Cardiovascular diseases are the major cause of death in developed countries. It is known that heart muscle can activates endogenous protective pathways in response to stress, thereby increasing resistance against ischemia/reperfusion (I/R) injury. Protective pathways involve many signaling molecules and reactive oxygen species (ROS) play an important role among them. ROS are applied in cardioprotection induced by various stimuli, such as chronic hypoxia, preconditioning and also physical exercise. It has been demonstrated that regular physical exercise naturally leads to the positive adaptation to protect heart against injury. The balance between production of ROS and their removal by antioxidant protection system is important for the right functioning of the heart. The overproduction of ROS occurs in pathological conditions such as an I/R leading to oxidative stress contributing to subsequent damage of heart. ROS may contribute not only to the injury but in the mild concentrations, resulting for example from physical exercise, ROS are important signaling molecules involved in series of events leading to cardioprotection. Slightly increased oxidative stress protects the heart by increasing the capacity of antioxidant system, stimulates angiogenesis, activates mitochondrial biogenesis and physiological hypertrophy. Exploring the precise role of ROS in the protection of heart induced by physical exercise could lead to improved prevention and treatment of cardiovascular diseases.