Effect of Melittin on Human Erythrocytes and Rat Liver Mitochondrial Membranes

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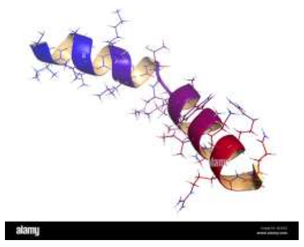
Introduction

Melittin as the major toxic component of the bee venom from *Apis mellifera* has previously been shown to be potent antibacterial, anti inflammatory agent with application in anticancer therapy. We analyze the action of melittin on human erythrocyte and rat liver mitochondria as test systems.

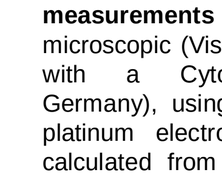
Apis mellifera

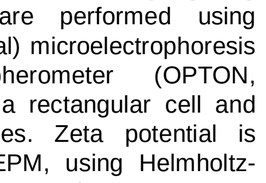


Melittin

Materials and methods

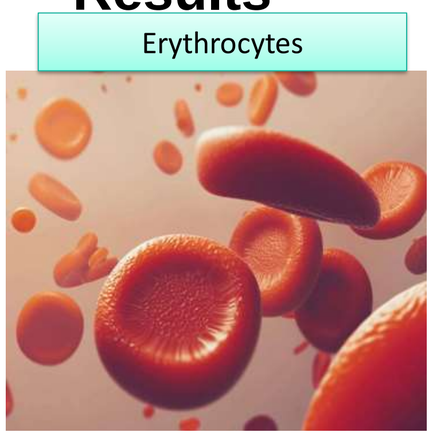
The **electrophoretic mobility (EPM) measurements** are performed using microscopic (Visual) microelectrophoresis with a Cytopherometer (OPTON, Germany), using a rectangular cell and platinum electrodes. Zeta potential is calculated from EPM, using Helmholtz-Smoluchowski equation. Human Erythrocytes are suspended in phosphate buffered saline (PBS, pH 7.4) to hematocrit Ht=15%. Intact liver mitochondria are isolated from male albino rats (Wistarstrain; 120-150 g) by method of Waseem et al. [1] with modifications. The resulting pellet was suspended in washing medium consisting of 0.25 M sucrose (to 1/3 of initial volume) and further centrifuged at the same conditions. The final mitochondrial pellet was suspended in 1–1.5 mL washing medium. EPM of mitochondrial membranes are performed at 2 x 10^8 mitochondria/ml. Melittin from honey bee venom (M-7391-10 MG, Sigma, are used as stock solution (mg/mL) and appropriate dilutions in PBS, pH 7.4 or Saline Sorbitol buffer, pH 7.2, respectively.



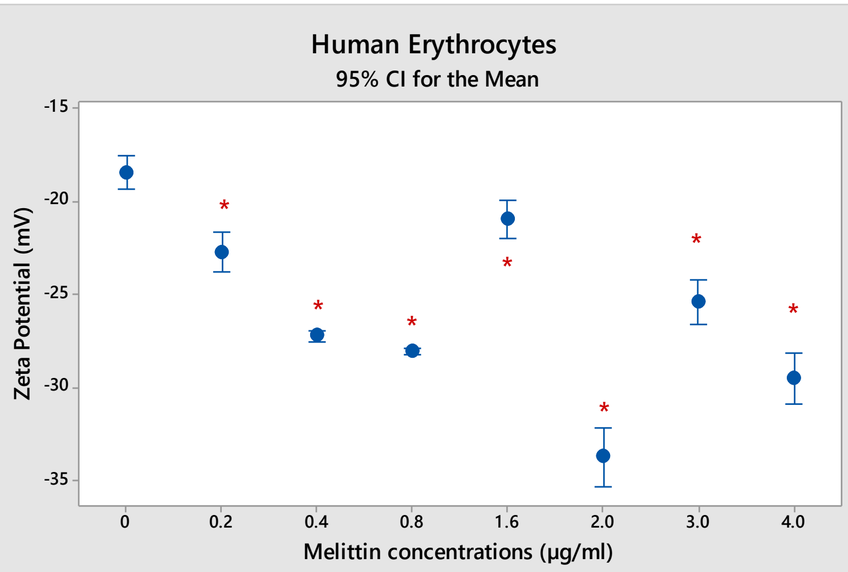


Results

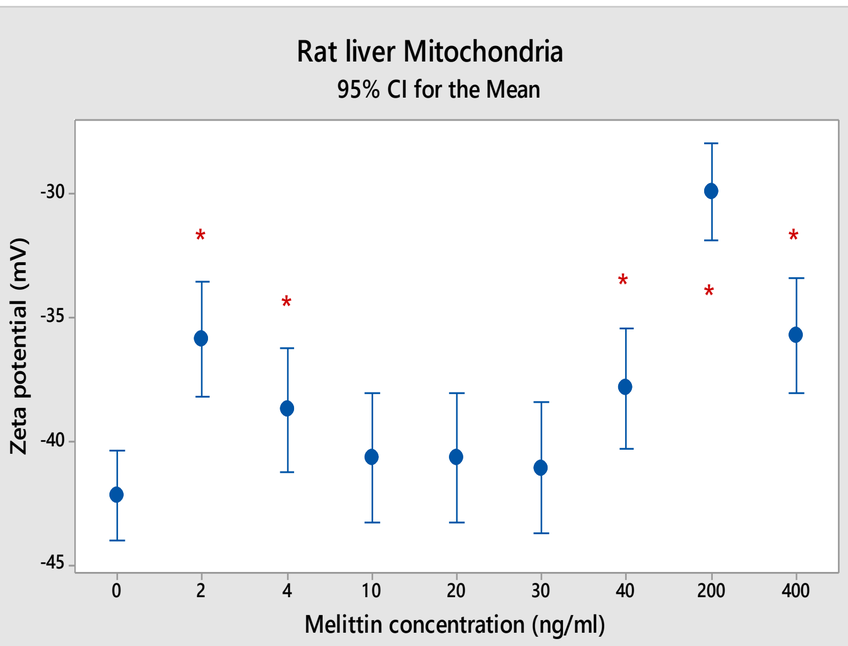
Erythrocytes



Mitochondria



Individual standard deviations were used to calculate the intervals.



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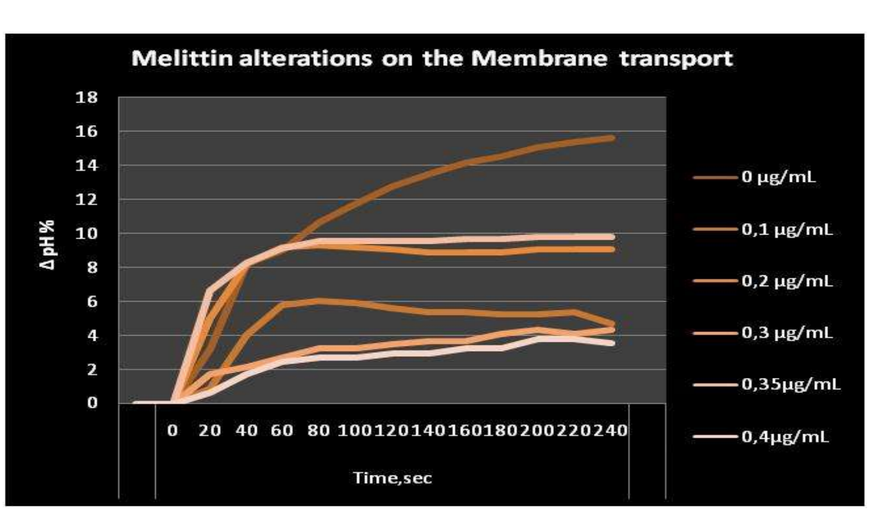

Conclusions

We demonstrate that the melittin increases the electrophoretic mobility (EPM), zeta potential and surface electrical charge of human erythrocyte membranes, suspended in phosphate buffered saline (PBS, pH 7.4). Melittin reduces the EPM of intact mitochondrial membrane, isolated from rat liver in saline sorbitol buffer (SSB, pH 7.2) and causes a decrease of negative electrokinetic potential at 2-4 ng/ml, 40-400 ng/mL because of decrease of negative charges on the outer surface of the membrane.

It is established that 0.2 µg/mL and 0.35 µg/mL Melittin accelerate the membrane transport of protons outflux the cells. There is a strong reduction of slope of membrane transport in the presence of 0.1, 0.3 and 0.4 µg/mL. Melittin disrupts the erythrocytes because of electrostatic interactions with membranes and changes in membrane transport via Band 3 alteration. It contributes to inhibition of membrane transport at the site of the transmembrane protein of Band 3 for proton and H^+/Cl^- cotransport across the erythrocyte membrane. The significance of melittin interaction with biological membranes increases its electrokinetic stability and provides a potential explanation of inflammatory diseases and anti-oxidative stress [2].

Literature cited

1. Waseem, M. Tabassum, H. Bhardwaj, M. Parvez,S. 2017. Ameliorate efficacy of quercetin against cisplatin-induced mitochondrial disfunction: Study on isolated rat liver mitochondria. *Mol Med Rep* 16(3):2939-2945.

2. Gasanoff, E., Liu, Y., Hanlon, P., Garab, G. 2021. Bee Venom Melittin Disintegrates the Respiration of Mitochondria in Healthy Cells and Lymphoblasts, and Induces the Formation of Non-Bilayer Structures in Model Inner Mitochondrial Membranes. Membranes *Int J Mol Sci* 22: 11122.

Acknowledgments

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FURTHER INFORMATION

The present study complies with the ethical regulations and legislation in both Europe and Bulgaria.
The experiments have been performed according to the "Directive 2010/63 / EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes".