To Characterise The Membrane Field Potential Changes Following Non-Immunological and Immunological Activations Of Rat Peritoneal Mast Cells

Jessica Ka-Yan Law¹, Chi-Kong Yeung², Sven Ingebrandt³, Hang-Yung Alaster Lau², John Anthony Rudd², Mansun Chan¹

¹The Hong Kong University of Science and Technology, Bioengineering Graduate Program, 852, Hong Kong, ²The Chinese University of Hong Kong, School of Biomedical Sciences, 852, Hong Kong, ³University of Applied Sciences Kaiserslautern, Department of Informatics and Microsystem Technology, 66482, Germany

Ionic movements (e.g. Ca²⁺, K⁺, and Cl⁻) across the cell membrane during mast cell degranulation can be modulated by different secretagogues [1]. While techniques such as patch-clamping and calcium imaging are often used to investigate such movements, they are tedious and unsuitable for high-throughput screening. In this study, we aimed to demonstrate the feasibility of using the microelectrode array (MEA) to detect the oscillation of rat peritoneal mast cell membrane field potential during degranulation.

Rat peritoneal mast cells were isolated and placed on top of an MEA chip with 64 detection electrodes [2]. They were then stimulated by the addition of either compound 48/80 (non-immunological) or anti-rat immunoglobulin E (anti-IgE, immunological). The stimulated extracellular field potential (exFP) of the cell membrane was measured, and the characterisation of respective exFPs in the presence of either agent was carried out using different ion channel blockers: charybdotoxin (ChTX, a BKca channel blocker) or 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB, a Cl⁻ channel blocker). The amount of histamine released from the mast cells was also measured in vitro.

The exFPs of the activated mast cells and the amount of histamine released were correlated to the concentrations of respective stimulants. Although both secretagogues were able to elicit mast cell degranulation, their underlying mechanisms were different. Compound 48/80 (0.01 – 1 μg/mL) elicited a rapid exFP change (latent period: 2.37 ± 0.07 s to 3.54 ± 0.24 s; temporal measurement: 1.56 ± 1.11 s to 5.37 ± 0.79 s, n = 5); while anti-IgE (1/10,000 – 1/500 dilution) elicited an exFP that had a slower onset time (latent period: 21.63 ± 2.83 s to 11.83 ± 1.09 s, n = 4, P < 0.001) and lasted longer (temporal measurement: 38.84 ± 5.36 s to 29.22 ± 2.40 s, n = 4, P < 0.001). By incubating the cells with ChTX (100 nM), the exFP in the presence of compound 48/80 (1 μg/mL) was reduced by 47.40 ± 8.56 % (n = 5); while by incubating the cells with NPPB (0.1 μM), the exFP of anti-IgE (1/500 dilution) stimulated mast cells was abolished (100%, n = 3). The results indicated that the responses to these compounds were K⁺ and Cl⁻ dependent.

The results show that immunological and non-immunological stimulations act via different mechanisms and that the Cl⁻ channels are more prominent than BKca channels in rat peritoneal mast cell degranulation. Furthermore, the changes in exFP were found to correlate well with the amount of histamine released. The present study demonstrates that the MEA can be used to investigate the relevant ionic movements involved during mast cell degranulation. The characteristics of ions involved in different mast cell activation mechanisms can also be compared.
