HYDROGEN PEROXIDE REGULATES THE RYANODINE RECEPTOR AND THE Na^+-Ca^{2+} EXCHANGER IN RAT (RATTUS NORVEGICUS) CARDIAC MYOCYTES

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Ca^{2+} plays an important role in cardiac muscle excitation-contraction coupling. The $[Ca^{2+}]_i$ is controlled by sarcolemmal Ca^{2+} channels and transporters. Voltage-gated, dihydropyridine-sensitive L-type Ca^{2+} channels serve as the first step in signaling of Ca^{2+} release from the sarcoplasmic reticulum (SR) (Nabauer et al., Science 244: 800-803, 1989). The Ca^{2+} extrusion process is controlled primarily by Na^+-Ca^{2+} exchanger which transports Ca^{2+} out of the cell against its concentration gradient utilizing the favorable gradient of Na^+ ions.

Reactive oxygen species (ROS) such as superoxide ($O_2\bullet^-$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (HO•) have been considered to be extremely reactive and inhibitory to biological molecules. Recent findings, however, showed that ROS also stimulate signal transduction pathways. For example, $O_2\bullet^-$ was found to enhance the IP_3-induced Ca^{2+} release from the vascular smooth muscle SR (Suzuki & Ford, Am. J. Physiol. 262: H114-H116, 1992). Therefore, ROS may be physiologically important signal transducing molecules in various tissues in diverse species including mammals, bacteria, plants and marine organisms.

ROS are implicated in myocardial ischemia-reperfusion injury, and a number of studies have examined the inhibitory effects of ROS on signal transduction components of cardiac muscle using high (mM) concentrations of ROS. In the present study, we have examined possible effects of physiological concentrations of ROS on Na^+-Ca^{2+} exchanger and Ca^{2+} release in isolated cardiac myocytes. We find that μM levels of H_2O_2 augment the activity of Na^+-Ca^{2+} exchanger and the Ca^{2+} channel-gated release of Ca^{2+} from the SR.

Ventricular myocytes were isolated from male Wistar rats using the collagenase/protease method as described (Mitra & Morad, Am. J. Physiol. 249: H1056-H1060, 1981). Whole cell clamped myocytes were dialyzed with 0.2 mM Fura-2, and voltage-dependent Ca^{2+} current (I_{Ca}) and SR Ca^{2+} release transients were simultaneously monitored (Cleemann & Morad, J. Physiol. 432: 283-312, 1991). Excitation wavelengths of 335 nm and 410 nm were used to monitor the fluorescence signals of Ca^{2+}-bound and Ca^{2+}-free Fura-2, and $[Ca^{2+}]_i$ was calculated as described by Cleemann & Morad (1991). Changes in $[Ca^{2+}]_i$ largely reflect changes in cytosolic Ca^{2+} in response to SR Ca^{2+} release (Cleemann & Morad, 1991). Since rapid application of caffeine causes intracellular Ca^{2+} release, and activates inward Na^+-Ca^{2+} exchange current, $I_{Na/Ca}$ (Callewaert et al., Am. J. Physiol. 257: C147-C152, 1989), SR Ca^{2+} release and $I_{Na/Ca}$ were simultaneously monitored. Extracellular solution contained (in mM): 137 NaCl, 5.4 KCl, 2 $CaCl_2$, 10 HEPES, 1 $MgCl_2$ and 10 glucose (pH 7.4). Intracellular solution contained (in mM): 110 CsCl, 30 TEA-Cl, 5 MgATP, 10 HEPES, 0.1 cAMP and 0.2 Fura-2. Cells were exposed to caffeine, H_2O_2 and/or dithiothreitol (DTT) using the rapid (<50 ms) perfusion system. The observed phenomena are presented by showing representative experimental results. Each

phenomenon was reproduced in at least five different rat or guinea pig ventricular myocytes. Efficacy of Ca^{2+}-induced Ca^{2+} release was calculated by the equation, $(dCa/dt)/I_{Ca}$.

Rapid exposure of cells to μM levels of H_2O_2 resulted in suppression of I_{Ca}, but enhancement of Ca^{2+} release, suggesting increased efficiency of Ca^{2+}-induced Ca^{2+} release mechanism. Fig. 1 shows voltage-dependent (A) I_{Ca} and (B) SR Ca^{2+}-transient as membrane potential was depolarized from -80 to 0 mV. Exposure of the cell to H_2O_2 (100 μM) inhibited I_{Ca} (Fig. 1A), but enhanced intracellular Ca^{2+} transients (Fig. 1B). According to the Ca^{2+}-induced Ca^{2+} release theory, a decrease in I_{Ca} would have resulted in decreased Ca^{2+} release. Thus, the enhancement of Ca^{2+} transients by H_2O_2 suggests increased efficacy of Ca^{2+} release mechanism gated by I_{Ca}. The data suggest that H_2O_2 increases the efficacy of Ca^{2+}-induced Ca^{2+} release by 2-fold.

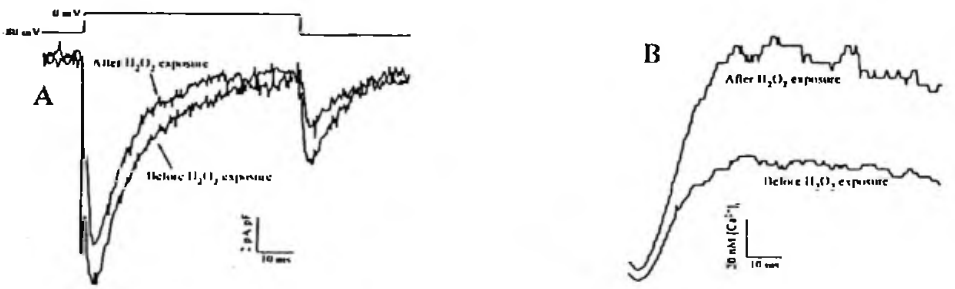

Fig. 1 Voltage-dependent (A) I_{Ca} and (B) Ca^{2+}-release before and after the H_2O_2 exposure

Consistent with this idea, in vitro studies of ryanodine receptor incorporated in lipid bilayers have shown that H_2O_2 enhances mean open time of the ryanodine receptor (Boraso & Williams, Am. J. Physiol. 267: H1010-1016, 1994). Figure 2 further supports this observation as acute exposure of the cell to H_2O_2 (100 μM) causes a rise in $[Ca^{2+}]_i$ and also activates $I_{Na/Ca}$. These Ca^{2+} transients appear quite similar to those induced by 5 mM caffeine.

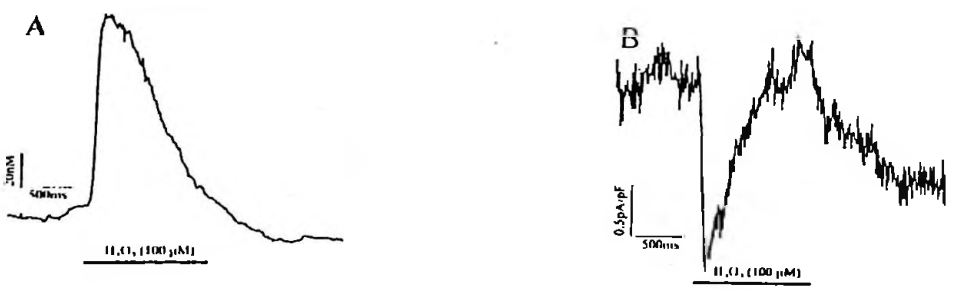

Fig. 2 H_2O_2-induced (A) Ca^{2+} release and (B) Na^+-Ca^{2+} exchange current

We conclude that, in intact rat cardiac myocytes, H_2O_2: (1) enhances the efficacy of Ca^{2+}-induced Ca^{2+} release; and (2) induces activity of the Na^+-Ca^{2+} exchanger. Our observations suggest that H_2O_2 may regulate Ca^{2+}-signaling in cardiac myocytes.

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