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INTRINSIC ACTIVITY

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MEETING ABSTRACT

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Pharmacological inhibition of endolysosomal Ca^{2+} channels in immune cells and related (ultra)structural and physiological implications

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Background: Pharmacological modifications of endolysosomal Ca^{2+} channels have led to a better understanding of the mechanisms of immune diseases. Targeting endolysosomal Ca^{2+} channels could potentially impact diseases such as anaphylaxis or viral infections. Only recently it was shown that endolysosomal two-pore channels (TPCs) play a major role in the interaction of endolysosomes and the endoplasmic reticulum (ER), thus maintaining interorganellar Ca^{2+} homeostasis. If TPCs were impaired, an increased anaphylactic reaction was observed in mice [1,2]. In addition, TPCs also play an important role, both in virus entry and during endolysosomal processes and potential endolysosomal virus escape. However, the underlying (ultra)structural alterations are still pending.

Methods: To shed some more light on this topic, we have implemented confocal laser scanning microscopy (CLSM) techniques as well as 2D, 3D and analytical transmission electron microscopy (TEM) methods. This range of methods should help to investigate the (ultra)structure of rat basophilic leukemia cells (RBL-1) treated with or without the plant alkaloid and potent TPC inhibitor tetrandrine as well as with the novel tetrandrine analogue SG-094.

Results: Our CLSM investigations showed that endocytosis and lysosomal activity as well as co-localization between lysosomes and ER in RBL-1 cells decreased significantly after pharmacological TPC inhibition. The 2D-TEM investigations showed that ER and endolysosomes formed direct interorganellar contact sites at the biomembrane level. Finally, 3D-TEM tomography revealed the full extent of the large contact envelopes between the two organelles. In comparison, ER–endolysosomal contact sites decreased significantly in cells treated with tetrandrine and SG-094, further supporting the hypothesis that TPC function is essential for interorganellar Ca^{2+} exchange.

Discussion: It is our goal to gain a better understanding of the role of TPCs in the crosstalk between ER and endolysosomes at an (ultra)structural level. The correlation of our results with analytical EM and molecular biological methods as well as the implementation of correlative light and electron microscopy (CLEM) methods could be crucial to clarify whether TPCs are indeed promising pharmacological targets for the treatment of several diseases such as allergic hypersensitivity or viral infections.

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