Oxidative stress induces cell death via the suppression of Orai1-mediated Ca^{2+} entry in brain capillary endothelial cells.

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Brain capillary endothelial cells (BCECs) form the blood-brain barrier (BBB) and play an essential role in maintaining BBB barrier function. Oxidative stress induces accumulation of excessive reactive oxygen species (ROS) and facilitates brain capillary cell death, leading to damage BBB. However, it remains still unclear how oxidative stress induces the cell death of BCECs. In this study, t-BBEC117 cells, an immortalized bovine brain endothelial cell line, were cultured under oxidative stress with 30 μM H_2O_2 for 24 hr. The protein expressions of Orai1 and STIM1 were not affected by oxidative stress in t-BBEC117 cells. However, the oxidative stress inhibited store-operated Ca^{2+} (SOC) entry and the suppression was rescued by the application of 10 mM N-acetyl-cycteine (NAC), a ROS scavenger. Ca^{2+} imaging study with Orai1 siRNAs revealed that SOC entry was mainly mediated by Orai1 channels under oxidative stress in t-BBEC117 cells. The application of 5 μM 2-Aminoethoxydiphenylborane (2-APB), an Orai channel activator, enhanced SOC entry under oxidative stress in t-BBEC117 cells and rescued H_2O_2-induced cell death. We show here that oxidative stress inhibits Orai1-mediated Ca^{2+} entry, and thereby facilitates the death of BCECs.