Biochemical Pharmacology, Vol. 39, No. 11, pp. 1633–1639, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00
Pergamon Press plc

COMMENTARY

MAITOTOXIN: A UNIQUE PHARMACOLOGICAL TOOL FOR RESEARCH ON CALCIUM-DEPENDENT MECHANISMS

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Maitotoxin (MTX), isolated from the marine dinoflagellate *Gambierdiscus toxicus*, is a water-soluble polyether with a molecular weight of 3424 as the disodium salt [1]. MTX would appear to contain a linear chain over 100 carbons in length; the chain contains four double bonds, many small ether rings, twenty-one methyl groups, and numerous tertiary hydroxyl groups. In addition, there are two sulfate esters and apparently a basic nitrogen. Thus, MTX is a highly polar substance that would not be expected to cross membrane lipid bilayers. The presence of the two sulfate ester groups appears essential to biological activity. Although the chemical structure is not elucidated completely, analogies to another potent marine toxin, palytoxin [2], have been noted [1]. MTX is a very toxic principle with an LD_{50} in mice of 0.17 μg/mL. Toxicity to cells after MTX administration has been reported for several tissues [3, 4], and the toxic effects are presumed due to influx of calcium. Such toxicity may complicate biochemical studies with cultured cells. Consonant with the high *in vivo* toxicity, pharmacological effects of MTX on cells and tissues are elicited in a dose-dependent manner at concentrations from 100 pM to 30 nM (0.3 to 100 ng/mL). These effects are diverse ranging from stimulation of calcium uptake, neurotransmitter/hormone release and phosphoinositide breakdown to contraction of smooth and skeletal muscle and stimulant effects on the heart. All the effects of MTX appear to be markedly dependent on extracellular calcium. Whether this reflects a calcium-dependent interaction of MTX with extracellular sites or an obligatory transport or influx of calcium remains a challenging question.

Calcium

MTX increases calcium content and/or calcium flux in a wide range of cell types (Table 1). In neuroblastoma-glioma hybrid cells and certain pituitary tumor cells, dihydropyridines block the influence of $^{45}Ca^{2+}$ elicited by MTX, whereas in smooth muscle BC_3H_1 cells, aortic myocytes and synaptosomes, these L-channel blockers have no effect on MTX-elicited calcium influx. Other organic calcium channel blockers, such as verapamil, diltiazem and certain local anesthetics, inhibit MTX-elicited changes in internal calcium or calcium flux in some, but not all, systems. Inorganic blockers of calcium channels, such as cobalt, manganese and lanthanum, block MTX-elicited calcium flux in virtually all preparations. The results clearly demonstrate that low concentrations of MTX can lead to activation of dihydropyridine-sensitive L-type calcium channels and to activation of other calcium channels that are not sensitive to dihydropyridines. It remains possible that activation of L-type channels by MTX in some cells may yield toxin-altered channels that are not sensitive to dihydropyridines. The mechanisms involved in the effects of MTX on calcium channels are unknown. MTX has no effect on binding of nitrendipine to sites associated with L-type calcium channels [11]. In two preparations, namely neuroblastoma-glioma hybrid cells [11] and pancreatic islets [16], a dihydropyridine L-type channel activator further increases the stimulation of calcium flux elicited by MTX. Remarkably, in pituitary 235-1 tumor cells, the inhibitory effect of a dihydropyridine on MTX-elicited calcium flux is lost after treatment with pertussis toxin [13]. In smooth muscle BC_3H_1 cells the MTX-elicited calcium influx, which is insensitive to dihydropyridines, is reduced greatly at temperatures of 20° or less and when the pH is reduced to 6 [10]. Interestingly, MTX increases intracellular levels of sodium in BC_3H_1 cells, while intracellular potassium is decreased. It is possible that such increases in internal sodium occur through MTX-activated calcium channels or indirectly through Na^+/Ca^{2+} exchange and that decreases in potassium reflect calcium-activation of potassium channels. Other studies have indicated that MTX has no effect on sodium flux in neuroblastoma-glioma hybrid cells [11] or on potassium flux in pancreatic islets [16]. In synaptosomes, MTX-elicited influx of $^{45}Ca^{2+}$ is augmented by dibytyryl cyclic AMP and by pertussis toxin [19, 20]. In 3T3 fibroblasts, MTX at 15–30 nM was reported to have *no* effect on $^{45}Ca^{2+}$ influx [11]. This is the only report of a cell system in which MTX does not increase calcium influx. Thus, MTX represents a rather general activator for calcium influx into cells, perhaps by interaction with high-affinity extracellular sites associated with calcium channels. Whether such sites are associated with all or only certain calcium channels is unknown. It is possible that L-type calcium channels and other voltage-dependent calcium channels are not acti-

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Table 1. Effects of maitotoxin on calcium, hormone/neurotransmitter release, phospholipid metabolism and muscle function

Preparation	Assay	Effective concentration of MTX (nM)	Inhibition by calcium channel blockers: Organic: DHP	Inhibition by calcium channel blockers: Organic: Other	Inhibition by calcium channel blockers: Inorganic	Effects of ionophores	Ref.
(A) Calcium							
Muscle (vas deferens, aorta, atria)	Ca^{2+} content	0.3–10	—	Y	Y (Co^{2+})	—	[3, 5–7]
Pheochromocytoma PC12 cells	$^{45}Ca^{2+}$	3	—	Y	Y (Mn^{2+})	—	[8, 9]
Smooth muscle BC_3H^1 cells	$^{45}Ca^{2+}$	1–3	N	N	Y (Zn^{2+},Co^{2+},Mn^{2+}) N (La^{3+})	—	[10]
Neuroblastoma-glioma hybrid cells	$^{45}Ca^{2+}$	3–30	Y	Y	Y (Cd^{2+})	—	[11]
Fibroblasts 3T3 cells	$^{45}Ca^{2+}$	Inactive	—	—	—	—	[11]
Pituitary GH_3 cells	$^{45}Ca^{2+}$	12	—	—	—	—	[12]
Pituitary 235-1 cells	Quin-2	0.3–3	Y	Y	—	—	[13]
Anterior pituitary cells	$^{45}Ca^{2+}$, Fura-2	0.3–12	—	—	—	—	[12, 14, 15]
Pancreatic islets	$^{45}Ca^{2+}$	0.01–0.3	—	—	—	—	[16]
Striatal neurons	$^{45}Ca^{2+}$	1	—	—	Y (Co^{2+})	—	[17, 18]
Synaptosomes	$^{45}Ca^{2+}$, Quin-2	30	N	—	Y (Co^{2+})	—	[19, 20]
Aortic myocytes	Fura-2	0.03–3	N	N	—	Y	[21, 22]
Frog oocytes	$^{45}Ca^{2+}$	3–10	—	Y	Y (La^{3+})	—	[23]
Cardiac myocytes	$^{45}Ca^{2+}$, Quin-2	0.3	—	—	—	—	[24]
Cardiac myocytes	Currents	0.3–1	—	—	Y (Cd^{2+})	—	[24, 25]
Neuroblastoma N1E-115 cells	Currents	3	—	Y	Y (La^{3+})	—	[26]
Heart	Action potentials	0.2–0.4	—	Y	Y (Mn^{2+})	—	[27]
Insect muscle	Action potentials	1.5–3	—	—	Y (Co^{2+})	—	[28]
HL-60 cells	Fura-2	0.3	—	—	—	Y	[29]
Parathyroid cells	$^{45}Ca^{2+}$	1.5	—	—	—	—	[30]
(B) Hormone/neurotransmitter release							
Pheochromocytoma PC12 cells	Norepinephrine	0.3–300	Y	Y	Y (Mn^{2+})	M	[8, 9]
Pheochromocytoma PC12 cells	ATP	0.6–6	Y	—	—	—	[31]
Anterior pituitary cells	LH, FSH, etc.	0.3–15	N	Y/N	Y (Mn^{2+})	—	[12, 15, 32]
Pancreatic islets	Insulin	0.01–0.3	—	—	—	—	[16]
Striatal neurons	GABA	0.03–3000	N	N	Y (Co^{2+}, Cd^{2+})	—	[17]
Phrenic nerve-diaphragm	Acetylcholine	0.3–3	—	—	Y (Co^{2+})	—	[33]
Vas deferens	Norepinephrine	1–10	—	Y	—	—	[7]
Parathyroid cells	Parathyroid hormone	0.03–1.5	—	—	—	—	[30]

Table 1—continued

Preparation	Assay	Effective concentration of MTX (nM)	Inhibition by calcium channel blockers: Organic: DHP	Organic: Other	Inorganic	Effects of ionophores	Ref.
(C) Phospholipid metabolism							
Pheochromocytoma							
PC12 cells	IP_3,IP_2,IP	0.03–0.6	N	N	N (La^{3+},Co^{2+},Mn^{2+}) Y (Cd^{2+})	N	[31, 34, 35]
	Arachidonate		N	N	N (Co^{2+}, Mn^{2+}) Y (Cd^{2+})	N	[36]
Aortic myocytes	IP_3,IP_2,IP	0.03–3	N	N	—	N	[21, 22]
Muscle BC_3H_1 cells	IP_3,IP_2,IP	0.3–3	—	—	—	—	[10]
Neuroblastoma hybrid NCB-20 cells	IP_3,IP_2,IP	0.15–1.5	N	N	N (Co^{2+},La^{3+},Cd^{2+},Mn^{2+})	M	[34, 35, 37]
Glioma C6, Kidney P23, fibroblast, RBL2H3, HL-60 and WRK cells, pituitary cells, astrocytes, striatal neurons	IP_3,IP_2,IP	0.15–3	—	—	—	—	[10, 29, 34]
Frog oocytes	PIP_2	30	—	—	—	—	[23]
Synaptoneurosomes	IP	0.15	—	—	—	—	[34]
(D) Muscle function and other functions							
Atria	Rate/force	0.3–10	—	Y	Y (Co^{2+},Mn^{2+})	—	[3, 24, 38–40]
Aorta	Contractions	0.03–3	—	Y	—	Y	[5]
Vas deferens	Contractions	1–10	—	Y	—	—	[7]
Mesenteric artery	Contractions	0.3	—	—	—	—	[11]
Duodenum	Contractions	0.3–5	—	—	—	—	[6, 38]
Cardiac myocytes	Rate/force	0.1–3	—	Y	—	—	[4, 24]
Frog oocytes	Cortical reaction	3–10	—	—	—	—	[23]

Preparations were from mammalian sources unless otherwise indicated. Abbreviations: DHP = dihydropyridine; LH, luteinizing hormone; FSH, follicle-stimulating hormone; IP, inositol phosphate; IP_2, inositol bis-phosphate; IP_3, inositol tris-phosphate; PIP_2, phosphatidyl inositol bis-phosphate; N, no inhibition or effect; Y, yes, inhibition or effect; and M, marginal effect.

vated directly by MTX, but open only as a result of depolarization caused by influx of calcium through another, ubiquitous, MTX-sensitive calcium channel. MTX lacks ionophoretic activity in studies with liposomes and mitochondria [9]. In addition, the highly polar structure of MTX should prevent passage across cell membranes, restricting its site of action to cell surfaces. The activation of calcium influx by MTX in virtually all cell types complicate any interpretation of other MTX-elicited alterations in cellular processes, since such alterations may only reflect the sequelae to MTX-elicited influx of calcium.

Electrophysiological studies with MTX have resulted in somewhat inconclusive results with regard to the nature of the calcium channels activated by MTX. In guinea pig cardiac myocytes, MTX sustains an inward depolarizing current that could be abolished by cadmium and enhanced by epinephrine [25]. No data were provided with respect to blockade by dihydrophyridines. It was proposed that MTX activates in these cells a new class of "voltage-independent calcium channels." The MTX-stimulated calcium currents in neuroblastoma cells are prevented by the addition of verapamil or lanthanum [26]. However, the characteristics of the currents induced by MTX were different from the voltage-dependent calcium currents. It was proposed that MTX may "create a pore in the membrane with pharmacological properties similar to those of calcium channels." Palytoxin, a structurally related potent marine toxin, has been proposed to convert Na^+/K^+ ATPase to a cation channel with selectivity for sodium or to aggregate in the membrane to form a sodium channel or pore (see Ref. 2). In rat heart, MTX increased the duration of action potentials, apparently through effects on calcium channels sensitive to blockade by verapamil or manganese [24, 27].

Neurotransmitter/hormone release

The increase in calcium influx elicited by MTX in pheochromocytoma PC12 cells, striatal neurons and pancreatic islets is accompanied by an increase in neurotransmitter/hormone release [8, 9, 16, 17]. The MTX-elicited release of norepinephrine/ATP in PC12 cells is sensitive to blockade by dihydropyridines [8, 9, 31] as is the MTX-elicited influx of $^{45}Ca^{2+}$ [36]. However, influx of $^{45}Ca^{2+}$ elicited by MTX is only reduced 50% by 1 μM nifedipine [36]. In contrast to the marked stimulation by MTX of norepinephrine release from PC12 cells, a calcium ionophore has only minimal effects on release. In striatal neurons, cobalt ions completely block MTX-elicited calcium flux, while only partially reducing MTX-elicited release of γ-aminobutyric acid (GABA), suggesting that increases in calcium influx are not obligatory to the effects of MTX on neurotransmitter release in this system [18]. However, the MTX-elicited release of neurotransmitter/hormone in all systems (Table 1) has been dependent to some extent on extracellular calcium. In medium with low sodium, the MTX-elicited release of norepinephrine from PC12 cells [8] or vas deferens [7] or of GABA from striatal neurons [18] is reduced markedly. The mechanism underlying this dependency on extracellular sodium is unclear, but may reflect a reduced ability of the cells to lower intracellular levels of calcium through the Na^+/Ca^{2+} exchange pathway in low sodium medium. In anterior pituitary cells MTX appears to enhance hormone release through activation of a calcium channel that also can be activated by gonadotropin-releasing hormone (GnRH) [32].

In contrast to the relatively short time for MTX actions in most preparations, usually 1–5 min to observe maximal effects, the MTX-elicited activation of spontaneous release of acetylcholine at the neuromuscular junction is apparent only after 25–40 min of incubation. The presence of tetrodotoxin greatly delays the onset of spontaneous release in this preparation [33].

The data on MTX-stimulated release of neurotransmitters and hormones strongly suggest a correlation with MTX-stimulated influx of calcium through either L-type channels or other channels. The partial dependence of the stimulation of release by MTX on sodium in some systems remains to be explained. The *very slow* time course in neuromuscular preparations and the delay by tetrodotoxin, a specific blocker of voltage-dependent sodium channels, are uncharacteristic of the effects of MTX in other systems.

In parathyroid cells, MTX *inhibits* release of parathyroid hormone and further enhances the inhibitory effect of a dihydropyridine-activator of L-type calcium channels [30]. The inhibitory effect of MTX on hormone release can be antagonized by dihydropyridine calcium channel blockers. In this system, MTX-elicited calcium influx clearly leads to *inhibition* of hormone release, as do other agents that increase intracellular calcium.

Phospholipid metabolism

MTX has been shown recently to cause remarkable stimulations of phosphoinositide breakdown in a variety of preparations (Table 1). This effect results in the generation of two second messengers: inositol 1,4,5-tris-phosphate, which induces the release of intracellular calcium, and diacylglycerol, which stimulates protein kinase C. MTX elicits the formation of inositol phosphates in rat aortic myocytes [21, 22], neuroblastoma hybrid NCB-20 cells [34, 35, 37], rat pheochromocytoma PC12 cells [31, 34, 35] and a wide variety of other cell types [10, 34]. Similar to results on release of neurotransmitters/hormones, the stimulatory effects of MTX on phosphoinositide breakdown are abolished in the absence of extracellular calcium [10, 22, 34, 35, 37]. However, the MTX-induced stimulation of phosphoinositide breakdown in almost all cases is unaffected by calcium channel blockers, either organic or inorganic [21, 31, 35, 37]. As yet, the sole exception appears to be the inhibition by cadmium and high concentrations of manganese of MTX-elicited phosphoinositide breakdown in PC12 cells [36]. In PC12 cells, where MTX-triggered release of catecholamines is antagonized by organic calcium channel blockers [8, 9], MTX-induced phosphoinositide breakdown is not affected by nifedipine and is stimulated at lower concentrations of MTX than those required to stimulate neurotransmitter release [31]. And in rat aortic myocytes

MTX stimulates calcium influx with an EC_{50} of about 300 pM, while stimulation of phosphoinositide breakdown occurs with an EC_{50} of about 80 pM [22]. Thus, either direct effects of MTX on calcium channels do not mediate the effects of MTX on phosphoinositide breakdown stimulation, or only a small influx of calcium through MTX-stimulated channels is necessary to fully activate phosphoinositide breakdown.

In HL-60 cells MTX-elicited phosphoinositide breakdown, but not that elicited by a chemotactic-peptide, is dependent on extracellular calcium [34]. In addition, the MTX-elicited stimulation of phosphoinositide breakdown is not sensitive to pertussis toxin, while chemotactic peptide-elicited phosphoinositide breakdown is inhibited by pretreatment with pertussis toxin [34]. Thus, at least in this cell line, MTX clearly affects phosphoinositide breakdown through a mechanism different from receptor-based mechanisms, both with respect to calcium-dependency and with respect to involvement of a pertussis toxin-sensitive guanyl nucleotide-binding protein. MTX does not appear to augment receptor-mediated stimulation of phosphoinositide breakdown [34]. Instead, the responses appear to be nearly additive.

Since MTX appears to induce phosphoinositide breakdown in every cell system tested [10, 34] and in each case the responses appear to be dependent on calcium, it is probable that the formation of inositol phosphates is secondary to calcium influx. However, while calcium channel blockers often prevent effects of MTX on calcium influx, such blockers virtually never prevent MTX-elicited stimulation of phosphoinositide breakdown. It might be proposed that MTX is an incredibly potent calcium ionophore, but that seems very unlikely. Indeed, MTX has been shown to lack ionophoretic activity [9]. Furthermore, established calcium ionophores, such as A23187 and ionomycin, have in several cells in which MTX markedly stimulates phosphoinositide breakdown no or only marginally effects on phosphoinositide breakdown [21, 29]. Because of its size and polar nature, it is unlikely that MTX will penetrate into cells, suggesting that its site of action is extracellular. As yet MTX has not been reported to have any effects on phosphoinositide breakdown in membrane preparations or in permeabilized cells [29]. An attractive hypothesis is that MTX interacts with high affinity with membrane component(s) that are part of calcium uptake mechanisms present in membranes of all cells. Such calcium uptake systems, must be closely associated to phospholipase C, the enzyme responsible for inositol phosphate formation, and do not seem to be sensitive to blockade by organic or inorganic calcium channel antagonists. Recently Bernard *et al.* [23] demonstrated that in frog oocytes MTX triggers phosphoinositide breakdown in a calcium-dependent manner. However, if the oocytes are microinjected with the calcium chelator ethyleneglycolbis (amino ethylether)tetra-acetate (EGTA), the response persists, suggesting that in this system calcium is an extracellular and not an intracellular requirement for MTX-induced phosphoinositide breakdown. Similarly, in HL-60 cells, pretreatment with 1,2-bis(σ-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA), which represents an intracellular calcium chelator, does not prevent stimulation of phosphoinositide breakdown by MTX [29]. These results raise the possibilities either that MTX requires calcium to bind to an extracellular site of action or that activation of phospholipase C by calcium occurs at an extracellular site or at a site within the plasma membrane. In PC12 cells, the presence of a calcium ionophore markedly inhibits the MTX-elicited stimulation of phosphoinositide breakdown [36]. Such inhibition of an MTX response by a calcium ionophore may be related to the biphasic effects of MTX on phosphoinositide breakdown. Thus, in most studies, MTX elicits a maximal stimulation of phosphoinositide breakdown at a concentration of 100–300 pM. At high concentrations the response to MTX declines and effects on phosphoinositide breakdown are virtually undetectable at concentrations >1 nM [31, 36, 37].

Muscle function

The effects of MTX on smooth and cardiac muscle were first described in the late seventies and early eighties: MTX induces contraction of a variety of isolated muscle preparations including rabbit aorta [5] intestinal smooth muscle [6], rabbit duodenum [38], guinea pig vas deferens [7], guinea pig and rat cardiac muscle [4, 24, 39, 40] and rat atria [27, 38]. The effects of MTX on muscle are suppressed in calcium-free solutions or by means of calcium channel antagonists. Thus, calcium entry seems to be involved in these physiological effects of MTX. However, since MTX elicits the formation of IP_3, internal release of calcium may also be involved.

Prospectus

Clearly, MTX is a unique and extremely potent substance that provides an extraordinary tool for pharmacological research on calcium and calcium-dependent mechanisms. Stimulations of L-type and non-L-type calcium channels by MTX have been characterized biochemically, but there is as yet no electrophysiological evidence, clearly demonstrating the activation of a voltage-dependent calcium channel by MTX. MTX-stimulated influx of calcium occurs relatively slowly ($T_{1/2} > 1$ min) and is sustained. This is in contrast to calcium ionophores, such as ionomycin and A23187, that cause almost instantaneous elevation in internal calcium. The stimulatory effects of MTX on neurotransmitter/hormone release appear dependent on activation of calcium channels, but the partial dependence on sodium ions remains unclear. The stimulatory effects of MTX on phosphoinositide breakdown appear to be elicited via a mechanism that either is independent of activation of calcium channels or requires only a very small influx of calcium. Certainly, stimulation of inositol phosphate formation by MTX is dependent on the presence of extracellular calcium. But stimulation of phosphoinositide breakdown by MTX may not require an increase in intracellular calcium.

The stimulation of phosphoinositide breakdown by MTX is seen in every system tested and, thus, MTX represents an invaluable tool for investigation of the effects of stimulation of phosphoinositide breakdown. In many cells receptors have no or only

minimal stimulatory effects on phospholipase C. In such cells MTX can be used to induce a profound stimulation of phosphoinositide breakdown to probe the sequelae of such stimulation. In a recent study in PC12 and NCB-20 cells we used MTX to examine the question as to whether MTX stimulation of phosphoinositide breakdown would, through formation of diacyglycerides, have the same effects on cyclic AMP-generating systems as phorbol esters [35]. Phorbol esters, presumably through the activation of protein kinase C, had been shown to augment responsiveness of cyclic AMP-generating systems in PC12 cells [41], while inhibiting receptor-mediated responses of cyclic AMP-generating systems in NCB-20 cells [42]. The results with MTX were consonant with predictions and represent the first use of MTX as a tool to probe the sequelae to stimulation of phosphoinositide breakdown by this toxin in intact cells. MTX caused an activation of protein kinase C in both PC12 and NCB-20 cells, presumably through diacylglycerides generated by MTX-induced activation of phospholipase C [35]. In the PC12 cells, responsiveness of cyclic AMP systems was augmented, while in the NCB-20 cells responses were inhibited, as had been the case with phorbol esters. MTX alone has no effects on cyclic AMP accumulation in PC12 or NCB-20 cells. MTX also has been used to investigate effects on cyclic GMP accumulations in PC12 cells [43]. In these cells neither MTX nor high concentrations of potassium ions have any effect on basal levels of cyclic GMP. However, MTX inhibits atrial natriuretic factor-mediated, but not sodium nitroprusside-mediated, accumulation of cyclic GMP. The inhibition of atrial natriuretic factor response appears due to stimulation of phosphoinositide breakdown by MTX and subsequent diacylglycerol formation, since phorbol esters induce similar inhibitory effects [43]. In addition, to being used to probe sequelae to phosphoinositide breakdown in various cells, MTX through a general stimulation of phosphoinositide breakdown provides a means to study possible inhibitory inputs to activated phospholipase C systems.

The effects of MTX on release of arachidonic acid from [^{3}H]arachidonate-labeled phospholipids also have been investigated in PC12 cells [36]. MTX appears somewhat more potent in stimulating arachidonate release than in stimulating breakdown of phosphoinositides. The effect of MTX on arachidonate is dependent on extracellular calcium and is blocked by cobalt, manganese and cadmium ions, while the effect on MTX on phosphoinositide breakdown is blocked effectively only by cadmium ions. In contrast to the inhibitory effect of a calcium ionophore on MTX-elicited phosphoinositide breakdown, the ionophore markedly enhances MTX-elicited arachidonate release [36]. The results suggest some differences in mechanisms whereby MTX stimulates phospholipase C and phospholipase A_2 systems in PC12 cells. But clearly further studies on the effects of MTX on phospholipid metabolism are needed.

Recently, two peptides, endothelin and sarafotoxin, were described as having actions on calcium movements in cells. Endothelin is an endogenous peptide found in mammals, which activates calcium channels and phosphoinositide breakdown in vascular smooth muscle [44]. Sarafotoxin S6b, found in the venom of the burrowing asp *Atractaspis engaddensis*, stimulates phosphoinositide breakdown in brain and heart preparations [45]. Sarafotoxin interacts with endothelin-binding sites as shown by displacement of radioactive ligands [46]. Whether a similar mechanism to that seen with MTX is involved in the action of these peptides remains to be elucidated.

Further research is needed to provide insights in the mechanisms underlying the potent effects of MTX on calcium transport and phospholipid metabolism and to establish the general usefulness of this toxin as a pharmacological tool. An important but difficult goal is the preparation of a radiolabeled derivative to establish the nature and distribution of membrane-binding sites for MTX.

Dedication

This review is dedicated to Professor Takeshi Yasumoto, whose work on marine toxins led him to the discovery of maitotoxin. Professor Yasumoto has played a major role in fostering pharmacological investigations of MTX, and is now meeting the difficult challenge of complete structure elucidation of this complex macromolecule.

REFERENCES

1. Yokoyama A, Murata M, Oshima Y, Iwashita T. and Yasumoto T, Some chemical properties of maitotoxin, a putative calcium channel agonist isolated from a marine dinoflagellate. *J Biochem (Tokyo)* **104**: 184–187, 1988.
2. Abrahim AR and Shier WT, Palytoxin: mechanism of action of a potent marine toxin. *J Toxicol Toxin Rev* **6**: 159–187, 1987.
3. Kobayashi M, Kondo S, Yasumoto T and Ohizumi Y, Cardiotoxic effects of maitotoxin, a principal toxin of seafood poisoning, on guinea pig and rat cardiac muscle. *J Pharmacol Exp Ther* **238**: 1077–1083, 1986.
4. Terao K, Ito E, Sakamaki Y, Igarashi K, Yokoyama A and Yasumoto T, Histopathological studies of experimental marine toxin poisoning. II. The acute effects of maitotoxin on the stomach, heart and lymphoid tissues in mice and rats. *Toxicon* **26**: 395–402, 1988.
5. Ohizumi Y and Yasumoto Y, Contractile response of the rabbit aorta to maitotoxin, the most potent marine toxin. *J Physiol (Lond)* **337**: 711–721, 1983.
6. Ohizumi Y and Yasumoto Y, Contraction and increase in tissue calcium content induced by maitotoxin, the most potent known marine toxin, in intestinal smooth muscle. *Br J Pharmacol* **79**: 3–5, 1983.
7. Ohizumi Y, Kajiwara A and Yasumoto Y, Excitatory effect of the most potent marine toxin, maitotoxin, on the guinea pig vas deferens. *J Pharmacol Exp Ther* **227**: 199–204, 1983.
8. Takahashi M, Ohizumi Y and Yasumoto T, Maitotoxin, a Ca^{2+} channel activator candidate. *J Biol Chem* **257**: 7287–7289, 1982.
9. Takahashi M, Tatsumi M, Ohizumi Y and Yasumoto T, Ca^{2+} channel activating function of maitotoxin, the most potent marine toxin known, in clonal rat pheochromocytoma cells. *J Biol Chem* **258**: 10944–10949, 1983.
10. Sladeczek F, Schmidt BH, Alonso R, Vian L, Tep A, Yasumoto T, Cory RN and Bockaert J, New insights into maitotoxin action. *Eur J Biochem* **174**: 663–670, 1988.

11. Freedman SB, Miller RJ, Miller DM and Tindall DR, Interactions of maitotoxin with voltage-sensitive calcium channels in cultured neuronal cells. *Proc Natl Acad Sci USA* **81**: 4582–4585, 1984.
12. Login IS, Judd AM and MacLeod RM, Activation of calcium channels by maitotoxin. *Methods Enzymol* **141**: 63–79, 1987.
13. Schettini G, Meucci O, Florio T, Grimaldi M, Landolfi E, Magri G and Yasumoto T, Pertussis toxin pretreatment abolishes dihydropyridine inhibition of calcium flux in the 235-1 pituitary cell line. *Biochem Biophys Res Commun* **151**: 361–369, 1988.
14. Anderson JM, Yasumoto T and Cronin MJ, Intracellular free calcium in rat anterior pituitary cells monitored by fura-2. *Life Sci* **41**: 519–526, 1987.
15. Schettini G, Koike K, Login IS, Judd AM, Cronin MJ, Yasumoto T and MacLeod RM, Maitotoxin stimulates hormonal release and calcium flux in rat anterior pituitary cells *in vitro*. *Am J Physiol* **247**: E520–E525, 1984.
16. Lebrun P, Hermann M, Yasumoto T and Herchuelz A, Effects of maitotoxin on ionic and secretory events in rat pancreatic islets. *Biochem Biophys Res Commun* **144**: 172–177, 1987.
17. Shalaby IA, Kongsamut S and Miller RJ, Maitotoxin-induced release of γ-[^{3}H]aminobutyric acid from cultures of striatal neurons. *J Neurochem* **46**: 1161–1165, 1986.
18. Pin J-P., Yasumoto T and Bockaert J, Maitotoxin-evoked γ-aminobutyric acid release is due not only to the opening of calcium channels. *J Neurochem* **50**: 1227–1232, 1988.
19. Ueda H, Tamura S, Fukushima N and Takagi H, Pertussis toxin (IAP) enhances maitotoxin (a putative Ca^{2+} channel agonist)-induced Ca^{2+} entry into synaptosomes. *Eur J Pharmacol* **122**: 379–380, 1986.
20. Ueda H, Tamura S, Harada H, Yasumoto T and Takagi H, The maitotoxin-evoked Ca^{2+} entry into synaptosomes is enhanced by cholera toxin. *Neurosci Lett* **67**: 141–146, 1986.
21. Berta P, Sladeczek F, Derancourt J, Durand M, Travo P and Haiech J, Maitotoxin stimulates the formation of inositol phosphates in rat aortic myocytes. *FEBS Lett* **197**: 349–352, 1986.
22. Berta P, Phaneuf S, Derancourt J, Casanova J, Durand-Clement M, Le Peuch C, Haiech J and Cavadore J-C, The effects of maitotoxin on phosphoinositides and calcium metabolism in a primary culture of aortic smooth muscle cells. *Toxicon* **26**: 133–141, 1988.
23. Bernard V, Laurent A, Derancourt J, Clement-Durand M, Picard A, Le Peuch C, Berta P and Doree M, Maitotoxin triggers the cortical reaction and phosphatidylinositol-4,5-bisphosphate breakdown in amphibian oocytes. *Eur J Biochem* **174**: 655–662, 1988.
24. Kobayashi M, Goshima K, Ochi R and Ohizumi Y, Arrhythmogenic action of maitotoxin in guinea pig and rat cardiac muscle. *Eur J Pharmacol* **142**: 1–8, 1987.
25. Kobayashi M, Ochi R and Ohizumi Y, Maitotoxin-activated single calcium channels in guinea pig cardiac cells. *Br J Pharmacol* **92**: 665–671, 1987.
26. Yoshii M, Tsunoo A, Kuroda Y, Wu CH and Narahashi T, Maitotoxin-induced membrane current in neuroblastoma cells. *Brain Res* **424**: 119–125, 1987.
27. Legrand AM and Bagnis R, Effects of highly purified maitotoxin extracted from dinoflagellate *Gambierdiscus toxicus* on action potential of isolated rat heart. *J Mol Cell Cardiol* **16**: 663–666, 1984.
28. Miyamoto T, Ohizumi Y, Washio H and Yasumoto T, Potent excitatory effect of maitotoxin on Ca channels in the insect skeletal muscle. *Pflugers Arch* **400**: 439–441, 1984.
29. Gusovsky F, Bitran JA, Yasumoto T and Daly JW, Mechanism of maitotoxin-stimulated phosphoinositide breakdown in HL-60 cells. *J Pharmacol Exp Ther*, in press.
30. Fitzpatrick LA, Yasumoto T and Aurbach GD, Inhibition of parathyroid hormone release by maitotoxin, a calcium channel activator. *Endocrinology* **124**: 97–103, 1988.
31. Gusovsky F, Daly JW, Yasumoto T and Rojas E, Differential effects of maitotoxin on ATP secretion and on phosphoinositide breakdown in rat pheochromocytoma cells. *FEBS Lett* **233**: 139–142, 1988.
32. Conn PM, Staley DD, Yasumoto T, Huckle WR and Janovick JA, Homologous desensitization with gonadotrophin-releasing hormone (GnRH) also diminishes gonadotrope responsiveness to maitotoxin: a role for the GnRH receptor-regulated calcium ion channel in mediation of cellular desensitization. *Mol Endocrinol* **1**: 154–159, 1987.
33. Kim YI, Login IS and Yasumoto T, Maitotoxin activates quantal transmitter release at the neuromuscular junction: evidence for elevated intraterminal Ca^{2+} in the motor nerve terminal. *Brain Res* **346**: 357–362, 1985.
34. Gusovsky F, Yasumoto T and Daly JW, Maitotoxin, a potent, general activator of phosphoinositide breakdown. *FEBS Lett* **243**: 307–312, 1989.
35. Gusovsky F, Yasumoto T and Daly JW, Calcium-dependent effects of maitotoxin on phosphoinositide breakdown and on cyclic AMP accumulation in PC12 and NCB-20 cells. *Mol Pharmacol* **36**: 44–53, 1989.
36. Choi OH, Gusovsky F, Yasumoto T and Daly JW, Maitotoxin: effects on calcium channels, phosphoinositide breakdown and arachidonate release in pheochromocytoma PC12 cells. *Mol Pharmacol*, in press.
37. Gusovsky F, Yasumoto T and Daly JW, Maitotoxin stimulates phosphoinositide breakdown in neuroblastoma hybrid NCB-20 cells. *Cell Mol Neurobiol* **7**: 317–322, 1987.
38. Legrand AM and Bagnis R, Effects of ciguatoxin and maitotoxin on isolated rat atria and rabbit duodenum. *Toxicon* **22**: 471–475, 1984.
39. Kobayashi M, Miyakoda G, Nakamura T and Ohizumi Y, Ca-dependent arrhythmogenic effects of maitotoxin, the most potent marine toxin known, on isolated rat cardiac muscle cells. *Eur J Pharmacol* **111**: 121–123, 1985.
40. Kobayashi M, Ohizumi Y and Yasumoto Y, The mechanism of action of maitotoxin in relation to Ca^{2+} movements in guinea pig and rat cardiac muscles. *Br J Pharmacol* **86**: 385–391, 1985.
41. Hollingsworth EB, Ukena D and Daly JW, The protein kinase C activator phorbol-12-myristate-13-acetate enhances cyclic AMP accumulation in pheochromocytoma cells. *FEBS Lett* **196**: 131–134, 1986.
42. Hollingsworth EB and Daly JW, Inhibition of receptor-mediated stimulation of cyclic AMP accumulation in neuroblastoma-hybrid NCB-20 cells by a phorbol ester. *Biochim Biophys Acta* **930**: 272–278, 1987.
43. Schulick A, Gusovsky F, Yasumoto T and Daly JW, Maitotoxin inhibits atrial natriuretic peptide-induced cGMP accumulation in PC12 cells. *Life Sci*, in press.
44. Yanasigawa M and Masaki T, Endothelin, a novel endothelium-derived peptide. *Biochem Pharmacol* **38**: 1877–1883, 1989.
45. Kloog Y, Ambar I, Sokolovsky M, Kochva E, Wollberg Z and Bdolah A, Sarafotoxin, a novel vasoconstrictor peptide: phosphoinositide hydrolysis in rat heart and brain. *Science* **242**: 268–270, 1988.
46. Gu X, Casley DJ and Nayler WG, Sarafotoxin S6b displaces specifically bound ^{125}I-endothelin. *Eur J Pharmacol* **162**: 509–510 1989.