MANGANESE SUPEROXIDE DISMUTASE INDUCED BY EXTRACELLULAR STRESS ENHANCES MYOCARDIAL TOLERANCE TO ISCHEMIA–REPERFUSION

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Summary. In this chapter, we describe our recent findings showing that an intrinsic radical scavenger, manganese superoxide dismutase (Mn-SOD), is induced in cardiac myocytes in response to various external stresses, such as ischemic (hypoxic) preconditioning, heat shock, and α1-adrenergic stimulation. The induction of Mn-SOD is well correlated with the acquisition of tolerance to ischemia–reperfusion injury of the myocardium. Because inhibition of Mn-SOD induction abolishes the tolerance to ischemia–reperfusion, Mn-SOD plays a pivotal role as a rescue protein in cardiac myocytes, and the induction of Mn-SOD could be an adaptation mechanism against ischemic heart disease.

INTRODUCTION

Oxygen radicals have been proposed as one of the culprits that cause myocardial injury during ischemia–reperfusion. In the late 1980s, several groups successfully detected the presence of oxygen radical species in myocardial tissue soon after reperfusion following ischemia [1,2] and at later phases of reperfusion [3]. Oxygen radicals were produced in myocardial tissue after reperfusion, and the production of oxygen radicals was augmented during the course of extended cardiac injury after reperfusion. Therefore, many studies were designed to scavenge radical species to prevent reperfusion injury to the myocardium [4]. Exogenous radical scavengers, such as superoxide dismutase (SOD) and catalase, successfully reduced reperfusion injury in in vivo models. Recent studies have shown, however, that the heart is not merely threatened by oxygen radicals but also has its own intrinsic radical scavenging system, which includes Mn-SOD, Cu,Zn-SOD, catalase, and the glutathione redox
system. These enzymes were revealed to be induced by exogenous stimuli such as endotoxin, cytokines, and hyperthermia [5–8]. Among these enzymes, Mn-SOD is located in the cardiac mitochondria and is supposed to play a major role in scavenging superoxide generated by the electron transport system on the front line. Therefore, we tried to examine whether or not the induction of mitochondrial Mn-SOD is responsible for the acquisition of tolerance to ischemia–reperfusion of the heart by scavenging superoxide generated in mitochondria.

**INDUCTION OF Mn-SOD AFTER ISCHEMIC PRECONDITIONING**

Firstly, we examined the relationship between the tolerance of myocardium to ischemia and Mn-SOD induction in a preconditioning model of canine LAD occlusion–reperfusion [9]. Mn-SOD protein was measured by enzyme-linked immunosorbent assay soon, 3 hours, 12 hours, and 24 hours after repeated ischemia. Mn-SOD content in the subendocardium increased gradually, with a peak observed 24 hours after sublethal ischemia (60% increase). At this peak point, myocardial Mn-SOD activity, simultaneously measured by the nitroblue tetrazolium method, had also increased by about 80% of normal control. In the experiment reported here, we could not see any differences in activity of other antioxidant enzymes, including Cu,Zn-SOD, catalase, and glutathione peroxidase. Next, we demonstrated that such an ischemic preconditioning protocol results in a delayed protective response against myocardial necrosis after a subsequent prolonged ischemia in the dog [10]. Immediately after, or 3, 12, and 24 hours after four five-minute occlusions of LAD, dogs were subjected to 90 minutes of occlusion followed by five-hour reperfusion. When the second ischemia was applied immediately after the first sublethal ischemia, the percent risk area infarcted was markedly decreased to 14% compared with the necrotic area in control animals. However, the reduction of myocardial infarction disappeared when the time interval between sublethal and sustained ischemia was 3 and 12 hours. Interestingly, the size of myocardial infarction was again reduced when the prolonged ischemia–reperfusion was applied 24 hours after ischemic preconditioning. The time course of the reappearance of tolerance to ischemia–reperfusion was identical with that of Mn-SOD induction in the preconditioned myocardium.

To investigate the role of enhancement of cardiac SOD activity in protection against ischemia–reperfusion injury, we examined whether the preconditioning phenomenon could be mimicked in cultured rat myocytes by exposing them to hypoxia (7 mmHg) and reoxygenation (143 mmHg) before exposure to sustained hypoxia–reoxygenation (hypoxic preconditioning) [11]. In control cells, which were subjected to normoxia instead of hypoxia for the first hour, Mn-SOD content and activity showed a slight decrease during the following 36 hours. On the other hand, in the cells exposed to hypoxia, both the activity and content of Mn-SOD increased markedly, with a peak at 24 hours after reoxygenation from hypoxia. We also examined the expression of Mn-SOD mRNA after hypoxia–reoxygenation by Northern hybridization using a rat Mn-SOD cDNA probe. Mn-SOD mRNA gives