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９月５日、学位申請者 李　迎霄 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。

Question 1): Why did you choose mouse musclin rather than rat musclin for your experiment?

Reply: Mouse musclin and rat musclin are very similar. Rat coding regions of cDNAs were cloned by using sequence homology with mouse musclin. Amino acid sequence identity between mouse and rat is 90.2%. The musclin used in my experiments was prepared by professor Kato (Faculty of Pharmaceutical Sciences, Hokuriku University). They synthesized mouse musclin alone.

Question 2): In the experiment of anti-NPR-C antibody on musclin-induced aorta contraction (Fig. 2), why did you use 0.01 nmol/L musclin rather than higher concentration musclin, even if 1.0 nmol/L musclin is more effective in Fig. 1?

Reply: Zero point zero one nmol/L is the concentration which significantly induces aortic contraction. Thus, it is suitable to apply as the indicator for reaction with antibody. Otherwise, it would need more antibodies to block the action of musclin induced by higher concentration.

Question 3): In SHR aortic rings without endothelium, musclin-induced vasoconstriction was 82.16 ± 1.96%, in aortic rings with endothelium (82.58 ± 2.62%, $P > 0.05$; $n = 8$). But in Fig. 1, the effect of musclin on aortic strips isolated from SHR is about 50% - 80% as compare with that of WKY. Please explain the differences between the two experiments.

Reply: The response of vasoconstriction was induced by musclin at 1 nmol/L, which is the most effective concentration. There is no difference with that in Fig. 1 at the same concentration.

Question 4): In Fig. 7 showing the effect of musclin on blood pressure in rats, musclin was used at the dose of 0.05 mg/kg. Why did you choose this dose?

Reply: Musclin at 1 nmol/L is the maximal concentration in the experiment of using isolated aorta (Fig. 1). The organ distribution of pharmaceuticals in the rat is usually estimated using 7% of body weight for blood volume. After the unit conversion, musclin at 1 nmol/L is similar to at 0.05 mg/kg, we chose this concentration to do the blood pressure experiment.

Question 5): According to the NPR-C hypothesis, musclin would be expected to bind NPR-C, which would decrease the ability of NPR-C to degrade natriuretic peptides, which would increase local concentrations of natriuretic peptides, which would decrease blood pressure. However, your experiment showed exactly the opposite result. How do you explain that?

Reply: In the *in vitro* studies, we observed that musclin could induce vasoconstriction in aortic strips and increased the calcium concentrations in A7r5 cells. Both results were obtained without the involvement of natriuretic peptides. Therefore, it is reasonable to consider that muslin could trigger blood vessel contraction. The *in vivo* study, blood pressure was raised by an intravenous injection of musclin into rats. This action of musclin was only partially reduced by NPR-C antibody Ab14335. Thus, musclin may have own specific receptor in addition to NPR-C.

Question 6): What do you think the clinical applications in the future?

Reply: Musclin blockers would be a new pharmacological tool in the treatment of hypertension.

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Question 7): Musclin was first identified in 2004. Please introduce the developments in musclin research.

Reply: The recent research progress shows the function of musclin regarding the response to insulin in vivo and insulin resistance in vitro. Also, musclin is introduced as a bone-active molecule that is highly expressed in cells of the osteoblast lineage of animals. Moreover, musclin can competitively bind to NPR-C with atrial natriuretic peptide.

Question 8): In Fig. 6B, the experiment of cANP induced constriction in aortic tissues, the sample sizes in both WKY and SHR are n=6, but in all the other experiments they are n=8. Please explain that.

Reply: The experiment of cANP regarding constriction in aortic tissues is a supplemental experiment.

Question 9): Why did you focus on aorta to design your experiment?

Reply: Musclin is secreted from muscle tissues, because aorta is the biggest artery in the body, which means that it contains lots of smooth muscle cells. Aorta is correlated with blood pressure regulation. Thus, I applied it in my research.

Question 10): Can musclin bind to all three kinds of natriuretic peptide receptors or to one specific receptor?

Reply: In 2009, it was reported that musclin binds to NPR-C with high affinity, but not to others.

Question 11): In Fig. 4 regarding the different effects between musclin and cANP on calcium influx, why is the effect of cANP lower than that of musclin under the same concentrations?

Reply: cANP is known to bind specifically to NPR-C. Maack et al. (1987) reported that cANP had no direct effects on any of the known biologic effect of ANP. Because cANP had no response to the vasoconstriction, it has the lower ability to calcium influx than musclin.

Question 12): Comparing with Fig. 4A, Fig. 5, showed the higher expression of musclin in SHR than in WKY. How much is the concentration endogenous of musclin?

Reply: We did not measure the concentration of endogenous musclin, because musclin ELISA kit is not available. Therefore, this concern will be investigated in the future.

Question 13): The paper demonstrates that musclin can bind to NPR-C. Does it mean that musclin only bind to G protein-coupling receptor?

Reply: Actually, musclin can bind to NPR-C, which is a G protein-coupling receptor. However, the data also suggest that musclin may have its own receptor that should be investigated in the future research.

Question 14): Musclin is originally from skeletal muscle. Why did not you investigate the performance of musclin in skeletal muscle? What is the difference between aorta tissues and skeletal muscles?

Reply: The present study is aimed of the role of musclin in vascular function. The action of musclin in skeletal muscle is also very important. Therefore, the study will be conducted in the future research.

Question 15): Have you measured the mRNA expressions of musclin and NPR-C in rats?

Reply: In the present study, the gene expression level of musclin or NPR-C was assessed by Western blot analysis. There is no doubt that RT-PCR is a suitable tool to quantify the expression of mRNA. This concern will be investigated in the future.

Question 16): In your experiments, what kind of antibody did you choose? Monoclonal antibody or polyclonal antibody?

Reply: NPR-C antibody (ab14355) is the rabbit polyclonal antibody. Musclin antibody (FL-133) is a rabbit polyclonal IgG.

Question 17): What happens if non-specific antibodies are injected or antibodies that have been pre-blocked with the musclin peptide are added to vessel preparations or whole animals?

Reply: The IgG was used as non-specific antibody. Treatment with IgG (100μg/ml) at 1:2500 dilution in aortic strips from SHR did not modify the musclin-induced vasoconstriction. Also, treatment with IgG at 1:500 dilution did not influence the blood pressure in SHR. These results showed that non-specific antibodies did not affect the outcomes in this report.

Question 18): What is the dosage of musclin antibody for each group in Fig. 8?

Reply: The volumes of musclin antibody diluent for intravenous injection into rats were 1ml/kg. The original concentration of musclin antibody was 200μg/ml, and then diluted with different ratios 1:5000, 1:1000, and 1:500.

以上の結果から、５名の審査委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。