Young Scientist Award

The novel TRPV2-selective blocker X10056 inhibits phagocytosis and lipopolysaccharide-induced migration of primary macrophages

R. Raudszus[1], M. Schaefer[1], K. Hill[1]
[1]Rudolf Boehm Institute of Pharmacology and Toxicology, Leipzig, Germany

Transient receptor potential channels (TRP) form a superfamily of mainly nonselective cation channels. Affected by various chemical and physical stimuli, these TRP channels are involved in a variety of physiological and pathophysiological processes, such as nociception or thermosensation. Within the about 30 mammalian members of the superfamily, TRPV2 is a Ca^{2+}-permeable ion channel, which is highly expressed in immune cells, including macrophages. However, due to a lack of potent and selective pharmacological tools, the function of TRPV2 in such cells is not very well understood. We have previously identified the dithiolane X10056 as a novel, potent and TRPV2-selective inhibitor. X10056 blocked TRPV2-mediated Ca^{2+} influx with an IC_{50} of 6.0 µM in HEK293 cells heterologously expressing rat TRPV2 (HEK_{TRPV2}), and inhibited TRPV2 currents in electrophysiological whole-cell recordings performed on HEK_{TRPV2} cells. Since TRPV2 is believed to play a role in controlling macrophage function such as phagocytosis and migration, we isolated and cultured primary bone marrow-derived macrophages (BMDM) and peritoneal macrophages from mice. Quantitative PCR (qPCR) revealed the expression of TRPV2 in BMDM and peritoneal macrophages. 2-APB elicited a Ca^{2+} influx in BMDM and peritoneal macrophages that was inhibited by X10056, confirming the functional expression of TRPV2 in both cell types. Besides, we validated a siRNA-mediated knockdown of TRPV2 in BMDM by qPCR and fura-2-assisted single cell Ca^{2+} assays. In phagocytosis assays using pHrodo-labelled *E. coli* and *S. aureus* bioparticles, phagocytosis was significantly decreased when TRPV2 was inhibited by X10056, valdecoxib or after siRNA-mediated knockdown. Furthermore, TRPV2 inhibition and siRNA-mediated knockdown caused significant inhibitory effects on macrophage migration in a lipopolysaccharide-induced trans-well assay. In contrast, TRPV2 activation with a combination of 2-APB and probenecid resulted in an increased number of migrated cells. Taken together we establish X10056 as novel and potent TRPV2-selective inhibitor. In addition, we provide evidence that TRPV2 plays an important role in two key functions of macrophages, phagocytosis and migration.