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Objectives Aims We previously documented that morphine upregulates Akt activity by inactivating protein Ser/Thr phosphatases via reactive oxygen species (ROS) may contribute to the protective effect of morphine on ischaemia/reperfusion injury, but the molecular mechanism underlying morphine-induced ROS production remains to be defined. Since receptor tyrosine kinases (RTKs) may contribute to the cardiac protection induced by the activation of opioid, bradykinin, or muscarinic receptors and could increase intracellular ROS production, the purpose of this study was to determine whether RTKs are involved in the protective effect of morphine against oxidant stress-induced mitochondrial damage and whether morphine could induce intracellular ROS production via RTKs, focusing on the roles of insulin-like growth factor 1-receptor tyrosine kinase (IGF-1RTK) and epidermal growth factor receptor tyrosine kinase (EGFR TK).

Methods Cardiac H9c2 cells were exposed to H₂O₂ for 20 min to induce mitochondrial oxidant damage and confocal microscopy was used to determine whether morphine could prevent the oxidant stress-induced loss of mitochondrial membrane potential ($\Delta\Psi_m$) by monitoring changes in TMRE fluorescence. ROS generation was measured by H₂DCFDA kit. Western blot and immunofluorescence assay were used to test Akt and EGFR TK phosphorylation.

Results Treatment of cardiac cells with such doses of morphine (0.1 or 1.0 μ M) showed a significant increase in TMRE fluorescence intensity compared to the control group, indicating that morphine could prevent oxidant stress-induced mitochondrial damage. However, this protective effect of morphine was reversed by the ROS scavenger N-(2-mercaptopyrrolyl) glycine (MPCG), suggesting that ROS accounts for the protective action of morphine. In support, morphine increased ROS generation significantly. Meanwhile, the protective effect of morphine was reversed both by the nonselective RTKs inhibitor genistein and the selective EGFR TK inhibitor AG1478, but not by the selective IGF-1RTK inhibitor AG1024, indicating that EGFR TK is involved in the protective effect of morphine. In addition, the effect of morphine on ROS generation was also reversed by genistein and AG1478, indicating that morphine produces ROS via EGFR TK. It has been reported that morphine activates Akt via ROS. Therefore, it is likely that morphine generates ROS via EGFR TK which then increases the activity of Akt. Based on the results of western blot and immunofluorescence assay, morphine markedly increased Akt phosphorylation which was blocked by AG1478.

Conclusions these findings demonstrate that morphine prevents oxidant stress-induced mitochondrial damage in cardiac H9c2 muscle cells via an EGFR TK-ROS-Akt signalling pathway.