

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、令和2年2月20日、学位申請者 Nguyen Thanh Trung 君面接し、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。

1. Do you think that 2-day fasting in mice is too long?

A: This method was implemented as previously described (Ahima., 1996, Gavrilova., 1999, Williams., 2012)

2. Whether the 7g body weight loss is good for animal welfare?

A. All experiments were carried out with male littermates or CD-1 mice that were approximately 8–13 weeks old. So, around 7g body weight loss (approx. 20% from original body weight) may not affect on animal welfare.

3. Where were AgRP and POMC expressed? Were they expressed in the PVH, VMH or ARC?

A. Co-expression of AgRP and NPY is found only in the ventromedial ARC neurons. In parallel, in the ventrolateral ARC, there are neurons that co-express CART and POMC. AgRP and POMC were expressed in ARC specifically, which means PVH and VMH do not express these neuropeptides. Even if we obtained the whole hypothalamus including PVH, VMH and ARC, AgRP and POMC expressions were originated from the ARC.

4. The mRNA levels of AgRP in PACAP (-/-) mice inhibited food intake 4 h after refeeding. However, food intake 4 h after refeeding was not changed between PACAP (-/-) and (+/+).

A. Although food intake of PACAP (-/-) mice 4 h after refeeding showed a tendency to decrease as compared with that of PACAP (+/+) mice, it should be noted that the reduction of food intake of PACAP (-/-) 8 h after refeeding showed statistically significant.

5. Are there any differences between daily and post fasting food intake?

A. As you mentioned, the food intake after fasting for 2 days do not occur in our daily life, thereby the situation of mice was different. Indeed, CRH, stress-related neuropeptide expression, was significantly increased in the hypothalamus of PACAP (-/-) mice, which suggested stress response might be involved in the fasting experiment. However, we need the further experiments to clarify the detail of the difference of daily and post-fasting food intake.

6. What is the different function between PACAP38 and PACAP27?

A. PACAP27 was shown to display the similar biological activity as compared with PACAP38, but they have a different peripheral distribution.

7. Can PACAP (-/-) mice grow normal?

A. PACAP (-/-) mice had previously been generated, backcrossed into a CD-1 background and had a high surviving rate. However, in a C57BL/6 background, PACAP (-/-) mice have high mortality during juvenile because of mal-adaptation to the ambient temperature.

8. Why you did not see any differences in body weight between two genotypes?

A. The reason why body weight is not changed between two genotypes may be due to the decreased energy expenditure in PACAP (-/-) mice. It was previously reported that the expressions of UCPI and Pgc1a, which contribute to the basal metabolism, were decreased in the brown adipocyte tissue of PACAP (-/-) mice than that of PACAP (+/+) mice (Bruce AA, 2008)

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9. Do PACAP inhibitors have any side effects? Is it possible for clinical application?

A. As far as we examined, any abnormal behavior were not observed after i.c.v. injection of PAC1R inhibitor, and it is possible to apply PACAP inhibition as a potential strategy for the development of anti-obesity drugs.

10. Did you check hourly food intake for 8 h after refeeding?

A. We examined it and these results indicated that food intake was significantly elevated at all-time points after refeeding as compared to that in non-fasted control mice. In addition, we have evidence that food consumption increased continuously for 8 h compared with 4 h after refeeding.

11. How about the circadian cycle? Were PACAP and PAC1R expression changed during the day?

A. In general, mice will eat more in the nocturnal and less in the diurnal periods regarding the circadian cycle. To the best of our knowledge, PACAP and PAC1R expression remained unchanged during the day.

12. How was the water intake? Did you allow mice to free access to water during fasting?

A. We did not examine the water intake in our studies. Feeding studies were performed in home cages with ad libitum access to water during fasting.

13. How about circadian or stress responses on feeding behavior?

A. It is reported that circadian cycle is normal in PACAP (-/-) mice (Dragich et al., 2010), but there is a possibility that the stress responses may be involved in feeding behavior.

14. Do you think overexpression is the good way to study endogenous functions of PACAP?

A. Yes, we demonstrated the benefit of overexpression for studying the endogenous PACAP in our studies.

15. What are the differences of exogenous injection of PACAP and AAV injection?

A. I think one clear difference would be a time course. PACAP injections can be used to see acute or transient effect of the drug while the overexpression can be used to test the chronic effect of gene product.

16. For elevated-plus maze test, how about the total entry?

A. We found the number of entries onto the open arms in the elevated-plus maze test was increased in PACAP (-/-) as compared PACAP (+/+) in our experiments.

17. Did you check the blood glucose or insulin level?

A. We did not check blood glucose or insulin level in our studies. Further experiments should be needed to clarify this point.

18. Did you check Orexin or CRH expression?

A. We found that CRH expression was significantly increased during the fasting and refeeding condition in PACAP (+/+) and (-/-) but no difference was observed between two genotypes. However, Orexin expression only significantly increased in (+/+) in our experiments.

19. How about the diurnal and nocturnal food intake in the mice applied with DREAD-suppression in VMH?

A. We checked the daily food intake but no difference was observed due to the short plasma half-life of CNO in mice.

20. Why did you refeed animals during the day? I guess you may see more enhanced effects during the night. What do you think?

A. We also thought that more enhanced effects will be clearly observed during the night. But further experiment is needed to clarify the effect of time of day with regard to the post-fasting feeding.

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。