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Contraction and increase in tissue calcium content induced by maitotoxin, the most potent known marine toxin, in intestinal smooth muscle

Yasushi Ohizumi & Takeshi Yasumoto*

Mitsubishi-Kasei Institute of Life Sciences, Machida, Tokyo 194 and Faculty of Agriculture,* Tohoku University, Sendai 980, Japan

Maitotoxin (MTX), the most potent known marine toxin, isolated from toxic dinoflagellates and poisonous fish, caused a dose-dependent contraction of the guinea-pig isolated ileum and taenia caeci at concentrations of 100 pg to 30 ng/ml. These contractile responses to MTX (3 ng/ml) in both tissues were abolished by incubation in Ca^{2+}-free solution and were markedly inhibited by treatment with methoxyverapamil (D600), but were not affected by tetrodotoxin and atropine. Furthermore, MTX markedly elevated tissue Ca^{2+} content of the taenia caeci. These results suggest that MTX activates Ca^{2+} channels in the smooth muscle membrane of both tissues to increase Ca^{2+} influx and thus induces contractions.

Introduction Numerous marine toxins such as tetrodotoxin (Narahashi, 1974), saxitoxin (Catterall, 1980), palytoxin (Ohizumi & Shibata, 1980) and ciguatoxin (Ohizumi, Shibata & Tachibana, 1981; Ohizumi, Ishida & Shibata, 1982) have been isolated from marine organisms and have been extensively studied. These studies have indicated that they may be very useful tools in pharmacology and physiology. Recently, it has been revealed that maitotoxin (MTX), isolated from toxic dinoflagellates and poisonous fish inhabiting tropical seas, is the most potent marine toxin yet known and that the minimum lethal dose of the toxin in mice is 0.17 μg/kg when injected i.p. (Yasumoto, Nakajima, Oshima & Bagnis, 1979; Yasumoto, 1980). We have shown that MTX produces a marked release of noradrenaline, which is accompanied by an increase in Ca^{2+} influx, in a rat phaeochromocytoma cell line (PC12h) (Takahashi, Ohizumi & Yasumoto, 1982). However, the effect of MTX on smooth muscles has not yet been studied. Therefore, the present experiments were undertaken to investigate the effects of the toxin on the guinea-pig isolated ileum and taenia caeci.

Methods Male guinea-pigs, weighing 250 to 350 g, were stunned and bled, and the taenia caeci was dissected from the caecum. The tissues were suspended in Krebs-Ringer solution of the following composition (mM): NaCl 120, KCl 4.8, $CaCl_2$ 1.2, $MgSO_4$ 1.3, KH_2PO_4 1.2, $NaHCO_3$ 25.2 and glucose 5.8; pH 7.4. Ca^{2+}-free solution was prepared by omitting $CaCl_2$ from the solution. The procedures for preparing the ileum and the taenia caeci, and the technique for measuring the mechanical response were as described previously (Ohizumi & Shibata, 1981). Tissue Ca^{2+} content of the taenia caeci was determined by the following method: 30 min after treatment with MTX, each tissue (about 20 mg) was blotted briefly, weighed and then ashed with 0.1 ml of 60% perchloric acid at 500°C for 6 h. The ashed samples were dissolved in 1.5 ml of solution containing 0.4% disodium edetate (EDTA) and 0.2% $SrCl_2$. The amount of Ca^{2+} was measured with an atomic absorption spectrophotometer. Purification of MTX was carried out as previously reported (Yasumoto *et al.*, 1979; Yasumoto, 1980). Dinoflagellates (*Gambierdiscus toxicus*) were extracted with methanol and the methanol extract was chromatographed on silicic acid and octadecyl silane columns to yield MTX. The minimum lethal dose of the purified toxin was 0.2 μg/kg (i.p.) in mice. The concentration of MTX was expressed on a g/ml basis since its molecular weight has not yet been determined. The following drugs were used: carbamylcholine chloride (Sigma), atropine sulphate (Tokyo Kasei), chlorpheniramine maleate (Sankyo), methysergide (Sandoz), indomethacin (Merck), tetrodotoxin (Sankyo) and methoxyverapamil (D600, Knoll).

Results In the guinea-pig isolated ileum, the addition of MTX in concentrations ranging from 100 pg to 30 ng/ml caused a dose-dependent contraction (Figure 1a). The contractile response induced by MTX at 30 ng/ml was comparable to the maximum response to carbamylcholine (3×10^{-7} M). As shown in Figure 1b, the MTX (3 ng/ml)-induced contraction increased with time, attained a maximum, and then

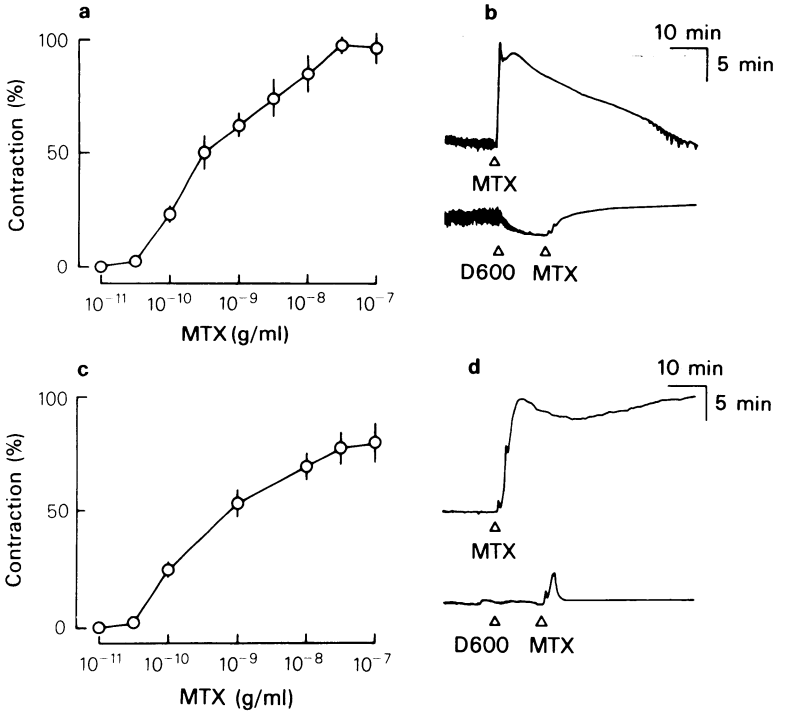

Figure 1 The log dose-response curve for maitotoxin (MTX) and the inhibitory effect of D600 (5×10^{-7} M) on the MTX (3 ng/ml i.e. 3×10^{-9} g/ml)-induced contraction in the guinea-pig isolated ileum (a and b) and taenia caeci (c and d). In (a) and (c), the maximum responses of the ileum and taenia caeci to carbamylcholine at 3×10^{-7} and 10^{-6} M are expressed as 100%, respectively. Symbols and vertical bars indicate the mean and s.e.mean of 6 experiments. In (b) and (d), D600 (5×10^{-7} M) was added before the administration of MTX (3 ng/ml); drugs were added at △.

gradually decreased. After 10 min exposure to MTX (3 ng/ml), washing with fresh medium 4 times for 1 min could not remove the effects of MTX. In the guinea-pig isolated taenia caeci, MTX at concentrations above 100 pg/ml produced a sustained contraction which lasted for at least 2 h. The MTX-induced contraction increased with concentration in the range 100 pg to 30 ng/ml (Figure 1c). The contractile response of the taenia caeci to MTX at 100 ng/ml was approximately 80% of the maximal contraction induced by carbamylcholine (10^{-6} M). The effect of MTX (3 ng/ml) on the taenia caeci was also irreversible after 10 min. The contractions induced by MTX (3 ng/ml) in both tissues were abolished during incubation in Ca^{2+}-free solution and markedly inhibited in the presence of D600 (5×10^{-7} M) (Figure 1b and d). In both tissues, treatment with atropine (10^{-6} M), tetrodotoxin (5×10^{-7} M), chlorpheniramine (10^{-6} M), methysergide (6×10^{-7} M) and indomethacin (3×10^{-6} M) had no effect on the contractile response to MTX (3 ng/ml). Furthermore, tissue Ca^{2+} content of the taenia caeci was increased by treatment with MTX at 3 ng and 30 ng/ml, from 1.05 ± 0.03 ($n = 12$) to 1.28 ± 0.04 ($n = 13$) and 1.59 ± 0.05 ($n = 10$) μmol/g wet weight (mean ± s.e. mean) or 22 and 51% increases, respectively, 30 min after application.

Discussion In the guinea-pig isolated ileum and taenia caeci, MTX elicited a marked contraction. The contractile responses in both tissues were inhibited or abolished by Ca^{2+}-free solution or a Ca-antagonist (D600). These responses were not affected by blockade of acetylcholine (muscarinic), histamine or 5-hydroxytryptamine receptors or Na^+ channels (atropine, chlorpheniramine, methysergide or tetrodotoxin), or by a prostaglandin synthesis-inhibitor (indomethacin). These data suggest that the MTX-induced contraction is not due to the activation of neurones or receptors for these chemical mediators. MTX may stimulate smooth muscle membranes directly to induce a contraction. Furthermore, MTX caused a significant increase in tissue Ca^{2+} content of the taenia caeci. In a previous paper, it was demonstrated that incubation of PC12h cells with MTX leads

to noradrenaline release and a profound increase in Ca^{2+} influx (Takahashi *et al.*, 1982). These effects of MTX have been observed even in the absence of external Na^+ and were blocked by Ca-antagonists but not by tetrodotoxin, probably indicating that MTX activates Ca^{2+} channels of PCl2h cells. Based on these observations, it is suggested that MTX increases Ca^{2+} influx into smooth muscle cells. It thus causes a rise in intracellular Ca^{2+} concentration, and therefore contractions of both the ileum and taenia caeci.

In smooth muscles, ouabain, a Na^+, K^+-APTase inhibitor, has been reported to produce contractions and to increase Ca^{2+} uptake and cellular Na^+ concentration, probably due to an inhibition of the Na^+, K^+ pump (van Breemen, Aaronson & Loutzenhiser, 1979). However, MTX (300 pg to 300 ng/ml) had little or no effect on Na^+, K^+-ATPase activity (unpublished data). These observations rule out possible involvement of inhibition of the Na^+, K^+ pump by MTX.

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