Reactive Oxygen Species (ROS) and Antioxidants 1. Introduction Cellular processes, such as mitochondrial respiration, result in the generation of reactive oxygen species (ROS) that contribute to the oxidative injury induced by diverse stressors including ischemia/reperfusion and inflammation. Excessive amounts of ROS can lead to the oxidation of nucleic acids, proteins, lipids, and carbohydrates. Tissues harbor numerous non-enzymatic and enzymatic antioxidants that work in concert to help maintain the pro-oxidative and antioxidative equilibrium. Enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase are involved in the direct detoxification of the three major reactive oxygen species. Non-enzymatic antioxidants scavenge ROS, convert them to metabolites, or repair the oxidative damage that has occurred to biomolecules. Failure of antioxidant systems results in the accumulation of ROS and subsequent oxidative flooding and cytotoxicity. As a result, antioxidants have been studied in a variety of clinical settings hoping that supplementation may exert a positive therapeutic effect. Yet, the potential utility of antioxidant therapy has been evidenced in only a small number of diseases that are associated with ROS overproduction. Differences in both antioxidant drug properties and patient populations confound the interpretation of contradictory results. Collectively, modulation of the endogenous antioxidant capacity of pathologically stressed tissues remains a promising therapeutic approach. 1.1. Background and Significance Mitochondria are cellular organelles with multiple functions in energy production and homeostasis in all eukaryotic cells. Mitochondrial functions rely on a multitude of interplaying metabolic, TCA cycle, and signaling pathways in organelles and the whole cell. Reactive oxygen species (ROS) are products of these pathways. ROS include nucleophilic and radical molecules such as superoxide radical anion, hydrogen peroxide, hydroxyl radical, and others. Next to these well-known signaling and toxic ROS, the mitochondrial electron transport chain was recently observed to produce several molecules termed “Mitochondrial ROS,” “Total O2-,” or “Supercomplex ROS.” These ROS species are considered beneficial, complexed with the antioxidative network, and affect all processes in cells, as mitochondria dynamically shape cell functions in real time. However, they may also cause pathophysiological effects leading to diseases, senescence, and aging. Antioxidants present in cells are a potent group of molecules that maintain cellular defenses against ROS. Antioxidants include both low molecular mass compounds such as glutathione, ketoglutarate, nitric oxide, ascorbate, and others, and a large pool of protein antioxidants also known as antioxidative status. Defenses of the cells against ROS are dynamic and respond to flux changes of ROS. We consider the most important of these low molecular mass antioxidants, their pathways, and their metabolisms in the network of mitochondrial metabolism. We pay special attention to the role of nicotinamide adenine dinucleotide phosphate and reduced nicotinamide adenine dinucleotide phosphate levels and discuss mitochondrially targeted low molecular mass antioxidants. We emphasize experiments with cells and mitochondria in model systems to allow a better prediction of the complementary outcome of ROS antioxidants in multitarget therapeutic approaches. 2. Reactive Oxygen Species (ROS) 2.1. Definition and Classification 3. Antioxidants 3.1. Role and Mechanisms of Action 4. Methods for Measuring ROS and Antioxidant Levels 5. Health Implications and Therapeutic Potential