# 論 文 要 旨

## Mechanisms underlying the modulation of L-type Ca2+ channel by hydrogen peroxide in guinea-pig ventricular myocytes

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**【序論および目的】**（適宜、項目をたてて、必ず２頁で記載する）

In the heart, ROS as highly reactive compounds accumulates in tissues during myocardial ischemia/reperfusion and cause peroxidation of lipids and proteins which play an important role in the pathogenesis of ischemia/reperfusion abnormalities, including myocardial stunning, irreversible injury, and reperfusion arrhythmias. The underlying mechanisms are not fully understood. In this study, we investigated effects of hydrogen peroxide (H2O2) on the Ca2+ channel using a patch-clamp technique in guinea-pig ventricular myocytes.

**【材料および方法】**

MgATP, Hydrogen peroxide (H2O2) tablet and 5,5'-dithiobis (2-nitrobenzoic acid) (DNTB) were purchased from Sigma-Aldrich, KN-93 (CaMKII inhibitor) and KN-92 (inactive analog of KN-93) from Calbiochem and Bay K 8644 (Ca2+ channel agonist) from Wako

Preparation of single cardiac myocytes and CaM and cell-attached and inside-out mode using the patch-clamp technique.

**【結　果】**

Fig. 1A and B, Ca2+ channel activity was rapidly increased without a change in the unitary current amplitude. In average of 6 patches, Ca2+ channel activity was increased to 206 ± 32 % of the control (Fig. 1D). This result confirmed the facilitating effect of H2O2 on LTCCs. Fig. 1C, KN-93 significantly attenuatedH2O2-mediated facilitation (132 ± 15 % (n=9) vs 206 ±32 % with no drug, $P<0.05$), while KN-92, an inactive analog of KN-93, partially attenuated the facilitation but it was not statistically significant (156 ± 13 % (n=5) vs 206 ± 32 % with no drug, $P=0.30$). These results suggested that not only CaMKII-dependent but also independent pathways were involved in H2O2-mediated facilitation of LTCCs.

Channel activity was changed by H2O2 from 144 ±32 % (control) to 272± 70 % in the absence of KN-93 (n=5), whereas from 139 ±21 % to 231 ±29 % in the presence of KN-93 (n=6). Although KN-93 seemed to slightly attenuate the increasing effect of H2O2 on channel activity, this difference was statistically insignificant. Thus KN93 did not significantly affect the H2O2-mediated facilitation of the Ca channel in the inside-out mode.

in Fig. 3A and B, after Ca2+ channel activity was maintained by intact (untreated with H2O2)

CaM + ATP in the inside-out patches, we substituted the oxidized CaM for the untreated CaM. Channel activity did not change significantly, suggesting that CaM was not oxidized or oxidized CaM,

in Fig. 4, 1mM DNTB significantly increased Ca2+ channel activity maintained by CaM (from 129 ±22 % up to 184 ± 21 %, n=5), suggesting that oxidation of cysteine residue(s) was responsible, at least partially, for the H2O2-mediated facilitation of LTCCs. we examined effects of H2O2 on LTCC after application of DTNB. Application of H2O2 + CaM after DTNB + CaM only slightly increased Ca2+ channel activity, which was statistically insignificant (Fig. 5)

**【結論及び考察】**

Thus our results indicate that, in addition to the CaMKII-dependent pathway, there is an additional CaMKII-independent pathway for the H2O2-mediated facilitation of LTCC. Since most of intracellular proteins are washed out in the inside-out patches, it is likely that direct oxidation of LTCC or its associated proteins by H2O2 might be involved in the facilitation of LTCC. Since H2O2-pretreated CaM does not mimic the facilitatory effect of H2O2, oxidation of CaM does not account for the mechanism of facilitation. It is consistent with that human CaM does not contain cysteine residue. Thus, it seems most likely that the Ca2+ channel protein itself undergoes direct oxidation by H2O2 as the CaMII-independent pathway of the LTCC facilitation.

Both cysteine and methionine residues are subject to oxidation by H2O2. The pore- forming subunit α1C of the cardiac LTCC is rich in cysteine and methionine residues in the cytoplasmic chains . Our findings that a specific cysteine oxidizing agent DTNB mimics the H2O2 effect and that the effect of subsequently applied H2O2 is largely occluded suggest that cysteine residues are involved in the H2O2-mediated facilitation. However, our data do not exclude a possible involvement of methionine residues. The future work should address to determine the oxidation sites responsible for the H2O2-mediated modulation of LTCC. In conclusion, LTCC may undergo ROS-mediated modification via the direct oxidation of LTCC as well as the indirect pathways involving CaMKII activation. This would be relevant for the understanding of ROS-mediated regulation of ion channels and Ca overload and arrhythmogenesis during oxidation stress on the heart.

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