H_2O_2 and paracetamol-induced oxidative stress initiates TRPM2 channel trafficking to the plasma membrane in rat hepatocytes

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Transient Receptor Potential Melastatin 2 (TRPM2) channel is a non-selective cation channel expressed in a variety of tissues. It is localized to both plasma membrane and lysosomes (Eisfeld & Luckhoff, 2007). Experimentally, TRPM2 channels are activated by extracellular application of H_2O_2, or intracellular application of ADP-Ribose (Eisfeld & Luckhoff, 2007). Opening of TRPM2 channels on the plasma membrane results in a sustained rise in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$), which may lead to the activation of Ca^{2+} dependent cell destructive enzymes and cell death (Fonfria *et al.*, 2004). We have previously shown that TRPM2 channels are expressed in rat hepatocytes and are activated by the treatment of hepatocytes with H_2O_2 or high concentrations of paracetamol (Kheradpezhouh *et al.*, 2012). It is likely that activation of TRPM2 channels by reactive oxygen species generated in the liver is a major contributor to hepatocellular death in paracetamol overdose (Kheradpezhouh *et al.*, 2012, Bajt *et al.*, 2011). Although we have a good evidence of TRPM2 presence in hepatocytes, it is not yet known how TRPM2 protein is distributed between plasma membrane and intracellular organelles, and whether there is any change in that distribution in oxidative stress.

To ascertain the relative expression levels of TRPM2 on the plasma membrane of rat hepatocytes we used biotinylation of surface proteins (Pierce Cell surface Isolation Kit, Thermo Fisher Scientific Inc.) with western blot analysis and the confocal microscopy using TRPM2 antibodies (ab63015, ABCAM) and plasma membrane staining (Anti-Pan Cadherin antibody, ab6528, ABCAM). The confocal images were analysed using Image J software.

The western blot analysis showed that in untreated hepatocytes just 3.6 ± 0.47% (n = 3) of total TRPM2 protein was expressed on the plasma membrane, while the rest of the protein was localised to intracellular organelles. In hepatocytes treated with 2 mM H_2O_2 for 30 min the amount of TRPM2 protein on the plasma membrane has increased approximately 4.4 folds to 16.17 ± 0.73% of the total protein (P = 0.0077, n = 3). The analysis of the confocal images of untreated hepatocytes and hepatocytes treated with either paracetamol (10 mM for 1 h) of H_2O_2 (1 mM for 30 min) showed a significant shift of TRPM2-specific immunofluorescence from intercellular space to the plasma membrane. Pearson Coefficient for TRPM2 immunofluorescence (green) and plasma membrane stain (red) overlap changed from 0.35 ±0.01 in untreated hepatocytes to 0.75 ± 0.01 and 0.73 ± 0.01 in H_2O_2-treated and paracetamol-treated hepatocytes respectively (P < 0.0001, n = 52).

In conclusion, we have shown that H_2O_2 or paracetamol induced oxidative stress initiates TRPM2 channels trafficking to the plasma membrane, which may contribute to the mechanism of TRPM2 current activation in hepatocytes.

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