Maitotoxin-induced membrane blebbing and cell death in bovine aortic endothelial cells

ABSTRACT

These results suggest that maitotoxin-induced Ca2+ influx in bovine aortic endothelial cells is followed by activation of COP. COP formation is associated with controlled membrane blebbing which ultimately gives rise to uncontrolled bleb dilation, lactate dehydrogenase release, and oncotic cell death.

INTRODUCTION

Background Maitotoxin (MTX), one of the most potent marine toxins known, is found in the "red-tide" dinoflagellate, Gambierdiscus toxicus, and is responsible in part for Ciguatera seafood poisoning. In all cells examined to date, MTX at subnanomolar concentrations causes a profound increase in cytosolic free Ca2+ concentration ([Ca2+]i). This occurs, not by release of Ca2+ from internal stores, but rather from activation of a ubiquitously-expressed, non-selective, Ca2+-permeable cation channel (CaNSC), present in the plasmalemma. Channel activation is followed after a short lag by the activation or formation of large endogenous pores that allow organic molecules with molecular weights of <800 Da to cross the plasma membrane. The activation of these pores can be determined experimentally by following the uptake of ethidium and propidium-based vital dyes. These dyes, of varying molecular weights, are normally excluded from the cytoplasm of intact viable cells, but gain access to the cell interior following pore formation where they bind to nucleic acids with a concomitant increase in dye fluorescence. The large pores activated by MTX have been referred to as cytolytic/oncotic pores, or COP, since their activation ultimately leads to the release of lactate dehydrogenase (LDH), an indication of necrotic or oncotic cell death. The cell death cascade activated by MTX is not unique to this toxin. Stimulation of P2X purinergic receptors by ATP causes similar changes in cytosolic Ca2+ and vital dye uptake suggesting that MTX activates a cell death cascade that is physiologically relevant, although the exact role of either the P2X- or MTX-induced cascade in normal cellular biology remains unknown. For the P2X receptor it has been suggested that the Ca2+-permeable channels grow in size to form the dye-permeable pores through either aggregation of channel subunits or through dilation of the existing channel pore structure. In support of this model it has been found that the kinetics of ATP-induced pore formation in HEK cells, heterologously expressing the P2X7 receptor, appears to depend on molecular size of the permeating ionic species. However, we recently showed that although MTX and ATP activate distinct channels, the characteristics of the ATP- and MTX-activated COP are indistinguishable. These results suggest that the channel and COP are unique molecular structures. The aggregation or dilation model make specific predictions concerning the kinetics of dye uptake. In particular, this model predicts a delay between channel activation and dye uptake and this delay should be directly proportional to the molecular size of the permeating dye. Furthermore, if pores grow in size, dye uptake should be nonlinear with time. In contrast, if channel activation causes the formation or activation of a molecularly unique COP with fixed pore dimensions, the delay between channel activation and dye uptake should be independent of molecular size, but the subsequent rate of dye uptake should be linear and inversely proportional to molecular weight. To distinguish between these two models, the effect of MTX on [Ca2+]i, vital dye uptake and LDH release was examined in bovine aortic endothelial cells, a cell line

particularly sensitive to the cytolytic effects of MTX. The results of the present study are consistent with the activation of an endogenous COP of fixed pore dimensions. The opening of large pores in the plasmalemma is expected to cause a dramatic change in the ionic concentration gradients that normally exist between the extracellular and intracellular milieus, i.e., loss of K+, and gain of Na+, Ca2+, and Cl- by the cell. The concomitant flow of water into the cell as a result of this ionic redistribution, will drive the cell towards the Gibbs-Donnan equilibrium. The change in osmotic pressure will produce cell swelling and ultimately membrane rupture and release of large macromolecules from the cytoplasm. This final phase in the cell death cascade can be monitored experimentally by measuring the release of the ubiquitous cytosolic enzyme, LDH. The role of COP and the biophysical mechanism associated with this rather violent cellular event remains unknown. However, many cell types undergo membrane blebbing in response to changes in osmotic pressure. Such membrane blebbing, which may in a sense represent a cellular safety valve, has been reported during both ATP- and MTX-induced cell death, but it is unclear if blebbing occurs before, during, or after COP formation or LDH release. In the present study, the effect of MTX on vital dye uptake in BAECs was correlated with changes in cell morphology using single cell fluorescence videomicroscopy. The videos presented demonstrate that COP activation as indicated by vital dye uptake correlates in time with the formation of membrane blebs, and that membrane lysis, i.e., LDH release, is associated with dramatic bleb dilation.

CONCLUSION

Conclusions In conclusion, MTX treatment of BAECs causes a specific sequence of events (i.e., a cell death cascade) that is triggered by the activation of CaNSC and a rise in [Ca2+]i. This is followed by formation or activation of COP which is correlated with the formation of membrane blebs. COP appears to be a unique molecule associated with the plasma membrane and to have a fixed pore geometry and conductance for each vital dye examined. Furthermore, activation of COP provides the initial driving force for osmotic swelling and bleb formation. LDH release, indicative of the final phase of MTX-induced cell death, is associated with massive bleb dilation. Although, the molecular mechanisms associated with each step in the cell death cascade remain unknown, MTX may prove to be an important tool for understanding the biochemical and biophysical links between channel activation, COP formation, and membrane blebbing.