MAITOTOXIN ACTIVATES A NON-SELECTIVE CATION CHANNEL IN CARDIAC MYOCYTES OF RATTUS NORVEGICUS AND SQUALUS ACANTHIAS

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Maitotoxin (MTX) isolated from the marine dinoflagellate Gambierdiscus toxicus is a water-soluble polyether that may be responsible for ciguatera seafood poisoning (Yokoyama et al., J. Biochem, 104:184, 1988). The toxic effects of MTX presumably result from calcium influx that evoke transmitter release, muscle contraction, and phosphoinositide breakdown in a variety of cell types (Gusovsky & Daly, Biochem. Pharm. 39:1633, 1990). Initial studies suggest that the MTX induced calcium influx is mediated by dihydropyridine (DHP) sensitive and insensitive pathways with the DHP insensitive pathway being blocked by cadmium and SK&F 96365 (a receptor and voltage activated Ca^{2+} channel antagonist) in some cell types (Soergel, et al, Mol. Pharm., 41:487, 1992). This lead to the idea of a ubiquitous MTX-sensitive calcium channel. However, more recent studies suggest that MTX activates non-selective cation, sodium or chloride channels in epithelial or ß-cell lines (Dietl & Völkl, Mol. Pharm., 45:300, 1994, Worley, et al., J. Bio. Chem., 269:32055, 1994). Thus the mechanism by which MTX stimulates Ca^{2+} entry is still poorly understood. In this study we show that MTX does not affect T- or L-type Ca^{2+} currents in dogfish cardiac myocytes. Rather, MTX activates a non-selective cation channel with a reversal potential near -6 mV under physiological conditions in both dogfish and rat cardiac myocytes.

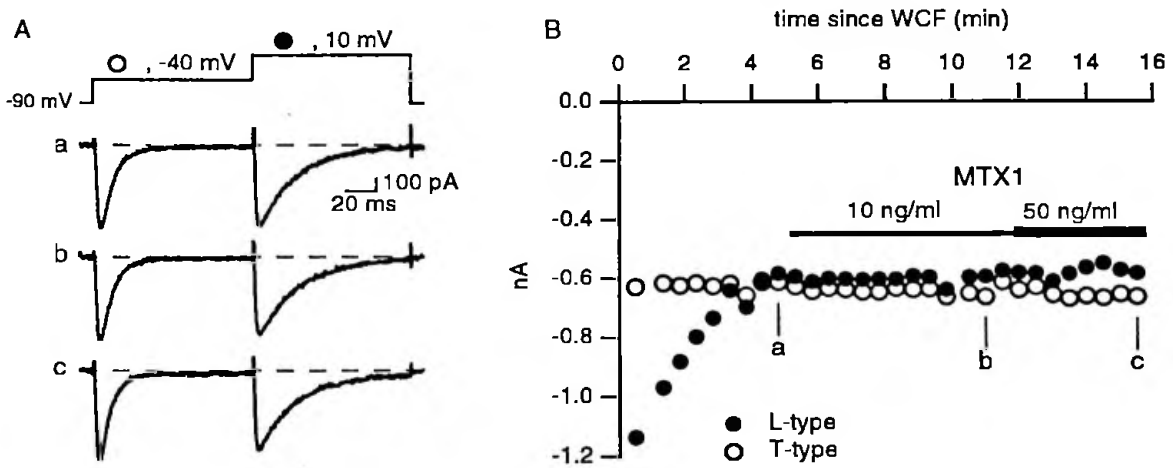

Figure 1. Lack of effect of MTX on Ca^{2+} currents in shark myocyte. A. T- and L-type I_{Ca} measured with a dual pulse protocol. The potential was stepped from a holding potential of -90 mV to -40 mV for 100 ms then to 10 mV for 100 ms (upper trace). Current traces a-c recorded in control solution, 10 ng/ml MTX , and 50 ng/ml MTX, respectively. Currents were leak corrected with a P/4 procedure. B. The peak inward current at -40 mV (closed symbols) and 10 mV (open symbols) is plotted versus time since WCF. The bars indicate exposure time to 10 and 50 ng/ml MTX, respectively. Letters correspond to traces in panel A.

The effect of MTX on Ca^{2+} currents (I_{Ca}) was tested in dogfish cardiac myocytes since this species expresses both T- and L-type calcium channels with approximately equal current density. Shark ventricular myocytes were isolated using established procedures and studied with the whole cell patch clamp configuration. To isolate Ca^{2+} currents from Na^+ and K^+ currents, the patch pipette contained (mM): CsCl 240, $MgCl_2$ 1, Urea 300, EGTA 20, HEPES 20, TMAO 70,

MgATP 5, pH 7.2. Cells were initially superfused with shark Ringer (mM): NaCl 270, KCl 4, $MgCl_2$ 3, KH_2PO_4 0.5, Na_2SO_4 0.5, Urea 350, HEPES 10, glucose 10, $CaCl_2$ 3, pH 7.2. Following formation of whole cell recording configuration (WCF) the external solution was switched to a Na^+ and K^+ free solution: TEA-Cl 275, $CaCl_2$ 5, $MgCl_2$ 5, HEPES 10, Urea 350, pH 7.2. Ionic currents were measured with an EPC-9 (HEKA). Figure 1 shows that MTX did not affect T- and L-type I_{Ca}. Ca^{2+} currents were measured with a double-step procedure in which the membrane potential was first stepped from a holding potential of -90 to -40 mV and then to 10 mV to sequentially activate T- and L-type I_{Ca}, respectively. The T-type I_{Ca}, activating at -40 mV, inactivated rapidly during the pulse and did not interfere with activation of the L-type channel during the second step to 10 mV. The plot of peak inward T- and L-type I_{Ca} as a function of time following WCF shows that the L-type I_{Ca} decreased during the first 4 min following WCF as a result of run-down (Fig. 1B). Addition of 10 or 50 ng/ml of MTX, diluted from a stock solution of 500 µg/ml in methanol, to the bath solution had no effect on T- or L-type I_{Ca} (Fig 1A, B). Similar results were observed in 7 out of 8 cells. However, in one cell 10 ng/ml of MTX decreased the T-type I_{Ca} by 17% and increased the L-type I_{Ca} 61%. In this experiment, MTX was diluted from a stock solution of 10 µg/ml in methanol. It was not determined whether the increase in L-type I_{Ca} in this cell was the result of 0.1% methanol which increases L-type I_{Ca}.

These results show that MTX does not affect T- or L-type Ca^{2+} channels in dogfish heart. To determine whether the lack of effect of MTX on cardiac Ca^{2+} channels is species specific, similar experiments were carried out on rat cardiac myocytes which express only L-type Ca^{2+} currents. Rat cardiac myocytes were isolated using established procedures and perfused with Tyrode solution containing 0.2 mM Ba^{2+} to block inward K^+ currents. The composition of the Tyrode solution was (mM): NaCl 140, KCl 5, $MgCl_2$ 1, $CaCl_2$ 2, HEPES 10, glucose 10, pH 7.4. The patch pipette contained (mM): CsCl 30, CsAsp 110, EGTA 20, HEPES 20, MgATP 5, pH to 7.2 with CsOH. Addition of MTX following whole cell formation appeared to have no effect on I_{Ca} in that no increase over the apparent run-down I_{Ca} was observed. Rather, the input conductance increased during perfusion with MTX. Figure 2A shows a family of current traces in control and 21 min after perfusion with 10 ng/ml of MTX. The plot of final current versus voltage shows that MTX activated a current with a linear current-voltage relation that reversed near -6 mV (panel B). That both Na^+ and Ca^{2+} currents were still observed suggest that a loss of patch pipette seal cannot account for the effect of MTX.

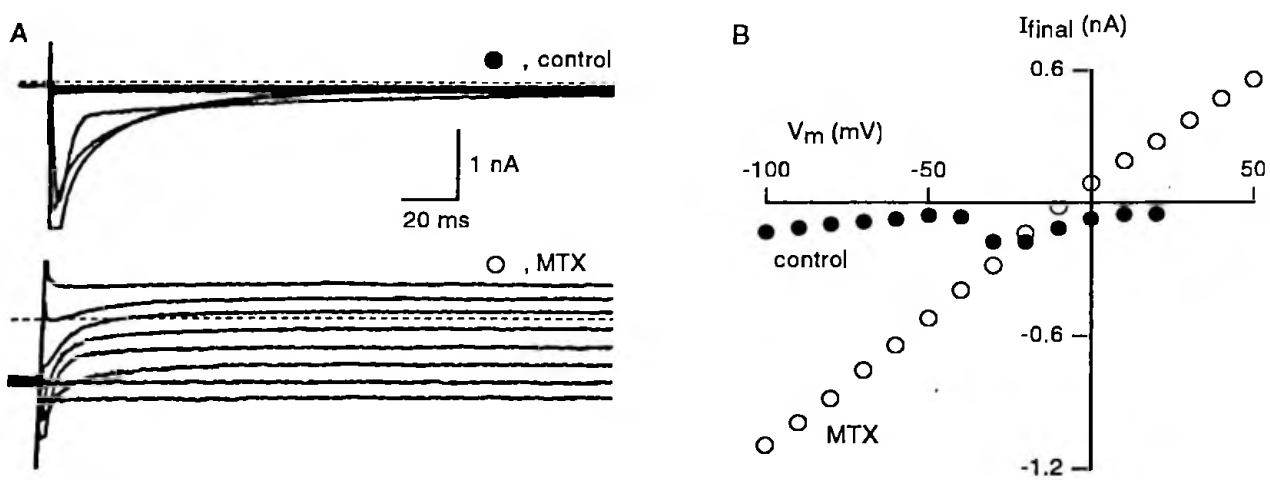

Figure 2. MTX activates a channel with a linear conductance in rat cardiac myocyte. A. Family of current traces in control (upper traces) and after 21 min perfusion with 10 ng/ml MTX (lower traces). Currents were recorded from a holding potential of -80 mV to test potentials between -100 and 20 mV (control) and -100 and 40 mV (MTX) in 20 mV increments. Dash line represents zero current. B. Plot of final current measured at the end of the test pulse versus test pulse potential.

The effect of MTX in rat was dose dependent with 10 ng/ml giving a greater increase in conductance than 1 ng/ml (Figure 3). The membrane potential was initially depolarized to 60 mV to inactivate Na^+ and Ca^{2+} currents and subsequently slowly repolarized to -120 mV over 1 s yielding a quasi steady state current-voltage relation. The control ramp shows residual Ca^{2+} currents that were absent in the MTX records as a result of run-down. During perfusion with MTX, the conductance increased 0.3 and 2.7 nS/min with 1 and 10 ng/ml MTX, respectively. MTX, 10 ng/ml, produced an increase in the steady state conductance within 1 min of its application and the average rate of increase in the conductance was 3.9 ± 0.8 nS/min (mean±SEM, n=6). The effect of MTX was reversible only with prolonged washout (>20 min). Steady state activation and a complete dose response of MTX was not determined.

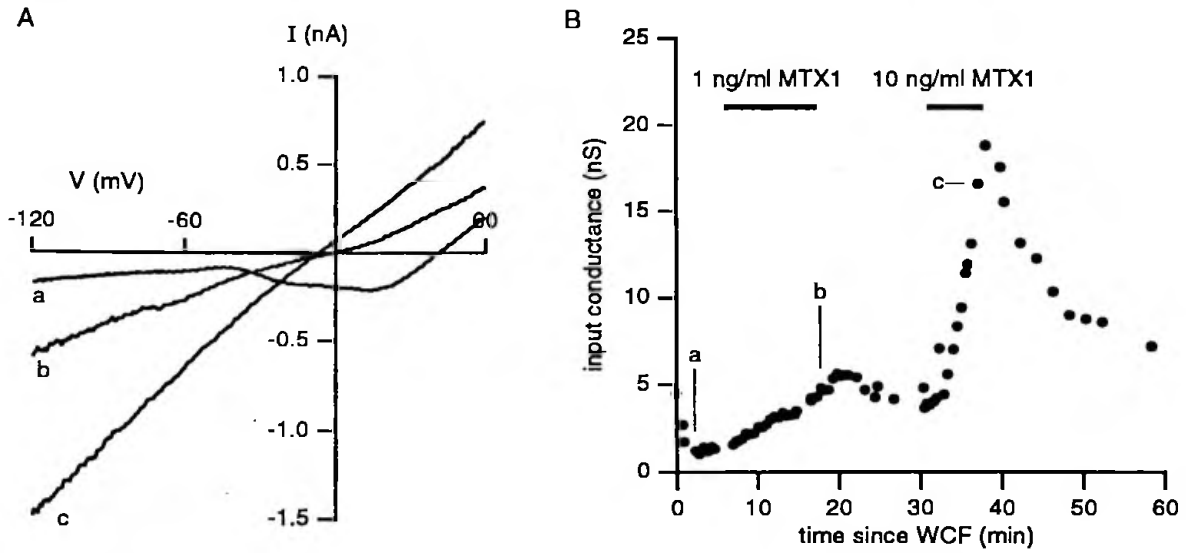

Figure 3. Ramp protocol to measure MTX activated current in rat cardiac myocyte. A. Quasi steady state current-voltage relation obtained from a voltage ramp of 60 to -120 mV in 1 s. Ramps a-c recorded in control solution, 1 ng/ml MTX, or 10 ng/ml MTX, respectively. B. Plot of input conductance versus time since WCF. Input conductance was measured with a -5 mV step from a holding potential of -50 mV. Letters correspond to ramps in panel A.

Ionic substitutions experiments were performed to determine the nature of the charge carrier of the MTX activated current in rat ventricular myocytes. To test whether Cl^- is a charge carrier, the external [Cl^-] was decreased from 153 to 11.4 mM (Cl^- replaced with methansulfonate); the Cl^- reversal potential would shift from -41 to 25 mV. The reversal potential of the MTX activated current was -6 mV in both normal and low Cl^-. Thus the MTX activated current is not carried by Cl^- ions in cardiac myocytes.

Rather, the reversal potential of ~-6 mV is more consistent with MTX activating a non-selective cation channel. The ion selectivity of the MTX activated current was determined under biionic conditions in which the bath solution was replaced with an external solution containing (mM): mannitol 280, HPES 1, BaCl 0.2, pH 7.4 with NaOH plus either NaCl 20, CsCl 20, or KCl 20. Following activation of the MTX sensitive current in normal Tyrode plus 0.2 BaCl, the external solution was switched to the 20 mM Cs-mannitol solution (Fig 4A). The reversal potential shifted from -9 in Tyrode to -45 mV in 20 Cs-mannitol; the predicted E_{Cs} for this solution is -56 mV and the measured reversal potential was not corrected for shifts in the junction potential. The reversal potential in the 20 Na- and 20 K-mannitol solution was -42.0 and -45 mV, respectively. The reduction in current in the Na- and K-mannitol solution is in part due to wash of MTX which was not included in the mannitol solutions. Fig 4B shows that the MTX activated current in the

Na-mannitol solution which does not contain Ca^{2+} is voltage and time independent. The permeability ratios calculated from the equation, $\Delta E_{rev} = 58.6\log(P_x[x]_o/P_{Na}[Na]_o)$ gave a value of 0.9 for both P_K/P_{Na} and P_{Cs}/P_{Na}. A second cell gave a permeability ratio of 0.94 for P_K/P_{Na} and P_{Cs}/P_{Na}. The permeability ratio for tetraethylammonium (TEA) was indirectly determined by replacing the NaCl and KCl in the normal Tyrode solution with TEA-Cl (Fig. 4C). The inward current was significantly reduced, shifting the reversal potential from -10 to -44 mV. The estimated P_{TEA}/P_{Na} was 0.25.

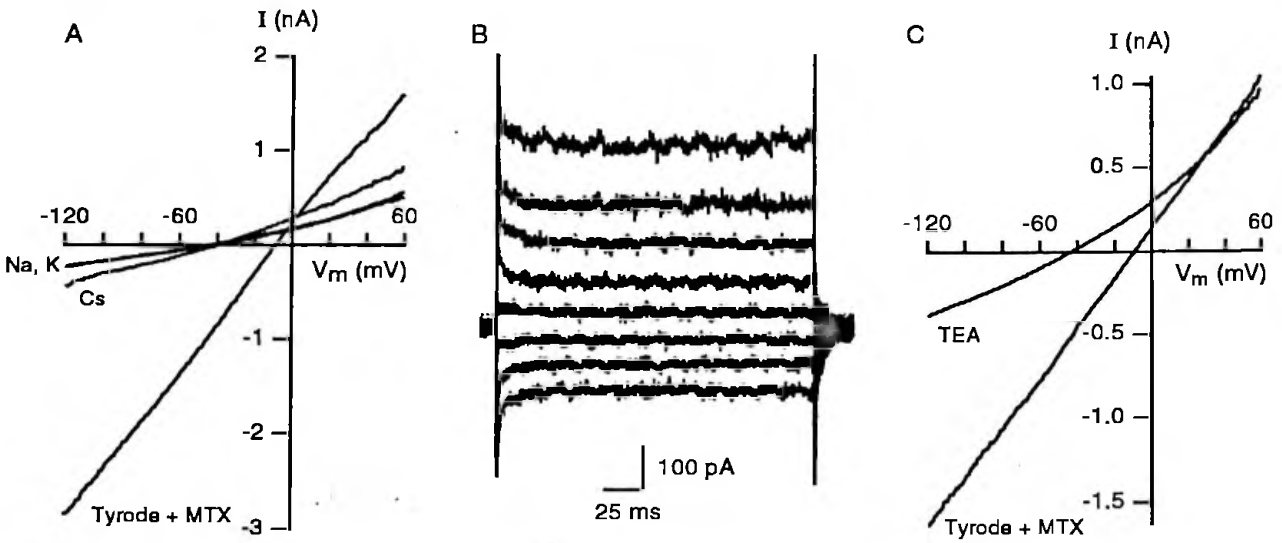

Figure 4. Cation selectivity of MTX activated current in rat cardiac myocyte. A. Current-voltage relations obtained from voltage ramps from 60 to -120 mV in Tyrode plus 10 ng/ml MTX, after 3 min perfusion with Cs-mannitol (Cs), 5 min with Na-mannitol (Na), and 2 min with K-mannitol (K). The IV for Na- and K-mannitol are overlapping. B. Family of current traces in Na-mannitol solution evoked from a holding potential of -50 mV to test potentials from -100 to 40 mV in 20 mV increments. C. Current-voltage relations obtained from voltage ramps from 60 to -120 mV in Tyrode plus 10 ng/ml MTX and after 2 min perfusion with TEA-Cl solution (TEA).

These results show that MTX activates a non-selective cation channel in rat ventricular myocytes. A similar non-selective cation current is activated by MTX in shark ventricular myocytes (data not shown). It is thus proposed that MTX stimulates Ca^{2+} entry into cardiac myocytes secondarily to activation of a non-selective cation channel. Activation of the non-selective cation channel depolarizes the membrane potential towards its reversal potential of ~-6 mV thus activating Ca^{2+} channels and reducing Na^+-dependent Ca^{2+} efflux from the cell by the electrogenic Na^+-Ca^{2+} exchanger. These effects will result in an increase in intracellular Ca^{2+} and muscle contraction. Consistent with this hypothesis is the observation that non-patched cells shorten when exposed to MTX.

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