Contractile Effects of Jellyfish Toxin Extracted from *Carybdea rastonii* on Isolated Rabbit Aorta

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Abstract—Effects of the toxic component of jellyfish (*Carybdea rastonii*) (pCrTX) on the smooth muscle tension of isolated rabbit thoracic aorta were examined. pCrTX, at concentrations higher than $10^{-7}$ g/ml, caused slowly developing tension that reached its maximum after about 1 hr. This contraction was partially inhibited by pretreatment of the tissue with phentolamine ($5 \times 10^{-6}$ M) or indomethacin ($10^{-5}$ M). The contraction induced by pCrTX was partially inhibited by nicardipine ($10^{-7}$ M) and markedly augmented by Bay k8644 ($10^{-6}$ M). In low-Na$^+$ solution, the rate of rise of the pCrTX-induced contraction was significantly reduced. Removal of external Ca$^{2+}$ inhibited the pCrTX-induced contraction by about 30%, while chlorpromazine, trifluoperazine, prenylamine and papaverine ($10^{-4}$ M) completely inhibited the contraction. pCrTX itself did not cause any contraction in saponin-skinned smooth muscle and had no effect on the Ca$^{2+}$-induced contractile tension. It has been reported that pCrTX-induced contraction is attributable to the release of endogenous catecholamines and also to the increase in Ca$^{2+}$ influx (Azuma et al., 1986). The present results confirmed the previous suggestion and further suggested that a portion of the contraction is due to release of prostaglandin(s) and also to the direct effect on smooth muscle which is not dependent on Ca$^{2+}$ influx.

The nematocyst of *Carybdea rastonii*, one of the species of box jellyfish ("Andon-kurage") living in the sea along the coast of Japan, is capable of producing severe cutaneous pain, erythema, wheeling and haemorrhagic skin lesions in humans who accidentally come into contact with its tentacles. A toxic component extracted from the tentacles of the jellyfish (pCrTX) has been shown to induce platelet aggregation (1). pCrTX has also been reported to cause a contraction in rabbit aorta by releasing noradrenaline from adrenergic nerve terminals and by increasing transmembrane Ca$^{2+}$ influx (2) and to inhibit the contraction in rat aorta by releasing a relaxant substance from the endothelium (3).

In the present study, we have investigated the more detailed mechanism of the contractile effects of pCrTX in the vascular smooth muscle of rabbit aorta.

Materials and Methods

Male rabbits, weighing 2–3 kg, were sacrificed by injection of an excess dose of pentobarbital. The thoracic aorta was dissected out and strips, 2–3 mm wide and 5–8 mm long, were prepared. In some experiments, the adventitial layer was removed according to the method of Karaki and Urakawa (4). The endothelium was removed by gently rubbing the endothelial surface with moistened finger. Each muscle strip was attached to the arm of a strain...
gauge transducer (Nihon Kohden), and the changes in contractile tension of the muscle strip were recorded isometrically. The magnitude of the contraction induced by 65.4 mM K+ was taken as a reference response (100%).

The normal physiological salt solution (PSS) was composed of 136.9 mM NaCl, 23.8 mM NaHCO₃, 1.5 mM CaCl₂, 1.0 mM MgCl₂, 5.4 mM KCl and 5.5 mM glucose, which was aerated with 95% O₂ and 5% CO₂ at 37°C, pH 7.4. High-K⁺ solution was made by replacing NaCl with equimolar KCl. Low-Na⁺ solution was made by replacing NaCl with equimolar tris(hydroxymethyl)aminomethane·HCl (tris). Ca²⁺-free solution was made by omitting CaCl₂ from PSS and adding ethylene glycol bis(β-aminoethyl ether)·N,N',N'·N'-tetraacetic acid (EGTA).

pCrTX was prepared as described by Azuma et al. (1). Tentacles obtained from jellyfish (Carybdea restoni) were lyophilized and stored at −80°C. An aliquot of the lyophilized tentacles was sonicated in 50 mM sodium acetate (pH 6.0) and centrifuged at 6000×g for 30 min. The separated supernatant was treated with 0–40% ammonium sulfate for 60 min and centrifuged at 6000×g for 30 min. This procedure was repeated again using 40–60% ammonium sulfate. Decanting the supernatant, the precipitate was dissolved in 50 mM sodium acetate (pH 6.0). pCrTX was obtained by dialyzing the solution with an Amicon YM-2. All the manipulations were carried out at 4°C. The concentration of pCrTX was expressed as g protein/ml. Protein was assayed by the Lowry method (5) using bovine serum albumin as the standard. pCrTX was stored at 4°C and used within 2 weeks. pCrTX was diluted prior to the experiments.

The experimental procedures for the chemical skinning of the tissue were the same as described by Saida and Nonomura (6). A thin bundle of rabbit aorta (0.3 mm in width and 2 mm in length) was prepared. After the contractility of the muscle was checked by high-K⁺ solution, the muscle was soaked in a skinnning solution of the following composition: 136.9 mM KCl, 5.0 mM MgCl₂, 2.0 mM EGTA, 20 mM tris-maleate, 5 mM ATP·Na₂ and 100 µg/ml saponin for 30–40 min at pH 6.8 and 22–24°C. Ca²⁺ concentrations were changed by adding an appropriate amount of CaCl₂ to EGTA. The apparent binding constant of EGTA for Ca²⁺ was considered to be 10⁶ M⁻¹ (7).

Drugs used in this experiment were Bay k8644 (Bayer), chlorpromazine hydrochloride (Sigma), indomethacin (Sigma), noradrenaline bitartrate (Wako), methylene blue (Wako), nicardipine (Sigma), sodium nitroprusside (Wako), papaverine hydrochloride (Sigma), phentolamine mesylate (Ciba-Geigy), pynylamine (Hoechst), saponin (ICN) and trifluoperazine hydrochloride (Sigma).

Results
pCrTX, at concentrations above 10⁻⁷ g/ml, produced long lasting contraction in isolated rabbit aorta. Prior exposure of the muscle to phentolamine (5×10⁻⁶ M) or indomethacin (10⁻⁵ M) resulted in a decrease in the magnitude of the pCrTX (10⁻⁶ g/ml)-induced contraction by 33.8% and 77.4%, respectively (Fig. 1). A combination of these inhibitors almost completely inhibited the contraction, and methylene blue (10⁻⁵ M) partially restored the contracting effect of pCrTX. In the muscle deprived of adventitia and endothelium, pCrTX still induced contraction, but it was about a half of that in the adventitia- and endothelium-intact tissue. Neither phentolamine nor indomethacin affected the pCrTX-induced contraction in this tissue.

The following experiments were conducted in muscle strips in which adventitia and endothelium were removed in order to determine the direct action of pCrTX on smooth muscle.

When applied to precontracted tissue with pCrTX (10⁻⁶ g/ml), nicardipine (10⁻⁷ M) or nitroprusside (10⁻⁶ M) reduced the tension by about 30%, and papaverine (10⁻⁴ M) reduced it to the initial level (Fig. 2). Pretreatment with nicardipine (10⁻⁷ M) reduced the rate of rise of the pCrTX-induced contraction, while Bay k8644 (10⁻⁶ M) resulted in a significant augmentation of the pCrTX-induced contraction (Fig. 3). In low-Na⁺, tris-substituted solution, the rate of rise of the contraction was decreased as shown in Fig. 4.

In a Ca²⁺-free solution with 0.5 mM
Fig. 1. Effects of pretreatment with phentolamine or indomethacin and with a combination of these agents on contraction induced by pCrTX (10^{-6} g/ml) in isolated rabbit aorta. Each pretreatment was performed for 10 min before application of pCrTX in adventitia- and endothelium-intact [left; ADV(+), ENDO(+)] and removed tissue [right; ADV(-), ENDO(-)]. (●), control (CONT). (○), phentolamine (PHENT, 5\times10^{-6} M). (■), indomethacin (INDO, 10^{-5} M). (□), phentolamine plus indomethacin (PHENT+INDO). Broken line indicates the response to pCrTX in the presence of methylene blue (10^{-5} M), phentolamine and indomethacin. Each point is the mean of 4–6 experiments (S.E.M. is not shown). Ordinate, relative contraction to that induced by 65.4 mM K+ solution (%). Abscissa, time after application of pCrTX (min).

Fig. 2. Effect of nicardipine (NICARD, 10^{-7} M) (A), nitroprusside (NITRO, 10^{-6} M) (B) and papaverine (PAPAV, 10^{-4} M) (C) on pCrTX (10^{-6} g/ml)-induced contraction in adventitia- and endothelium-removed tissue.

Fig. 3. Effect of nicardipine, Bay k8644 and removal of Ca^{2+} on the contraction induced by pCrTX (10^{-6} g/ml) in adventitia- and endothelium-removed tissue. Tissues were pretreated with these agents for 5 min before addition of pCrTX. (●), control. (○), nicardipine (10^{-7} M). (□), Bay k8644 (10^{-6} M). (■), Ca^{2+} removal. Each point is the mean of 4–6 experiments (S.E.M. is not shown). Ordinate, relative contraction to that induced by 65.4 mM K+ solution (%). Abscissa, time after application of pCrTX (min).
EGTA, pCrTX (10^{-6} g/ml) produced a contraction with a slower rate of rise than that in normal PSS, reaching approximately 50% of the contraction induced in normal PSS during a 70 min observation period. In the presence of Ca^{2+} and nicardipine (10^{-7} M), pCrTX showed a similar time course of contraction (Fig. 3). When the pCrTX-contracted tissue was treated with Ca^{2+}-free solution, the developed tension decreased, reaching a new steady level within 30 min which was approximately 80% of the contraction before the Ca^{2+} removal. Chlorpromazine, trifluoperazine, prenylamine or papaverine (10^{-4} M) completely inhibited the pCrTX-induced contraction in the Ca^{2+}-free solution (Fig. 5).

In saponin-treated skinned muscle, pCrTX (2 \times 10^{-6} g/ml) had no contractile effect and had no effect on the relaxing process upon reducing the external Ca^{2+} concentration (Fig. 6A). Application of 3 \times 10^{-6} M Ca^{2+} caused a rapid increase in tension, and the second application of Ca^{2+} caused slightly lesser contraction. pCrTX had no appreciable effects on the rate of decay of the Ca^{2+}-induced contraction (Fig. 6B).

**Discussion**

In the present experiment, we confirmed previous results that a portion of the contraction induced by pCrTX is due to release of endogenous catecholamines (2). Further, pCrTX-induced contraction was partially inhibited by indomethacin only in the pre-

![Fig. 4](image-url). Contractile effect of pCrTX (10^{-6} g/ml) in normal (●) and low-Na+, tris-substituted solution (○) in adventitia- and endothelium-removed tissue. Each point is the mean of 4–6 experiments (S.E.M. is not shown). Ordinate, relative contraction to that induced by 65.4 mM K^{+} solution (%). Abscissa, time after application of pCrTX (min).

![Fig. 5](image-url). Effect of chlorpromazine (CPZ, 10^{-4} M) (A), trifluoperazine (TFP, 10^{-4} M) (B) and prenylamine (PREN, 10^{-4} M) (C) on the contraction induced by pCrTX (10^{-6} g/ml) in the absence of external Ca^{2+} and presence of EGTA (0.5 mM) in adventitia- and endothelium-removed tissue.

![Fig. 6](image-url). Effect of pCrTX (2 \times 10^{-6} M) on contractile response in saponin-skinned muscle.
paration in which adventitia and endothelium are intact, suggesting that part of the pCrTX-induced contraction is attributed to prostaglandin(s) released from nerve terminals and/or endothelial cells. In rabbit aorta with endothelium, methylene blue augmented the pCrTX-induced contraction. It is known that a relaxant substance derived from vascular endothelium inhibits muscle contraction by increasing cellular cGMP (8), and methylene blue, a guanylate cyclase inhibitor, inhibits this (9). In rat aorta, pCrTX induced an endothelium-dependent relaxation of noradrenaline-induced contraction (3). These results suggest that pCrTX releases a relaxing substance from the endothelium and decreases tension development caused by pCrTX itself in rabbit aorta.

Our previous report showed that the pCrTX-induced contraction was partially inhibited by verapamil (2). In the present experiment, we re-examined this in the tissue from which adventitia and endothelium had been removed. The contraction, the amplitude of which had been reduced by the removal of adventitia and endothelium, was also partially inhibited by nicardipine, a Ca\(^{2+}\) channel inhibitor, and it was markedly augmented by Bay k8644 (10), a Ca\(^{2+}\) channel activator, and it was significantly reduced by lowering external Na\(^{+}\). pCrTX has been reported to increase the inward movement of \(^{22}\)Na\(^{+}\) and cause membrane depolarization in platelets (1). Based on these results, it seems likely that pCrTX increases Ca\(^{2+}\) influx, possibly through voltage-dependent Ca\(^{2+}\) channels.

In a Ca\(^{2+}\)-free solution, pCrTX still induced a slowly developing contraction reaching approximately 50% of the contraction induced in the presence of Ca\(^{2+}\) in 70 min. This contraction was not affected by nicardipine. Since pCrTX had no contractile effect on saponin-treated skinned muscle, pCrTX may increase the intracellular free Ca\(^{2+}\) concentration by mobilizing the Ca\(^{2+}\)-store. When external Ca\(^{2+}\) was removed during the pCrTX-induced contraction, muscle tension also decreased, reaching a new steady level within 30 m:n. However, the residual tension in a Ca\(^{2+}\)-free solution (approximately 80%) was greater than that induced by the addition of pCrTX in a Ca\(^{2+}\)-free solution. The residual contraction in a Ca\(^{2+}\)-free solution was inhibited by calmodulin antagonists and papaverine. These results suggest that pCrTX may also decrease Ca\(^{2+}\) sequestration and/or extrusion.

From previous (2, 9) and present results, it is suggested that in rabbit aorta, the pCrTX-induced contraction is due to indirect action through the release of catecholamines from adrenergic nerve terminals and prostaglandin(s) from the adventitia and/or endothelium, and it is also due to the direct action on the smooth muscle through the opening of voltage-dependent Ca\(^{2+}\) channels, which results from an increase in Na\(^{+}\) permeability, and a mechanism which is not dependent on Ca\(^{2+}\) influx. pCrTX also seems to release a relaxant substance from aortic endothelium.

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