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Palytoxin-induced contraction and release of endogenous noradrenaline in rat tail artery

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1 The mechanism of the contractile effect of a potent marine toxin, palytoxin (PTX) on the rat isolated tail artery was examined.

2 PTX (10^{-7} M) induced a contraction in the tail artery which was dependent on external Ca^{2+}. This contraction was inhibited (by 75% or more) by 10^{-6} M prazosin, 2.4×10^{-5} M bretylium and 10^{-4} M 6-hydroxydopamine (6-OHDA), and partially (by 40%) by 10^{-5} M indomethacin. However, this contraction was not affected by 10^{-6} M tetrodotoxin (TTX), 10^{-6} M nifedipine or reserpine treatment. The PTX-induced contraction in reserpine-treated artery was partially inhibited by nifedipine and indomethacin but not by prazosin.

3 Transmural electrical stimulation induced a transient contraction which was dependent on external Ca^{2+}. The contraction induced by electrical stimulation was inhibited by TTX, prazosin, bretylium, reserpine treatment and 6-OHDA but not by nifedipine or indomethacin.

4 PTX increased the release of noradrenaline from this artery. However PTX did not release noradrenaline from reserpine-treated arteries. PTX-induced noradrenaline release was only partially inhibited by TTX or by Ca^{2+}-free solution.

5 These results suggest that PTX has pre- and postsynaptic effects in the rat tail artery. PTX may stimulate adrenergic nerves and release noradrenaline mainly by a TTX-insensitive and Ca^{2+}-independent mechanism and partially by a TTX-sensitive and Ca^{2+}-dependent mechanism. Further, PTX may also release prostaglandins and depolarize smooth muscle cell membrane to induce a contraction.

Introduction

Palytoxin (PTX, Cha *et al.*, 1982; Moore *et al.*, 1982) is one of the most potent toxins isolated from a coelenterate *Palythoa spp.* living on the coasts of southern Japan (Ryukyu islands), Hawaii, Tahiti and Jamaica (Hashimoto, 1979). The toxic action of PTX has been attributed to its profound vasoconstrictor effect (Ito *et al.*, 1976). PTX depolarizes the membrane of various excitable cells (Rayner *et al.*, 1975; Deguchi *et al.*, 1976; Ito *et al.*, 1976; 1979; Dubois & Cohen, 1977; Kudo & Shibata, 1980; Muramatsu *et al.*, 1984), possibly by increasing the permeability to Na^+ and K^+ ions (Ozaki *et al.*, 1983; 1984; Nagase *et al.*, 1986). Mitani & Ito (1986) have studied the effect of PTX on cardiac cell membrane using the patch-clamp technique and found that the membrane depolarization is attributable to the opening of an ion channel which is different from any channel ever studied. This effect may be the major mechanism of PTX-induced contraction in the isolated aorta of the rabbit (Ito *et al.*, 1977) or guinea-pig (Ozaki *et al.*, 1983). In rat aorta, however, PTX-induced contraction is partly due to the release of prostaglandins (Nagase & Karaki, 1987). Further, PTX releases endothelium-derived relaxing factor at concentrations lower than those needed to induce contraction in rat aorta (Nagase *et al.*, 1987). These results suggest that PTX has multiple sites of action and that the effect of PTX is different in different vascular beds. In the present experiments, we examined the mechanism of PTX-induced contraction in the rat isolated tail artery and found that PTX releases endogenous noradrenaline to induce vascular smooth muscle contraction.

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Methods

Muscle contraction

Male Wistar rats (250–300 g) were killed by a blow on the neck and exsanguination. The tail artery was removed and cut into spiral strips of 0.3–0.5 mm width and 5–7 mm length. These vascular strips did not contain functionally intact endothelium. Muscle tension was recorded isometrically with a force-displacement transducer connected to a polygraph (Nihon Kohden). Passive tension of 0.5 g was initially applied and tissues were allowed to equilibrate in a 10 ml bath for 60 min before beginning the experimental period. Inhibitors were added 10 min before the addition of PTX or transmural electrical stimulation. The electrical stimulation was applied through a pair of platinum wire electrodes with a duration of 3 ms at a frequency of 5 Hz at supramaximum intensity (approximately 90 V). The normal physiological salt solution contained (mM): NaCl 120.3, KCl 4.8, glucose 5.8, $NaHCO_3$ 25.2, $CaCl_2$ 1.2, $MgSO_4 \cdot 7H_2O$ 1.3 and KH_2PO_4 1.2. Ca^{2+}-free solution was made by omitting $CaCl_2$ and adding 0.1 mM ethyleneglycol *bis*(beta-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA) to the normal solution. These solutions were aerated with 95% O_2 and 5% CO_2 at 37°C (to pH 7.4).

Reserpine and 6-hydroxydopamine treatment

For functional denervation, reserpine treatment and treatment with 6-hydroxydopamine (6-OHDA) were used. Reserpine, 0.5 mg kg^{-1}, was given i.p. once a day for 2 days and rats were killed on the third day. For the 6-OHDA treatment, muscle strips were treated with a solution containing 10^{-4} M 6-OHDA and 100 mg l^{-1} ascorbic acid for 30 min followed by a wash with normal solution.

Measurement of endogenous noradrenaline release

Noradrenaline released from the tail artery was measured as described by Ohizumi *et al.* (1983) and Ishida *et al.* (1985). After incubation of the muscle strips with PTX for 30 min, noradrenaline released in the medium was absorbed with alumina and desorbed with 0.2 M $HClO_4$. The amount of noradrenaline was measured by the combination of a high-liquid chromatograph and an electrochemical detector (Yanaco, Kyoto).

Measurement of [^{3}H]-noradrenaline release

Release of ^{3}H from tissue previously loaded with [^{3}H]-noradrenaline was measured as described by Karaki *et al.* (1984). Muscle strips were incubated in a solution containing 1.5×10^{-7} M [^{3}H]-noradrenaline (approximately 10^4 d.p.m. μl^{-1}) for 60 min. At the end of the incubation, the tissue was dipped and transferred sequentially to vials containing 2 ml of aerated non-radioactive solution at 1 min intervals for the duration of the washout. Upon completion of the 9 min washout, liquid scintillation cocktail (ACS II, Amersham, U.K.), 4 ml, was added to these vials and radioactivity was counted in a scintillation spectrometer (Packard, U.S.A.).

Statistics

Results of the experiments are expressed as means ± s.e.mean. Values were considered to be significantly different when the P value was less than 0.05, by use of Student's t test.

Drugs and chemicals

Palytoxin, isolated from *Palythoa tuberculosa*, was kindly donated by Dr Y. Hirata, Meijo University, Nagoya. The following drugs and chemicals were used: (−)-noradrenaline bitartrate (Wako, Tokyo), tetrodotoxin (TTX, Sankyo, Tokyo), prazosin (Pfizer, Brooklyn, NY), nifedipine (Pfizer), 6-OHDA (Sigma, St Louis, MO), reserpine (Apoplone Injection, Daiichi, Tokyo), bretylium tosylate (Burroughs-Wellcome, U.K.), indomethacin (Merck, Sharp & Dohme, West Point, PA) and (±)-[7-^{3}H]-noradrenaline (specific activity 17.4 μCi $mmol^{-1}$, Amersham Japan, Tokyo).

Results

Contractile effect of PTX

The threshold concentration of PTX to induce a contraction in the rat tail artery was between 10^{-9} and 10^{-8} M. PTX, 10^{-7} M, induced a contraction averaging 0.94 ± 0.08 g (n = 18), which was approximately 65% of the maximum contraction induced by 10^{-5} M noradrenaline. Further increasing the concentration of PTX to 10^{-6} M did not induce a greater contraction. From these results, 10^{-7} M PTX was used in the following experiments. Transmural electrical stimulation induced a rapid, transient contraction in rat tail artery. The maximum contractile tension induced by electrical stimulation was approximately 26% of that induced by 10^{-7} M PTX (Figure 1).

Effects of Ca^{2+} removal and nifedipine

As shown in Figure 1 and Table 1, 10^{-7} M PTX-induced contraction was completely inhibited in

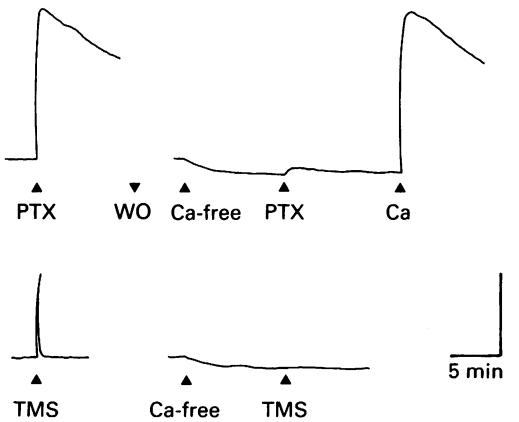

Figure 1 Contractile effects of 10^{-7} M palytoxin (PTX) and transmural electrical stimulation (TMS; 3 ms, 5 Hz) in the presence and absence of external Ca^{2+} in rat isolated tail artery. Vertical scale indicates 0.5 g tension for upper trace and 0.25 g tension for lower trace. WO: washout. Ca: 1.2 mM $CaCl_2$ was added.

Ca^{2+}-free solution. Readdition of Ca^{2+} in the presence of PTX induced a contraction with a similar shape and magnitude to that induced in normal solution. Contraction induced by transmural electrical stimulation was also completely inhibited by Ca^{2+}-free solution. Preincubation of the muscle with 10^{-6} M nifedipine did not inhibit the PTX-induced contraction. Nifedipine had no effect on the contraction induced by electrical stimulation.

Effects of inhibitors

As shown in Table 1, contractions induced by 10^{-7} M PTX were markedly inhibited by 10^{-6} M prazosin and 10^{-5} M bretylium. Contractions induced by electrical stimulation were abolished by prazosin and bretylium. Although 10^{-6} M TTX did not inhibit the PTX-induced contraction, this inhibitor completely inhibited the contraction induced by electrical stimulation. Indomethacin, 10^{-5} M, inhibited the PTX-induced contraction by approximately 40% but had little effect on the contraction induced by electrical stimulation.

Effects of reserpine and 6-OHDA-treatment

In tail arteries isolated from reserpine-treated rats, 10^{-7} M PTX induced a contraction with a similar magnitude as those isolated from normal rats. PTX-induced contractions in reserpine-treated arteries were not inhibited by prazosin. However, these contractions were significantly inhibited by 10^{-6} M nifedipine (by 31%, $P < 0.05$) and by 10^{-5} M indomethacin (by 35%, $P < 0.05$). In arteries pretreated with 6-OHDA, PTX-induced contractions were only approximately 25% of those in normal tissue and electrical stimulation did not induce a contraction. PTX-induced contractions in the 6-OHDA-treated arteries were not inhibited by prazosin (Table 1).

Release of noradrenaline

In normal solution, 12.4 ± 4.0 ng noradrenaline was released from 1 g of artery in 30 min ($n = 4$). In the

Table 1 Effects of various treatments on the contraction of rat tail artery induced by palytoxin (PTX) or transmural electrical stimulation

	PTX (10^{-7} M)	*Electrical stimulation* (3 ms, 5 Hz)
Control	0.94 ± 0.08 (18)	0.24 ± 0.03 (18)
Ca^{2+}-free	0 ± 0** (4)	0 ± 0** (6)
Nifedipine 10^{-6} M	1.02 ± 0.11 (7)	0.16 ± 0.02 (4)
Prazosin 10^{-6} M	0.07 ± 0.01** (7)	0 ± 0** (5)
Bretylium 2.4 × 10^{-5} M	0.03 ± 0.03** (4)	0 ± 0** (3)
TTX 10^{-6} M	0.91 ± 0.05 (3)	0 ± 0** (6)
Indomethacin 10^{-5} M	0.55 ± 0.02* (4)	0.20 ± 0.03 (6)
Reserpine	0.90 ± 0.10 (4)	0 ± 0** (4)
Reserpine + prazosin	0.88 ± 0.06 (4)	—
Reserpine + nifedipine	0.65 ± 0.02* (4)	—
Reserpine + indomethacin	0.61 ± 0.04* (4)	—
6-OHDA	0.23 ± 0.02** (4)	0 ± 0** (4)
6-OHDA + prazosin	0.20 ± 0.03** (4)	—

Results are given as mean contractile tension (g) ± s.e.mean. Number of experiments is shown in parentheses. * and **: significantly different from control with $P < 0.05$ and 0.01, respectively. — indicates not determined. TTX = tetrodotoxin and 6-OHDA = 6-hydroxydopamine.

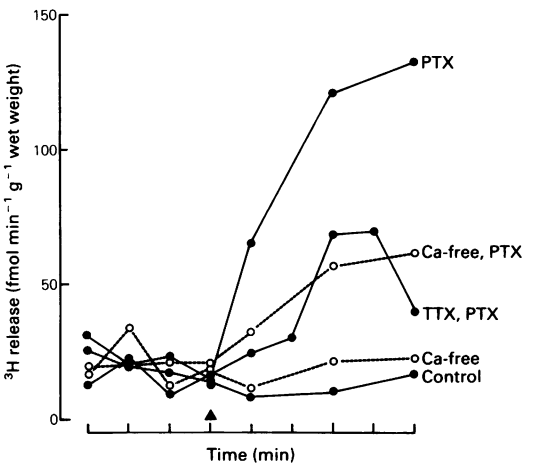

Figure 2 Effects of 10^{-7} M palytoxin (PTX) on the release of ^{3}H from rat tail artery loaded previously with [^{3}H]-noradrenaline. Last 8 min of the 9 min washout period is shown. PTX was added at ▲. Tetrodotoxin (TTX) 10^{-6} M, or Ca^{2+}-free solution was added 3 min before the addition of PTX.

presence of 10^{-7} M PTX, release of noradrenaline increased to 332.8 ± 53.2 ng g^{-1} ($n = 4$). In reserpine-treated arteries, however, release of noradrenaline was only 9.0 ± 1.7 ng g^{-1} 30 min^{-1} ($n = 4$) even in the presence of 10^{-7} M PTX.

As shown in Figure 2, release of ^{3}H from arteries previously loaded with [^{3}H]-noradrenaline was also increased by 10^{-7} M PTX. TTX, 10^{-6} M, and Ca^{2+}-free solution only partially inhibited the effect of PTX. Transmural electrical stimulation also increased the release of ^{3}H which was abolished by TTX and also by Ca^{2+}-free solution (data not shown).

Discussion

In rat tail artery, PTX induced a contraction. The threshold concentration of PTX to induce a contraction (10^{-9}–10^{-8} M) was higher than that in other isolated blood vessels; 10^{-11} M in dog coronary and mesenteric arteries (Ito *et al.*, 1976) and 10^{-10} M in rabbit aorta (Ito *et al.*, 1977), guinea-pig aorta (Ozaki *et al.*, 1983), human umbilical artery (Ishida *et al.*, 1985) and rat aorta (Nagase & Karaki, 1987).

PTX-induced contractions of the rat tail artery were inhibited in the absence of external Ca^{2+} and restored on the readdition of Ca^{2+}. However, these contractions were not inhibited by the Ca^{2+} channel blocker, nifedipine. These results suggest that PTX-induced contractions in the rat tail artery are attributable to Ca^{2+} entry which is mediated by a Ca^{2+} channel blocker-insensitive pathway.

PTX-induced contractions were strongly inhibited by prazosin and bretylium. Functional sympathectomy with 6-OHDA also inhibited the PTX-induced contractions. Furthermore, PTX increased the release of noradrenaline from the rat tail artery by approximately 27 fold. These results strongly suggest that the contractile effect of PTX in the rat tail artery is mainly due to the release of endogenous noradrenaline and subsequent opening of receptor-linked Ca^{2+} channels, which are less sensitive to Ca^{2+} channel blockers (Karaki & Weiss, 1984).

The contraction induced by transmural electrical stimulation was inhibited by prazosin, bretylium, 6-OHDA and reserpine treatment, supporting previous results that electrical stimulation releases endogenous noradrenaline and induces contraction (Vanhoutte *et al.*, 1981). There are several results indicating that the mechanisms of noradrenaline release due to PTX and electrical stimulation are different. PTX-induced contraction was much greater than that induced by supramaximum electrical stimulation. Although TTX inhibited the contraction induced by electrical stimulation, this toxin did not inhibit PTX-induced contraction. It has been shown that the release of noradrenaline induced by electrical stimulation from adrenergic nerve terminals in the vascular wall is inhibited by TTX and by Ca^{2+} removal (Vanhoutte *et al.*, 1981). We confirmed this in the present experiments. However, PTX-induced noradrenaline release was only partially inhibited by TTX and by Ca^{2+} removal. Since PTX induces TTX-insensitive membrane depolarization in skeletal muscle (Deguchi *et al.*, 1976) and nerve (Dubois & Cohen, 1977; Kudo & Shibata, 1980; Muramatsu *et al.*, 1984), a portion of the PTX-induced noradrenaline release may not be due to a physiological exocytotic mechanism. Detailed mechanisms of the PTX-induced noradrenaline release remain to be clarified.

In reserpine-treated arteries, PTX induced a contraction but not the release of endogenous noradrenaline. However, PTX only induced a small contraction in 6-OHDA-treated arteries. PTX-induced contractions in these arteries were not inhibited by prazosin. These results suggest that PTX has not only a presynaptic effect but also a postsynaptic effect to induce vascular contraction. Since PTX-induced contractions in rat tail arteries treated with reserpine were inhibited partially by nifedipine and partially by indomethacin, these contractions may be induced by membrane depolarization and release of prostaglandins. Reserpine treatment makes postsynaptic membranes more permeable to Ca^{2+} and smooth muscle highly sensitive to stimulants (Hudgins & Harris, 1970), and this may be the

reason why PTX-induced noradrenaline release was inhibited and yet contraction was not inhibited in reserpine-treated arteries.

In rabbit aorta (Ito *et al.*, 1976) and human umbilical artery (Ishida *et al.*, 1985), PTX-induced contractions were inhibited by Ca^{2+} channel blockers suggesting that these contractions are mainly due to membrane depolarization. In contrast to this, PTX-induced contractions in the rat aorta were partially inhibited by indomethacin and also partially by Ca^{2+} channel blockers, suggesting that both membrane depolarization and release of prostaglandins are involved in the contractile effect of PTX (Nagase & Karaki, 1987). The present experiment indicated that the PTX-induced contraction in the rat tail artery is mainly due to the release of noradrenaline. PTX also releases noradrenaline in rabbit aorta, although the amount of noradrenaline released is so small that it has only a slight effect on muscle contraction (Nagase & Karaki, 1987). In smooth muscle other than blood vessels, Ohizumi & Shibata (1980) found, in guinea-pig vas deferens, that PTX releases noradrenaline and Shibata *et al.* (1986) showed, in rabbit urinary bladder, that PTX releases metabolites of arachidonic acid. Thus, PTX seems to have multiple sites of action in vascular and other smooth muscle tissue.

In conclusion, PTX seems to have pre- and post-synaptic effects in the rat tail artery. PTX may stimulate the adrenergic nerve and release noradrenaline mainly by a TTX-insensitive and Ca^{2+}-independent mechanism and partially by a TTX-sensitive and Ca^{2+}-dependent mechanism. Further, PTX may also release prostaglandins and depolarize smooth muscle cell membrane to induce contraction.

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