Critical Role of the Sodium Hydrogen Exchanger in Maitotoxin-Induced Neuronal Cell Death in Cultured Rat Cortical Neurons

Yushan Wang, M. Tracy Weiss, Catherine C. Tenn, Robert Frew, Cory Vair, and Thomas W. Sawyer
Casualty Management Section
Defence Research & Development Canada-Suffield
Medicine Hat, Alberta, Canada
Phone 1-403-544-4922, Fax 1-403-544-4714

Yushan.Wang@drdc-rddc.gc.ca

ABSTRACT

Maitotoxin (MTX) is one of the most potent toxins known to date. It causes massive calcium influx and necrotic cell death in various tissues. However, the exact mechanisms underlying its cellular toxicity are not fully understood. In the present study, the roles of extracellular acidosis and the sodium hydrogen exchanger (NHE) in MTX-induced increase in intracellular Ca^{2+} and subsequent cell death were investigated in cultured rat cortical neurons. Intracellular Ca^{2+} ($[Ca^{2+}]_i$) concentrations were measured fluorimetrically using fura-2 as a fluorescence indicator. Total cell death was measured with the alamarBlue™ cell viability assay and the vital dye ethidium bromide (EB) uptake assay. Results showed that MTX increased, in a concentration dependent manner, both $[Ca^{2+}]_i$ and cell death in cultured rat cortical neurons. Decreasing the pH of the treatment medium from 7.5 to 6.0 diminished MTX-induced cell death. The protection offered by lowering extracellular pH was not due to MTX degradation, because it was still effective even if the cells were treated with MTX in normal pH and then switched to pH 6.0. Pre-treatment of cells with the specific NHE inhibitor 5-(N-ethyl-N- isopropyl)-amiloride (EIPA) prevented MTX-induced increase in $[Ca^{2+}]_i$ as well as cell death in a concentration dependent manner. These results indicate that NHE is activated by MTX in cultured rat cortical neurons. Because EIPA offered complete protection against MTX-induced cell death, we conclude that NHE plays a major role in MTX neurotoxicity. NHE inhibitors may potentially be used to treat MTX induced neurotoxicity.

1.0 INTRODUCTION

Maitotoxin (MTX) is a potent marine toxin produced by the dinoflagellate *Gambierdiscus. Toxicus,* and is a principle toxin of ciguatera seafood poisoning (Yasumoto, 2001). Because of its extremely toxic nature, it has the potential to be employed as a chemical warfare agent by enemies in the battle field and/or terrorists. The toxin evokes massive calcium (Ca^{2+}) increase in the intracellulr space in both excitable and non excitable cells (Escobar et al., 1998; Cataldi et al., 1999). The increased cytosolic Ca^{2+} triggers a variety of responses, including phosphoinositide breakdown (Berta et al*.,* 1988*;* Gusovsky et al*.,* 1989), arachidonic acid release (Choi et al*.,* 1990), muscle contraction (Ohizumi and Yasumoto, 1983; Kobayashi *et al.,* 1985), and neurotransmitter release (Takahashi *et al.,* 1982; Kakizaki et al., 2006. Regardless of the down stream events after Ca^{2+} influx, MTX ultimately leads to necrosis in a variety of cell types, including bovine aortic endothelial cells (Estacion and Schilling, 2001); Chinese hamster ovary cells (Cataldi et al., 1999), murine macrophages (Verhoef et al., 2004); brain stem neurons (Kakizaki et al., 2006) and cultured rat hippocampal neurons (Zhao et al., 1999).

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Because of its ability to increase intracellular Ca^{2+} concentrations, MTX has been used as a unique pharmacological tool for research on calcium–dependent mechanisms and pathological mechanisms of necrosis for a long time (Takahashi *et al*., 1982; Gusovsky and Daly, 1990). However, the specific mechanisms by which MTX triggers Ca^{2+} entry and subsequent cytotoxicity have not been fully elucidated (Sorrentino et al., 1997; Yi et al., 2006; de la Rosa et al., 2007). Previous reports showed that MTX's actions involved the formation of a non-selective, cation channel that resembles the purinergic $P2X_7$ receptor and allows massive influx of Ca^{2+} (Schilling et al., 1999; Lundy et al., 2004). More recently, an MTX-induced cell death cascade has been proposed involving three sequential changes in membrane permeability: 1) activation of a non selective cation channel (NSCC) permeable mainly to Na^+ and K^+, but also Ca^{2+} and other divalent cations. The activation of NSCC causes a large increase in $[Ca^{2+}]_i$; 2) opening of a cytolytic/oncolytic pore that permits molecules < 800 Da (e.g., ethidium bromide) to enter the cell; and 3) formation of a glycine-sensitive cytolytic pore resulting in cell lysis and release of lactate dehydrogenase (Estacion *et al.,* 2003, Wisnoskey *et al.,* 2004). However, the nature of the NSCC and the exact mechanisms by which this channel is activated remains to be understood. Several studies have suggested the existence of a MTX receptor, which could act as an NSCC or serve as the first step to activate the NSCC (Lundy et al., 2004; de la Rosa et al., 2007).

The sodium hydrogen exchangers (NHE) are a group of membrane bound proteins that are important in regulating intracellular pH, cell volume, cell growth and differentiation (Putney et al., 2002). In the central nervous system (CNS), NHE activation has been reported to play important roles in ischemic injuries, stroke and other excitotoxic events (Yao and Haddad, 2004). NHE inhibitors have been shown to block glutamate induced intracellular Ca^{2+} increase and subsequent cell death in cultured rat cortical neurons (Matsumoto et al., 2004). Because MTX increases both intracellular Ca^{2+} and Na^+, it is reasonable to speculate that NHE may play a role in MTX-induced responses in the CNS. The current study was undertaken to examine the role of NHE in MTX induced increases in $[Ca^{2+}]_i$ and necrotic cell death in cultured rat cortical neurons.

2.0 MATERIALS AND METHODS

2.1 Materials

Female timed pregnant Sprague Dawley rats (Charles River, Montreal, QC, Canada) were maintained on a 12/12 hr light/dark cycle and had free access to food and water. In conducting this research the authors adhered to the "Guide to the Care and Use of Experimental Animals" and "The Ethics of Animal Experimentation" published by the Canadian Council on Animal Care. MTX was purchased from Wako (Richmond, VA). Ethylisopropylamiloride (EIPA) and ethidium bromide (EB) were purchased from Sigma-Aldrich (Saint Louis, MO) and dissolved in water. The initial stock solutions of MTX were made in methanol at a concentration of 10 mM and stored at -20ºC. FURA-2 AM ester was purchased from Invitrogen (Carlsbad, CA) and dissolved in water. All other chemicals were of chemical grade and purchased from Sigma-Aldrich.

2.2 Primary Culture of Rat Cortical Neurons

Dissociated cultures of cortical neurons were prepared from 17-19 day old rat embryos as described previously (Wang et al., 2004) with some modifications. Briefly, cortices were removed into ice cold Hank's Balanced Salt Solution (HBSS, Ca^{2+}, Mg^{2+} free, Invitrogen, Burlington, ON, Canada). The tissues were minced and trypsinized (0.25%, 10 min at 37^0C), titrated and plated on poly-D-lysine coated 12-well plates at a density of 10^6 cells per well. For microscopic studies, cells were plated onto 18-mm round poly-D-lysine coated coverslips (Harvard Apparatus, Quebec, Canada). For fluorimetric measurement of $[Ca^{2+}]_i$, cells were plated onto poly-D-lysine coated 28 × 12 mm rectangle glass coverslips at a density of

10^6 cell per cover slip. After plating, cells were grown in Neurobasal medium supplemented with B-27 and 0.5 mM glutamax (Invitrogen). Cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. The maintenance medium was replaced every 3 to 4 days and cells were kept for 11 to 14 days *in vitro* (DIV) before being used. This procedure has been reported to yield neuronal cultures of more than 95% purity (Lesuisse and Marin, 2002).

2.3 Treatment of Cultured Neurons with MTX

To assess the toxicity of MTX on cultured cortical neurons, cells (11-14 DIV) were incubated in medium containing varying concentrations (0 to 200 pM) of MTX. After 30 min incubation at 37°C, the treatment medium was replaced with fresh maintenance medium and the culture returned to the incubator for 18 to 24 h prior to cell death assays. To test the effect of the NHE inhibitor, EIPA, on MTX-induced neurotoxicity, EIPA, at varying concentrations (1 to 10 µM), was introduced into the treatment medium 30 min before MTX treatment. EIPA was present thereafter until ethidium bromide (EB) uptake or cell viability assays.

2.4 Fluorimetric Measurement of $[Ca2+]_i$

Cells plated on the rectangular coverslips were washed once with maintenance medium and then incubated with 1 µM FURA-2 AM in fresh medium for 30 min at 37°C. After the incubation, cells were washed twice with HEPES buffer (10 mM HEPES, 140 mM NaCl, 25 mM glucose, 5.4 mM KCl, 1.3 mM $CaCl_2$, pH 7.35, osmolarity 310–320 mosM). The cover slip was then gently inserted into a fluorimeter cuvette so that it was aligned at 45° to the excitation beam. The cuvette containing the cover slip and 2 ml HEPES buffer was kept at 37°C and under continuous magnetic stirring. A fluorescence ratio of 340/380 nm excitation was obtained by measuring the fluorescence intensity at 495 nm emission. $[Ca^{2+}]_i$ was calculated according to the following equation, as described by Beani et al. (1994):

$$[Ca^{2+}]_i = K_d\,(R\text{-}R_{min}) / (R_{max} - R)\,(F_o/F_S)$$

Where K_d is 225 nM, R the ratio 340/380 of fluorescence of the indicator, R_{min} the ratio in the absence of extracellualr Ca^{2+}, R_{max} the ratio in the presence of saturating Ca^{2+} concentration, and F_o/F_S the ratio of 380 nm excitation fluorescence at zero and saturating Ca^{2+} levels. This procedure yield a $[Ca^{2+}]_i$ of 10-15 nM under basal conditions, which is consistent with what was previously (Beani et al., 1994).

2.5 Cell Viability Assay

Cell viability was assessed using alamarBlue™ (AccuMed International Inc., Westlake, OH). Briefly, 24 h after the initial treatment, the alamarBlue™ dye was added to cultures (10% v/v) and left for 3-4 h in the incubator before the supernatant was transferred to a 96 well titer plate. Fluorescence was measured on a Bio Tek FL600 Microplate Fluorescence Reader (MTX Lab Systems, Inc., Vienna, VA) using an excitation wavelength of 530-560 nm and an emission wavelength of 590 nm. Cell viability was calculated and plotted as a percent of control cells.

2.6 Microscopic Imaging of EB Uptake

Cells plated on 18-mm coverslips were pre-incubated for 30 minutes with fresh medium or medium containing various concentrations of EIPA. After incubation, the cover slips were transferred to a holder for microscopic imaging. Ethidium bromide (1µg/ml) was loaded prior to image acquisition. Collection of data was performed using a Wave-FX spinning disc confocal microscope (Quorum Technologies, Guelph, Canada) with a laser excitation wavelength of 491 nm and an emission filter of 593 nm at a magnification of 20x. Images were collected at 1 minute intervals for 31 minutes, with MTX being added after the first

time point. Images collected at time 0 (the second image for the experiment) for each group were designated as background and were subtracted from each time point after MTX treatment.

3.0 RESULTS

3.1 MTX triggers a rapid increase in $[Ca^{2+}]_i$ and causes cell death in cultured rat cortical neurons

Cultured cortical neurons kept at 37^0C showed a basal $[Ca^{2+}]_i$ of 15.2 ± 2.1 nM. This basal concentration is in agreement with previously published reports (Beani et al., 1994). Treatment of these neurons with varying concentrations of MTX induced a dramatic increase in the levels of intracellular calcium, as seen by the increases in both the absolute concentrations and the normalized ratio of 340/380, (Figure 1).

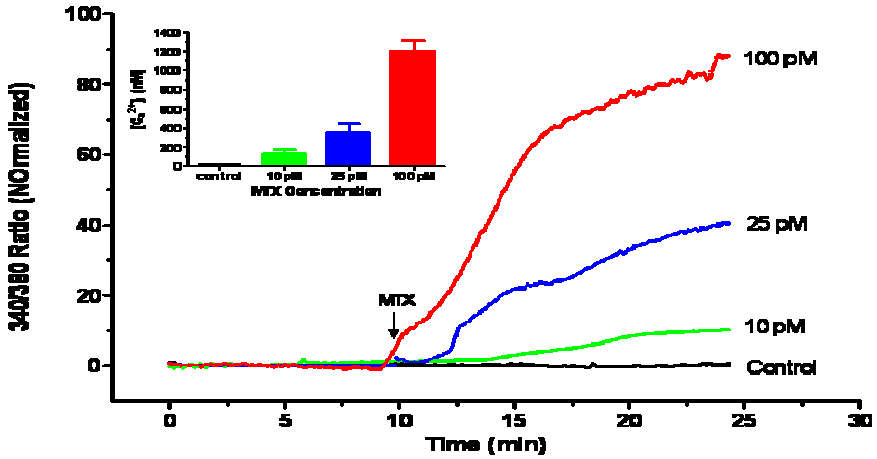

Figure 1. MTX triggers rapid intracellular calcium increase in cultured rat cortical neurons. Main graph shows representative traces of normalized fluorescence ratios of 340/380 nm excitation. Inset: $[Ca^{2+}]_i$ calculated using the formula shown in Materials and Methods. Values represent Mean ± SEM from four experiments.

We next examined the uptake of EB into cultured rat cortical neurons treated with MTX. As shown in Figure 2, treatment of cultured rat cortical neurons with varying concentrations of MTX caused a dramatic increase of EB uptake within 30 min. The uptake could be detected in live cells as early as 5 min after MTX treatment (data not shown). Furthermore, cell viability assay (measured approximately 20 h after MTX treatment) revealed that MTX induced significant cell death in a concentration dependent manner (Figure 2, right panel). It should be noted that, the concentration of MTX required to cause both $[Ca^{2+}]_i$ increase and cell death in cultured rat cortical neurons is much lower than that required for rat aortic small muscle cells, endothelial cells (unpublished observation; Wisnoskey et al., 2004) and Chinese hamster ovary (CHO) cells (Cataldi et al., 1999; Morales-Tlalpan and Vaca, 2002).

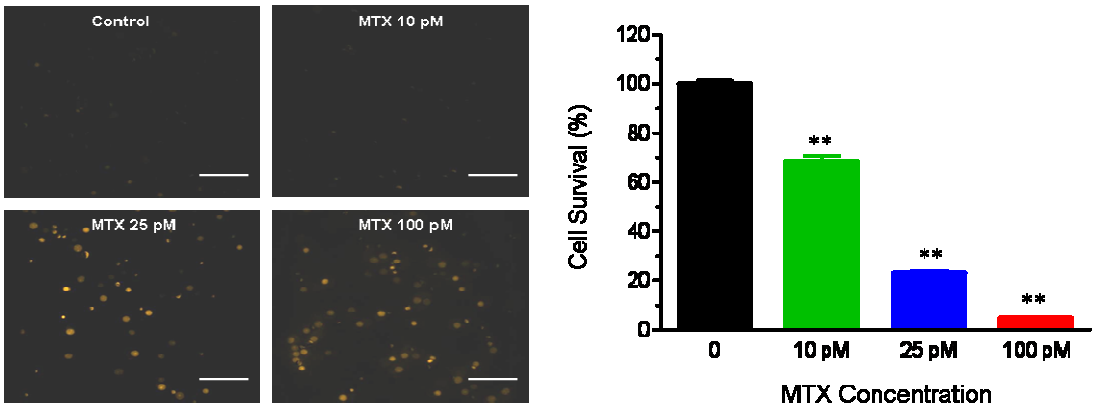

Figure 2. MTX induced ethidium bromide (EB) uptake and cell death in cultured rat cortical neurons. Left panel shows representative photographs of EB uptake 30 min after MTX treatment, captured with a confocal microscope. Magnification: 20X; scale bars: 50 µm. Right panel shows MTX-induced total cell death assayed using the almarBlue™ method. Values are Mean ± SEM from four experiments. ** $p < 0.01$ compared with control

3.2 Protection of MTX-induced cell death by extracellular acidosis

To test if changes in extracellular pH affects MTX-induced responses in cultured rat cortical neurons, cells were treated with 25 pM MTX in medium with a pH of 6.0, and then left overnight at pH 6.0. As shown in Figure 3 (left panel), exposure of cultured rat cortical neurons to pH 6.0 medium alone did not affect cell survival ($p > 0.05$, compared to pH 7.5). On the other hand, extracellular acidosis significantly diminished MTX-induced cell death (Figure 3, left panel). To exclude the possibility that MTX is degraded in acidic conditions, cells were first treated with MTX in normal medium (pH 7.5), and then switched to and maintained in low pH (6.0) medium prior to cell death assay. Figure 3 (right panel) showed that this strategy was also effective at protecting against MTX induced cell death, as compared to the group that was treated with MTX and maintained in pH 7.4 medium throughout the experimental period (Figure 3).

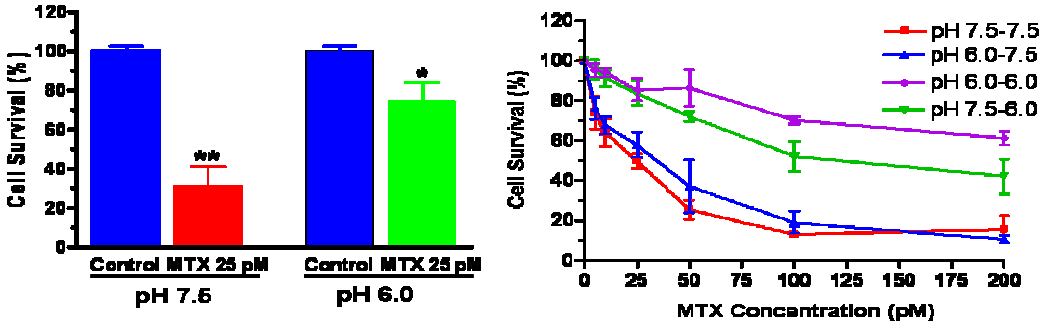

Figure 3. Extracellular acidosis protects MTX-induced cell death in culture rat cortical neurons. Values represent Mean ± SEM from four experiments. Left panel, effect of low pH medium on 25 pM MTX-induced cell death, * $p < 0.05$, ** $p < 0.01$ compared with their respective control. right panel, effect of different treatment procedures on MTX-induced cell death. pH 7.5-7.5 (red): cells were treated with varying concentrations of MTX in pH 7.5 medium for 30 min, then switched to and maintained in pH 7.5 medium; pH 6.0-7.5 (blue): cells were treated with MTX in pH 6.0 medium, then switched to and maintained in pH 7.5 medium; pH 6.0-6.0 (purple): cells were treated with MTX in pH 6.0 medium, then switched to and maintained in pH 6.0 medium; pH 7.5-6.0 (green): cells were treated with MTX in pH 7.5 medium, then switched to and maintained in pH 6.0 medium.

3.3 Effect of EIPA on MTX-induced $[Ca^{2+}]_i$ increase and cell death in cultured rat cortical neurons

Because extracellular acidosis has been shown to inhibit the cell membrane NHE activity (Grinstein et al., 1989; Vornov et al., 1996), we tested the ability of the NHE inhibitor, EIPA to protect MTX-induced events. As shown in Figure 4, pretreatment of cells with 5 µM EIPA significantly attenuated MTX-induced $[Ca^{2+}]_i$ increase.

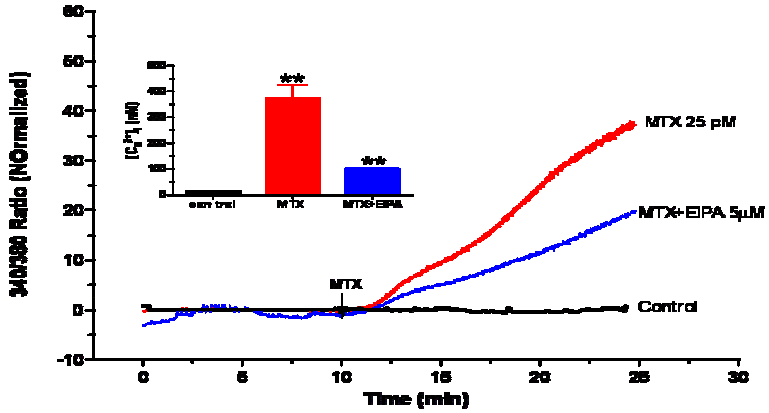

Figure 4. Effect of the EIPA on MTX-induced intracellular calcium increase in cultured rat cortical neurons. Main graph shows representative traces of normalized 340/380 nm ratio after MTX treatment. Inset: Calculated intracellular calcium concentration using the formula shown in Materials and Methods. Values represent Mean ± SEM from four experiments. ** $p < 0.01$ compared with control.

Figure 5 showed the effect of EIPA on MTX-induced EB uptake and cell death in cultured rat cortical neurons. Pretreatment of cells with varying concentrations of EIPA dramatically reduced both MTX-induced EB uptake (Figure 5, left panel) and total cell death (Figure 5, right panel).

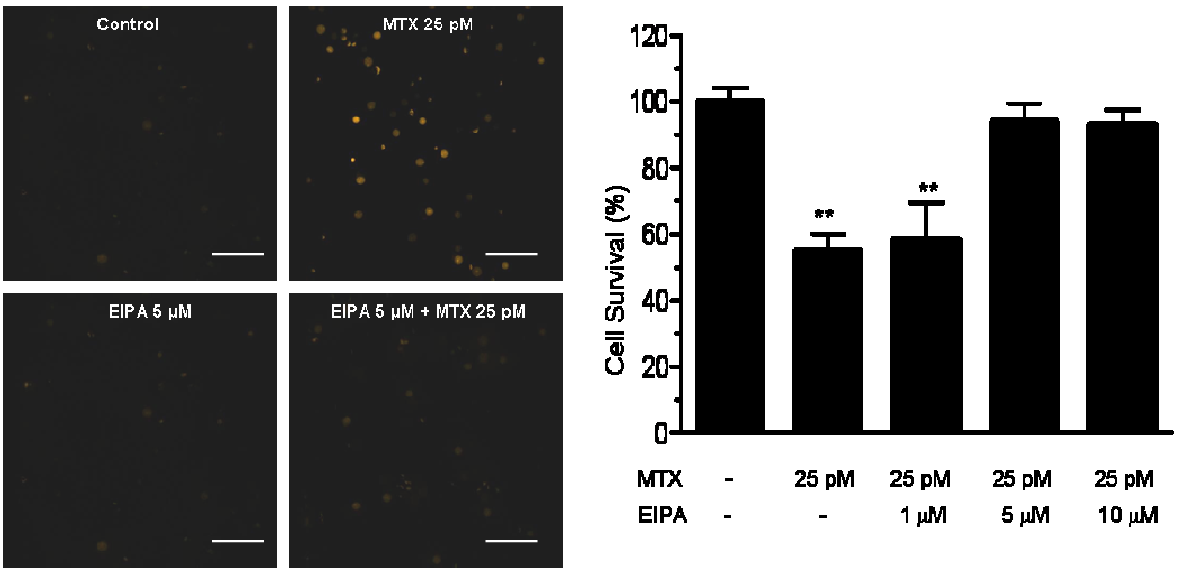

Figure 5. Effect of EIPA on MTX-induced cell death in cultured rat cortical neurons. Left panels shows representative images of EB uptake 30 min after MTX treatment. Magnification: 20X; scale bars: 50 µm. Right panels shows the effect of EIPA on MTX-induced cell death assayed with the alamarBlue™ method. Values are Mean ± SEM from four experiments. ** $p < 0.01$ compared with control.

4.0 DISCUSSION

In the present study we demonstrate that MTX caused dramatic increases in $[Ca^{2+}]_i$ and cell death, in a concentration dependent manner in cultured rat cortical neurons. The MTX effects were significantly attenuated by extracellular acidosis or pharmacological inhibition of the cell membrane NHE, suggesting that NHE may play an important role in MTX-induced neurotoxicity.

MTX's ability to trigger necrotic cell death in a variety of tissues has been extensively studied in the past decade (de la Rosa, et al., 2007). It is now clear that MTX triggers a series of events that involves the opening of the NSCC. Although it is known that NSCC is a cation channel, which allows multiple ions, including Na^+ and Ca^{2+} to enter the cell, its molecular structure is currently unknown. In addition, the pharmacological characteristics of the NSCC are not well defined, mainly due to the lack of specific inhibitors (Weber et al., 2000). Numerous studies have suggested that MTX may directly interact with the NSCC to make it permeable to Na^+ and Ca^{2+}, and activation of the NSCC is likely the first step in a series of MTX-triggered events (Wisnoskey et al., 2004; Schilling et al., 2006). However, it is possible that MTX binds to its own receptor(s) other than NSCC, which then regulate the opening of the NSCC and other channels. In fact, studies have suggested the existence of a MTX receptor on the cell membrane, although the identity of the receptor remains a mystery (Lundy et al., 2004; de la Rosa et al., 2007). Our finding that the NHE inhibitor EIPA almost completely blocked MTX-induced Ca^{2+} influx and cell death in cultured cortical neurons suggests that the activation of NHE precedes Ca^{2+} influx. Therefore, the activation of NHE, may, in fact, be the first step of MTX's actions in these neurons. This finding could shed some light in the search for MTX receptor(s).

Previous reports indicate that NSCC may be the primary route of Ca^{2+} entry in most cell types (de la Rosa et al., 2007). However, it is obvious that NSCC is not the only channel that allows Ca^{2+} entry after MTX treatment, as it has been reported that voltage sensitive calcium channel (VSCC) blockers can attenuate MTX induced $[Ca^{2+}]_i$ increases in excitable cells (Kakizaki et al., 2006). Interestingly, it has been suggested that distinct pathways of Ca^{2+} entry play different roles in Ca^{2+} responses. Specifically, Sattler et al (1998) reported that the source of Ca^{2+} entry, not the Ca^{2+} load, is the main determinant of the neurotoxic potential of Ca^{2+}. Because Ca^{2+} entry through VSCCs is not generally toxic to excitable cells (Sattler et al., 1998), it is unlikely that VSCCs contribute significantly to MTX-induced cell death in cultured rat cortical neurons. In addition to its ability to regulate intracellular pH, NHE is tightly coupled to other membrane channels such as the Na^+, K^+-ATPase, and the Na^+/Ca^{2+} exchanger (NCX) (Putney et al., 2002; Matsumoto et al., 2004). Based on our results that EIPA blocks MTX-induced Ca^{2+} influx and cell death, it is tempting to speculate that Ca^{2+} may enter the cells through the two membrane channels mentioned above. Indeed, our preliminary experiments with cultured rat aortic endothelial cells indicate that MTX-induced Ca^{2+} influx can be blocked by an inhibitor of the reversed mode NCX. Therefore, it is plausible that NHE activation, as an initial response after MTX exposure, causes a massive increase in intracellular Na^+. In order to extrude the excessive Na^+, NCX is then activated, resulting in a massive influx of Ca^{2+}. Whether NHE activation regulates the activation of the NSCC remains to be investigated.

MTX has been shown to cause uptake of the vital dye ethidium bromide into a variety of cell types, presumably through the NSCC (Estacion and Schilling, 2002; Lundy et al., 2004). Ethidium bromide, when bound to the cell nucleus, fluoresces, which is detectable at wavelengths of 600 nm or longer. Under physiological conditions, the intact cell membrane is not permeable to EB. However, when cells are challenged with toxic stimuli, the integrity of the membrane can be compromised, thus allowing EB to enter the cells and bind to the nucleus. Its uptake is initiated by an increase in $[Ca^{2+}]_i$ and typically precedes total cell lysis (Schilling et al., 2004). Our finding that EB uptake occurs as early as 5 min after MTX treatment, yet cell death can be attenuated even if the pH of the extracellular medium is lowered after the initial 30 min treatment, suggests that EB uptake may be a reversible process. It can be inferred from these results that MTX neurotoxicity may be alleviated even if the NSCC is already activated.

In conclusion, our results suggest that the activation of NHE plays an important role in MTX-induced responses in cultured rat cortical neurons. NHE inhibitors may be developed as medical counter measures against MTX toxicity in the future.

REFERENCES

Beani L, Tomasini C, Govoni BM, Bianchi C. Fluorimetric determination of electrically evoked increase in intracellular calcium in cultured cerebellar granule cells. J Neurosci Meth. 1994; 51: 1-7.

Berta P, Phaneuf S, Derancourt J, Casanova J, Durand-Clement M, le Peuch C, Haiech J, Cavadore JC. The effects of maitotoxin on phosphoinositides and calcium metabolism in a primary culture of aortic smooth muscle cells. Toxicon. 1988; 26:133-41.

Cataldi M, Secondo A, D'Alessio A, Taglialatela M, Hofmann F, Klugbauer N, Di Renzo G, Annunziato L. Studies on maitotoxin-induced intracellular Ca(2+) elevation in chinese hamster ovary cells stably transfected with cDNAs encoding for L-type Ca(2+) channel subunits. J Pharmacol Exp Ther. 1999; 290: 25-730.

Choi OH, Padgett WL, Nishizawa Y, Gusovsky F, Yasumoto T, Daly JW. Maitotoxin: effects on calcium channels, phosphoinositide breakdown, and arachidonate release in pheochromocytoma PC12 cells. Mol Pharmacol 1990; 37: 222-230

Escobar LI, Salvador C, Martinez M, Vaca L. Maitotoxin, a cationic channel activator. Neurobiol. 1998; 6: 59-74.

Estacion M, Schilling WP. Maitotoxin-induced membrane blebbing and cell death in bovine aortic endothelial cells. BMC Physiol 2001; 1:2 http://www.biomedcentral.com/1472-6793/1/2.

Estacion M, Schilling WP. Blockade of maitotoxin-induced oncotic cell death reveals zeiosis. BMC Physiol 2002; 2:2 http://www.biomedcentral.com/1472-6793/2/2

Estacion M, Weinberg JS, Sinkins WG, Schilling WP. Blockade of maitotoxin-induced endothelial cell lysis by glycine and L-alanine. Am J Physiol Cell Physiol. 2003; 284: C1006-20.

Grinstein S, Rotin D, Mason MJ. Na^+/H^+ exchange and growth factor-induced cytosolic pH changes. Role in cellular proliferation. Biochim Biophys Acta 1989; 988: 73-97.

Gusovsky F, Yasumoto T, Daly JW. Calcium-dependent effects of maitotoxin on phosphoinositide breakdown and on cyclic AMP accumulation in PC12 and NCB-20 cells. Mol Pharmacol. 1989 Jul;36:44-53.

Gusovsky F, Daly JW. Maitotoxin: a unique pharmacological tool for research on calcium-dependent mechanisms. Biochem Pharmacol. 1990; 39: 1633-1639.

Kakizaki A, Takahashi M, Akagi H, Tachikawa E, Yamamoto T, Taira E, Yamakuni T, Ohizumi Y. Ca2+ channel activating action of maitotoxin in cultured brainstem neurons. Eur J Pharmacol. 2006; 536: 223-231.

Kobayashi M, Ohizumi Y, Yasumoto T. The mechanism of action of maitotoxin in relation to Ca2+ movements in guinea-pig and rat cardiac muscles. Br J Pharmacol. 1985; 86:385-91.

Lesuisse C, Martin LJ. Immature and mature cortical neurons engage different apoptotic mechanisms involving caspase-3 and the mitogen-activated protein kinase pathway. J Cerebr Blood Flow & Metab. 2002; 22: 935-950.

Lundy PM, Nelson P, Mi L, Frew R, Minaker S, Vair C, Sawyer TW. Pharmacological differentiation of the $P2X_7$ receptor and the maitotoxin-activated cationic channel. Eur J Pharmacol. 2004; 487: 17-28.

Morales-Tlalpan V, Vaca L. Modulation of the maitotoxin response by intracellular and extracellualr cations. Toxicon 2002; 40: 493-500.

Ohizumi Y, Yasumoto T. Contraction and increase in tissue calcium content induced by maitotoxin, the most potent known marine toxin, in intestinal smooth muscle. Br J Pharmacol. 1983; 79:3-5.

Putney LK, Denker SP, Barber DL. The changing face of the N^+/H^+ exchanger, NHE1: structure, regulation and cellular actions. Annu Rev Pharmacol Toxicol. 2002; 42: 527-552.

de la Rosa LA, Alvarez-Parrila E, Martinez-Martinez A. Biochemistry of maitotoxin. In: Phycotoxins: Chemistry and Biochemistry. Eds, LM Botana, Blackwell Publishing, 2007.

Sattler R, Charlton MP, Hafner M, Tymianski M. Distinct influx pathways, not calcium load, determine neuronal vulnerability to calcium neurotoxicity. J Neurochem. 1998; 71: 2349-2364.

Sawyer TW, Vair C, Nelson P, Shei Y, Bjarnason S, Tenn C, Mcwilliams M, Villanueva M, Burczyk A. Ph dependent toxicity of sulphur mustard in vitro. Toxicol Appl pharmacol. 2007; 221: 363-371.

Schilling WP, Wasylyna T, Dubyak GR, Humphreys BD, Sinkins WG. Maitotoxin and P2Z/P2X(7) purinergic receptor stimulation activate a common cytolytic pore. Am J Physiol. 1999; 277(4 Pt 1):C766-76.

Schilling WP, Snyder D, Sinkins WG, Estacion M. Palytoxin-induced cell death cascade in bovine aortic endothelial cells. Am J Physiol Cell Physiol. 2006; 291: C657-C667.

Sorrentino G, Monsurro MR, Singh IN, Kanfer JN. Membrane depolarization in LA-N-1 cells. The effect of maitotoxin is Ca(2+) and Na(+)-dependent. Mol Chem Neuropathol. 1997; 30: 199-211.

Takahashi M, Ohizumi Y, Yasumoto T. Maitotoxin, a Ca^{2+} channel activator candidate. J Biol Chem. 1982; 257: 7287-7289.

Verhoef PA, Kertesy SB, Estacion M, Schilling WP, Dubyak GR. Maitotoxin induces biphasis interleukin-1β secretion and membrane blebbing in murine macrophages. Mol Pharmacol. 2004; 66: 909-920.

Vornov JJ, Thomas AG, Jo D. Protective effects of extracellular acidosis and blockade of sodium/hydrogen ion exchange during recovery from metabolic inhibition in neuronal tissue culture. J Neurochem. 1996; 67: 2379-2389.

Wang Y, Ju W, Liu L, Fam S, D'Souza S, Taghibiglo C, Wang YT. α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid subtype glutamate receptor (AMPAR) endocytosis is essential for N-methyl-D-aspartate-induced neuronal apoptosis. J Biol Chem. 2004; 279: 41267-41270.

Weber WM, Popp C, Clauss W, Driessche WV. Maitotoxin induces insertion of different ion channels into the xenopus oocyte plasma membrane via Ca^{2+}-stimulated exocytosis. Eur J Physiol. 2000; 439: 363-369.

Wisnoskey BJ, Estacion M, Schilling WP. Maitotoxin-induced cell death cascade in bovine aortic endothelial cells: divalent cation specificity and selectivity. Am J Physiol Cell Physiol. 2004; 287: C345-56

Yao H, Haddad GG. Calcium and pH homeostasis in neurons during hypoxia and ischemia. Cell Calcium 2004; 36: 247-255.

Yasumoto T. The chemistry and biological function of natural marine toxins. Chem Record 2001; 1: 228-242.

Yi SJ, Kim KH, Choi HJ, Yoo JO, Jung H, Han JA, Kim YM, Suh IB, Ha KS. [Ca^{2+}]-dependent generation of intracellular reactive oxygen species mediates maitotoxin-induced cellular responses in human umbilical vein endothelial cells. Mol Cells. 2006; 21: 121-128.

Zhao X, Pike BR, Newcomb JK, Wang KKW, Posmantur RM, Hayes RL. Maitotoxin induces Calpain but not caspase-3 activation and necrotic cell death in primary septo-hippocampal cultures. Neurochem Res 1999; 24: 371-382.