

## 最終試験の結果の要旨

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主査および副査の5名は、平成25年7月10日、学位申請者 楊 磊 君に面接し、学位申請論文の内容について説明を求めると共に、関連事項について試問を行った。具体的には、以下のような質疑応答がなされ、いずれについても満足すべき(回答)を得ることができた。

質問1) Why did you use  $Ba^{2+}$  instead of  $Ca^{2+}$  for the recording of  $Ca^{2+}$  channel currents?

(回答) Because  $Ba^{2+}$  is more permeable to the channels than  $Ca^{2+}$ , and prevents  $Ca^{2+}$ -dependent inactivation of the channel. Our experiments were thereby facilitated very much.

質問2) In your internal solution, why did you use a high concentration of  $K^+$  and low concentrations of  $Na^+$  and  $Ca^{2+}$ ?

(回答) We aimed to make the internal solution whose ionic composition was similar to that of cytoplasm. Then, the resting membrane potential could be fixed to zero.

質問3) Can you record  $Ca^{2+}$  current without Bay K 8644 ( $Ca^{2+}$  channel agonist)?

(回答) Yes. However, we have used Bay K in our experiments, because Bay K prolongs the open time of the channel that makes a stable and reliable recording of the channel activity.

質問4) Does KN-93 have any effects on activity of the  $Ca^{2+}$  channel?

(回答) It is reported that a CaMKII inhibitor KN-93 can directly inhibit  $Ca^{2+}$  channels. However, low concentrations of KN-93 (<10  $\mu$ M) do not significantly inhibit the channel.

質問5) In your experiment, guinea-pig myocytes were used, but CaM was from human. How about the similarity between human CaM and guinea-pig CaM.

(回答) Homology between human CaM and guinea-pig CaM is very high. There are small difference in the nucleotide sequence, but no difference in the amino acid sequence.

質問6) Is  $H_2O_2$  permeable to membrane? Does  $H_2O_2$  directly pass through the cell membrane or via some other channels?

(回答) Yes,  $H_2O_2$  is permeable to cell membranes. It is suggested that  $H_2O_2$  does not pass through the lipid phase, but through aquaporins, water-permeable channels.

質問7) Do you think that the effect of  $H_2O_2$  is reversible? Can the channel be reduced by any reagent?

(回答) We think the effect may be reversible. It is reported that the effect of thiol-oxidizing agents on the  $\text{Ca}^{2+}$  channel is reversed by dithiothreitol (DTT). However, we have not tested the effect of DTT in this study.

質問8) Please explain production and metabolism of  $\text{H}_2\text{O}_2$ .

(回答) First, superoxide ( $\text{O}_2^{\cdot-}$ ) is generated from oxygen mainly by NADPH oxidase and the mitochondrial electron transport chain, and then,  $\text{O}_2^{\cdot-}$  is catalyzed by superoxide dismutase (SOD) to  $\text{H}_2\text{O}_2$ .  $\text{H}_2\text{O}_2$  is degraded to  $\text{H}_2\text{O}$  by catalase and glutathione peroxidase.

質問9) You treated CaM with  $\text{H}_2\text{O}_2$ . So, do you think that CaM can be oxidized by  $\text{H}_2\text{O}_2$ ? Is there any evidence in your experiment or some reports for oxidation of CaM by  $\text{H}_2\text{O}_2$ ?

(回答) Yes, it has been reported that the methionine residues in CaM is oxidized by  $\text{H}_2\text{O}_2$ . However, our aim was to examine whether a short time (10 min) was enough for  $\text{H}_2\text{O}_2$  to oxidize CaM, which was enough for  $\text{H}_2\text{O}_2$  to manifest the facilitation of the channels.

質問 10) Did you determine which cysteine residues is responsible for the  $\text{H}_2\text{O}_2$ -mediated facilitation of the  $\text{Ca}^{2+}$  channel?

(回答) No, not yet. In this study, we have found only that some cysteine residues are involved in the  $\text{H}_2\text{O}_2$ -mediated facilitation of the channel. In the future study, we would like to identify the cysteine residues responsible for the facilitation.

質問 11) Please explain how CaMKII facilitates the L-type  $\text{Ca}^{2+}$  channel?

(回答) CaMKII facilitates  $\text{Ca}^{2+}$  channel activity through phosphorylation of the channel protein. Both the main ( $\alpha 1\text{C}$ ) and accessory ( $\beta$ ) subunits of the channel are suggested to be phosphorylated, but it is still in discussion.

質問 12) How are you sure that oxidation of  $\alpha 1\text{C}$ , but not other subunits, is responsible for the  $\text{H}_2\text{O}_2$  effect.

(回答) We are not sure for this. We cannot exclude the possibility that  $\beta$ -,  $\alpha 2\delta$ -, and/or  $\gamma$ -subunit(s) is oxidized and thereby modulates activity of the channels.

質問 13) Is it possible that  $\text{H}_2\text{O}_2$  facilitates the  $\text{Ca}^{2+}$  channel through modulation the effect of Bay K 8644, but not through direct oxidation of  $\text{Ca}^{2+}$  channel?

(回答) We could not rule out this possibility. However, it is difficult to think that agonistic effect of Bay K 8644 is potentiated by oxidation, because the effects of dihydropyridines, such as nifedipine and Bay K 8644, are easily lost by photo-oxidation.

質問 14) Did you check whether a  $\text{Ca}^{2+}$  channel blocker influences the  $\text{H}_2\text{O}_2$ -induced facilitation of the  $\text{Ca}^{2+}$  channel?

(回答) No, not yet. We would like to examine this point in the future study.

質問 15)  $\text{H}_2\text{O}_2$  can oxidize proteins and lipids in the membrane. Which one is more sensitive to oxidation?

(回答) Generally speaking, proteins may be more sensitive to redox modification. However, for example, unsaturated lipids are very sensitive to the attack of the hydroxyl radical ( $\cdot\text{OH}$ ).

以上の結果から、5名の審査員は申請者が大学院博士課程修了者としての学力・識見を有しているものと認め、博士(医学)の学位を与えるに足る資格を有するものと認定した。