

EQUINATOXIN II-INDUCED LYSIS OF THE CULTURED ENDOTHELIAL CELL LINE ECV-304

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Abstract: Equinatoxin II (EqT II) is a pore-forming actinoporin. Its lethality in rat tissue is due to cardio-respiratory effects. The toxin contracts the vascular smooth muscle only in the presence of intact endothelium. In our study, its effects on the endothelial cell culture ECV-304 were tested. The EqT II effects were dose-dependent and were influenced by calcium ions and sucrose. The obtained results support the conclusion that calcium ions are the intracellular messengers of the EqT II effects on the isolated endothelial cells.

Key Words: EqT II, Endothelium, Calcium, Sucrose, Nicardipine, Lysis

INTRODUCTION

Equinatoxin II (EqT II) is a toxin isolated from the sea anemone *Actinia equina* L [1]. Cardiotoxic effects are considered to be the key mechanism of the toxin's lethality [2]. EqT II decreased the coronary flow in isolated guinea-pig hearts in a concentration-dependent manner (10 nM-1 μ M) [3]. It caused a contraction of the large epicardial porcine coronary artery only in the presence of intact endothelium, while nicardipine, a calcium channel blocker, diminished those contractions [4]. Morphological changes in various cell cultures incubated with EqT II were due to calcium entry into the cells through newly formed pores in the cell membranes [5]. Although endothelial cells are the first cells exposed to the toxin after an intravenous application, the effects of EqT II on those cells have not yet been studied. The main aim of this study is to evaluate the effects of EqT II on the endothelial cell line ECV-304.

MATERIALS AND METHODS

The endothelial cell line ECV-304 was used in all the experiments. Cells (80-100 cells per plate) cultured in MEM, transferred in Krebs-Henseleit (K-H) solution before the experiments, were treated by one of the following EqT II concentrations (nM): 0.5, 1, 10, 100. Morphological changes in the cells were observed using laser scanning confocal microscopy (Leica, Germany). In the

other group of experiments, K-H solution was substituted by K-H without calcium, or 1 μ M nicardipine was added to normal K-H solution. In one group of the experiments, K-H solution was substituted with 320 mM isotonic sucrose.

RESULTS AND DISCUSSION

The results (Fig. 1 and 2) are reminiscent of the results obtained on smooth muscle cells and NG 15-108 cells, where the lytic effects of the toxin were dose-dependent. At 0.5 nM of EqT II, no morphological changes in the cells were observed, even several hours after the addition of the toxin. At 1 nM and 10 nM of EqT II, the diameter of the cells rose to 150-250 % of the control values ($0=178\pm 12$; $p < 0.01$). 60 min after the addition of 1-10 nM of EqT II all the cells were lysed. At 100 nM of EqT II, all the cells were lysed within three minutes of the addition of the toxin. Less than 5% of the cells were lysed after addition of 100 nM of the toxin in case of K-H without calcium medium, but after the addition of calcium ions the lysis of the cells proceeded in a dose-dependent manner (see above). Nicardipine did not affect the EqT II induced changes in cell morphology. In isotonic sucrose, only 1.8 \forall 0.1 % of cells were lysed after the addition of 100 nM of the toxin. The inhibition of the toxin's action by sucrose may be explained by the prevention of osmotic shock and thus, the consequent lytic action of the toxin.

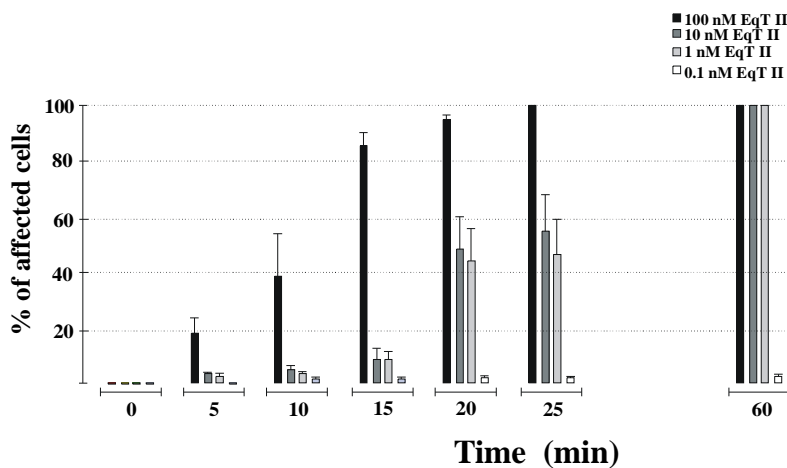


Fig.1. Time course of cell lysis at different concentrations of EqT II.

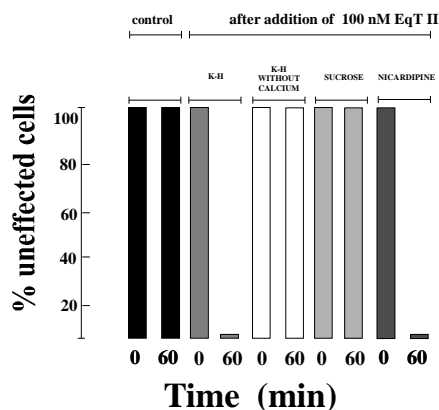


Fig. 2. The effect of extracellular fluid composition on cell lysis after EqT II addition (0 and 60 min).

The threshold concentration for the toxin effects was 1 nM. Calcium ions are essential for toxin action. Apparently Ca^{2+} enters the cell through newly formed pores [5] which are not sensitive to the action of nicardipine.

Acknowledgements. This work was financed by the MŠZŠ (MESS), RS, Research Grants J3-2389-0381-00 and 0381-520.

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