50 (3)	2.128 (0.382)

The value in the parenthesis shows the activity after heating at 75°C for 5 min. ¹⁾ Coagulation time of egg yolk caused by addition of 1 mg of the venom fraction. ²⁾ Absorbance at 280 nm of hydrolyzed casein caused by 1 mg of the venom fraction.

that the increase in vascular permeability by venom B could be due to prostaglandin production. Taking together, harmful symptoms seen in human who contacts with the starfish spines may mostly come from prostaglandin produced by the venom.

We examined the isolation of the muscle contractile factor from venom B and obtained DE-1 fraction. This fraction showed the activities of phospholipase and proteinase. These enzymes almost lost their activities after heating at 75°C for 5 min. This is in accordance with the decrease of venom B and DE-1-induced uterus contraction after the heat treatment. Therefore, it is presumed that these activities may contribute to the contraction. Judging from the depression of G-100-2-induced uterus contraction by indomethacin, it was strongly suggested that DE-1 contracts the smooth muscle via prostaglandin releasing. In considering that phospholipase A can release arachidonic acid from biological membranes as the first step of prostaglandin synthesis,

it is reasonable that phospholipase activity is present in DE-1 fraction. However, we can not exclude a possibility that other protein than phospholipase A may contribute to the contraction since DE-1 fraction contains several proteins as judging from SDS-polyacrylamide gel electrophoresis (data not shown). Further purification of the contractile factor is needed.

Taira *et al.* (1975) reported that the lethal factor of the spine venom is involved in the venom A. Shiomi *et al.* (1988) purified the lethal factor through CM-cellulose which is a cation exchanger. Thus, it is clear that DE-1 fraction which has been adsorbed on an anion exchanger DEAE-cellulose is different from the lethal factor of the spine venom.

In summary, the starfish venom causes uterus contraction via producing prostaglandins and phospholipase A in the venom may partly contribute to the contraction.

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