Maitotoxin stimulates the formation of inositol phosphates in rat aortic myocytes

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Maitotoxin is the most potent of the known marine toxins. The effect of maitotoxin on muscle contraction or hormone release was consistent with its action on the voltage-sensitive channel. Indeed, calcium antagonists such as nifedipine or diltiazem were able to reverse the maitotoxin effects. Using smooth muscle cells, we have analysed the effects of maitotoxin on the inositol phosphate metabolism. Maitotoxin stimulates the inositol phosphate formation (5 ± 1.8-fold in the presence of 10 mM LiCl). Moreover, this effect is not reversed, even partially by calcium antagonists, by α_1 antagonists and is not mimicked by Ca^{2+} ionophores such as A23187 or calcium agonists such as Bay-K 8644. The action of maitotoxin is further discussed in this paper.

Maitotoxin *Polyphosphoinositide* *Myocyte* *(Rat aorta)*

1. INTRODUCTION

Ciguatera, a food poisoning caused by the ingestion of tropical reef fish, is a grave nutritional problem in certain Pacific archipelagoes. Furthermore, several cases of ciguatera have recently been reported in France due to the consumption of deep-frozen fish of tropical origin [1]. The toxic compounds responsible for these intoxications, CTX and MTX, are produced by the dinoflagellate *Gambierdiscus toxicus* and are transmitted to the tropical reef fish via their normal food chain. CTX appears to increase the sodium permeability [2] of plasma membranes, whereas MTX, the most potent of the known marine toxins, may stimulate the opening of the VSCCs. While the effects of MTX on Ca^{2+} fluxes and other Ca^{2+}-dependent phenomena (e.g., muscle contraction [3–7] or hormone release [8–11]) are consistent with its alleged action at the VSCCs, there may be another equally valid explanation for its mechanism of action. It has recently been shown that inositol 1,4,5-trisphosphate (IP_3) liberates Ca^{2+} from intracellular storage sites [12] and thus stimulates Ca^{2+}-dependent phenomena such as the elevation of the fertilization membranes of sea urchin [13] and starfish [14] oocytes. We therefore examined the effect of MTX on the metabolism of IPs and found that this toxin is a very potent stimulus for formation of IPs. This study suggests that in myocytes MTX elevates the level of IPs, possibly through a direct action on the metabolism of IPs.

Abbreviations: CTX, ciguatoxin; MTX, maitotoxin; VSCC, voltage-sensitive calcium channel; IP_1, IP_2, IP_3, the mono-, bis- and trisphosphates of inositol; IP, inositol phosphate

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2. MATERIALS AND METHODS

2.1. *Preparation of aortic myocytes*

Aortic myocytes were prepared from adult male rat aortic medias as described [15]. Briefly, aortae were dissected from 12-week-old male Wistar rats and digested for 30 min by treatment with collagenase. After elimination of the adventitia and the endothelium, the myocytes were disaggregated by incubating them in a collagenase-elastase mixture for 90 min. The separated myocytes were grown on collagen-coated micro-carriers while being stirred for 13–14 days in modified Eagle's medium (MEM) supplemented with vitamins, amino acids and 5% foetal calf serum.

2.2. *Preparation of maitotoxin*

MTX was purified from a methanol extract of *Gambierdiscus toxicus* as described [8].

2.3. *Measurement of phospho[^{3}H]inositide hydrolysis*

Cells were preincubated for 2 days in culture medium complemented with 2.5 μCi/ml *myo*-[^{3}H]inositol. Cells were then washed 3 times with a PBS buffer (composition in mM: 138 NaCl, 2.7 KCl, 8 Na_2HPO_4, 1.5 KH_2PO_4, 0.5 $MgCl_2$ and 0.9 $CaCl_2$) and distributed in test tubes. After MTX stimulation, the reaction was stopped by adding 400 μl of 10% PCA. ^{3}H-labelled *myo*-inositol-phosphates were extracted and separated by ion-exchange chromatography (Dowex 1-10, formate form, 100–200 mesh) as described [16].

3. RESULTS AND DISCUSSION

Rat aortic myocytes were used 13–16 days after seeding. 3–4 days prior to the experiment the culture medium was supplemented with 2.5 μCi/ml of *myo*-[^{3}H]inositol. Cells were preincubated for 10 min in PBS medium containing 10 mM LiCl and then exposed for 45 min to MTX. MTX stimulated IP formation in a dose dependent manner (fig.1). The magnitude of the MTX-induced response saturated at 5 ± 1.8-fold the basal level determined in the absence of MTX. The EC_{50} ($3.25 \times 10^{-10} \pm 0.83$ g/ml; $n = 3$) for the accumulation of IP was similar to the apparent EC_{50} found for the MTX-induced contraction of rabbit aorta [4] and intestinal smooth muscle [7] and even

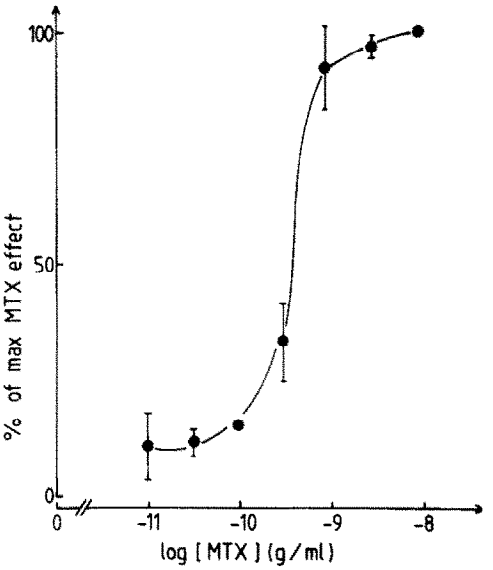

Fig.1. Dose response curve of maitotoxin. After two washings with a PBS buffer (as described in section 2), the cells were distributed in test tubes and incubated for 10 min with 1 ml PBS buffer supplemented with 10 mM LiCl. Increasing concentrations of MTX were added and the reaction was stopped after 45 min by adding 400 μl of 10% PCA. The IPs were extracted and separated as described under section 2. Data shown are the means, expressed as a percentage of the response induced by 10^{-8} g/ml MTX, ± SE of triplicate determinations and are representative of 3 such studies.

lower than that found for the MTX-induced release of hormones [9,10]. Since our primary culture system appears to be free of fibroblasts, epithelial cells and nerve endings, the origin of this MTX-induced formation of IP can be attributed solely to the cultured myocytes.

It is now generally accepted that the first step in the phosphatidylinositol (PI) response is the degradation of phosphatidylinositol-4,5-bisphosphate (PIP_2) into two second messenger molecules, diacylglycerol (DAG), which activates the protein kinase C [17], and IP_3, which liberates Ca^{2+} from intracellular Ca^{2+} pools [12]. IP_3 should therefore be the first IP generated whereas IP_2 and IP_1 are considered to be degradation products of IP_3. The results of studies on the kinetics of IP formation in the absence of LiCl are shown in fig.2 and are consistent with this model. Upon MTX stimulation the accumulation of IP_3 did not show a marked lag period. The rate of IP_3 accumulation was either

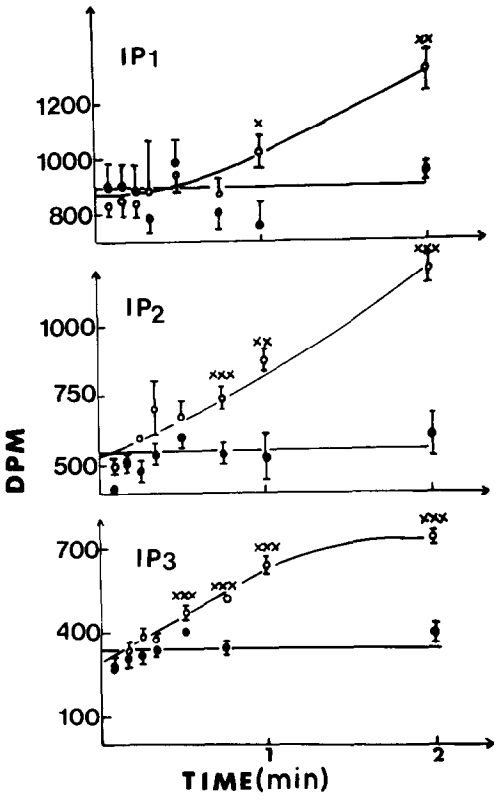

Fig.2. Time course of MTX-induced IP_1, IP_2, and IP_3 formation in the absence of LiCl. 10^{-8} g MTX (○) or buffer (●) was added at time 0 and the formation of IP_1, IP_2 and IP_3 was measured at the indicated time points after the MTX addition. Values were significantly different (x, $p \leq 0.05$; xx, $p \leq 0.01$; xxx, $p \leq 0.005$) from control as determined by Student's t-test.

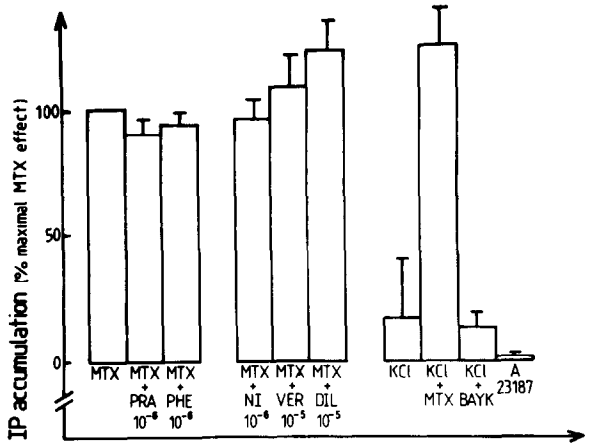

Fig.3. Effect of α-adrenergic and Ca^{2+} channel antagonists on MTX-induced IP formation and the effect of Bay-K 8644 and A23187 on the level of IP. Cells were incubated with prazosin (PRA), phentolamine (PHE), nifedipine (NI), diltiazem (DIL) and verapamil (VER) at the indicated concentrations, 15 min prior to stimulation with 10^{-8} g/ml of MTX. The formation of IPs was measured as described in section 2. Results are the means of 3 independent experiments and are expressed as the percentage of the response induced by 10^{-8} g MTX. 10^{-6} M Bay-K 8644 and 2×10^{-6} M A23187 were added after preincubation of the cells with LiCl. The experiments with Bay-K 8644 were done in a high K^+ (15 mM) PBS buffer. At this potassium concentration, 10^{-6} M Bay-K 8644 was shown to induce a maximal contraction of the isolated rabbit aorta [20]. Results are the means, expressed as a percentage of the response induced by 10^{-8} g/ml MTX, ± SE of at least 3 independent experiments on separate culture preparations, performed in triplicates.

equivalent to or greater than the rates of MTX-induced Ca^{2+} uptake reported in various cell types [8,10,18].

The hypothesis that MTX stimulates the opening of Ca^{2+} channels is based mainly on the fact that Ca^{2+} channel antagonists can inhibit the MTX-induced responses [4,8,11]. However, in our system neither nifedipine (10^{-6} M), diltiazem (10^{-5} M) nor verapamil (10^{-5} M) significantly depressed the MTX-stimulated IP response (fig.3). Furthermore, neither the Ca^{2+} channel agonist Bay-K 8644 nor the Ca^{2+} ionophore A23187 was able to mimic this action of MTX. Our results obtained with drugs acting on VSCCs argue against the possibility that the MTX-induced IP formation is a consequence of Ca^{2+} influx through VSCCs. Moreover, the A23187 data suggest that this response is not consequent to an increase in intracellular Ca^{2+} levels due to other unforeseen effects of MTX on the Ca^{2+} permeability of the plasma membrane. As already studied in model systems [9], MTX does not present any ionophoretic activity. Therefore, it is unlikely that the IP stimulation is a consequence of Ca^{2+} entry in the cell. However, it is uncertain whether the Ca^{2+} influx is due to the stimulation of IPs or if the two events are independent.

The fact that Ca^{2+} channel blockers inhibit the MTX-induced contractile response in smooth muscle preparations [4,8] does not prove that MTX interacts directly with Ca^{2+} channels. In fact, the MTX-induced contraction in these tissues may

result from its action at more than one locus. We demonstrate here that one effect of MTX might be the direct stimulation of IP formation. Based on the data presented here it is reasonable to hypothesize that one way MTX may stimulate smooth muscle contraction is via the triggering of IP formation.

However, it has previously been found that 10^{-6} M phentolamine (PHE) [4] and 10^{-6} M prazosin (PRA) (N. Vidal and J. Haiech, unpublished), both α-adrenergic antagonists, partly inhibit the MTX-induced arterial contractile response. The same concentration of these drugs did not affect the IP formation in our system (fig.3). One explanation for this difference is that the fraction of the contractile response of rabbit aorta blockable by α-adrenergic antagonists could be due to noradrenaline release from remaining nerve endings in this tissue preparation.

Earlier work [19], done with α-latrotoxine, gave similar results and also favoured the hypothesis that such toxins may act on intracellular Ca^{2+} concentration through stimulation of IP metabolism.

MTX appears to bind on a finite number of high affinity sites, the nature of which will have to be defined in order to understand better the mechanism of action of these toxins.

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REFERENCES

[1] Baylet, R., Beccaria, C., Niaussat, P.M., Boyer, F. and Guillaut, M. (1978) Pathol. Biol. 26, 95–97.
[2] Bidard, J.-N., Vijverberg, H.P.M., Frelin, C., Chungue, E., Legrand, A.-M., Bagnis, R. and Lazdunski, M. (1984) J. Biol. Chem. 259, 8353–8357.
[3] Miyahara, J.T., Akau, C.K. and Yasumoto, T. (1979) Res. Commun. Chem. Pathol. Pharmacol. 25, 177–180.
[4] Ohizumi, Y. and Yasumoto, T. (1983) J. Physiol. 337, 711–721.
[5] Miyamoto, T., Ohizumi, Y., Washio, H. and Yasumoto, Y. (1984) Pflügers Arch. 400, 439–441.
[6] Legrand, A.M. and Bagnis, R. (1984) Toxicon 22, 471–475.
[7] Ohizumi, Y. and Yasumoto, T. (1983) Br. J. Pharmac. 79, 3–5.
[8] Takahashi, M., Ohizumi, Y. and Yasumoto, T. (1982) J. Biol. Chem. 257, 7287–7289.
[9] Takahashi, M., Tatsumi, M., Ohizumi, Y. and Yasumoto, T. (1983) J. Biol. Chem. 258, 10944–10949.
[10] Schettini, G., Koike, K., Login, I.S., Judd, A.M., Cronin, M.J., Yasumoto, T. and Macleod, R.M. (1984) Am. J. Physiol. 247, E520–E525.
[11] Login, I.S., Judd, A.M., Cronin, M.J., Koike, K., Schettini, G., Yasumoto, T. and Macleod, R.M. (1985) Endocrinology 116, 622–627.
[12] Berridge, M.J. and Irvine, R.F. (1984) Nature 312, 315–321.
[13] Whitaker, M. and Irvine, R.F. (1984) Nature 312, 636–639.
[14] Picard, A., Giraud, F., Le Bouffant, F., Sladeczek, F., Le Peuch, C. and Dorée, M. (1985) FEBS Lett. 182, 446–450.
[15] Bucher, B., Travo, P., Laurent, T.P. and Stoclet, J.C. (1982) Cell Biol. Int. Rep. 6, 113–123.
[16] Bone, E.A., Fretten, P., Palmer, S., Kirk, C.J. and Michell, R.M. (1984) Biochem. J. 221, 803–811.
[17] Nishizuka, Y. (1984) Nature 308, 693–698.
[18] Freedman, S.B., Miller, R.J., Miller, D.M. and Tindall, D.R. (1984) Proc. Natl. Acad. Sci. USA 81, 4582–4585.
[19] Vicentini, L.M. and Meldolesi, J. (1984) Biochem. Biophys. Res. Commun. 121, 538–544.
[20] Schramm, M., Thomas, G., Torwart, R. and Franckoviak, G. (1983) Nature 303, 535–537.