学位論文内容の要旨
Abstract of the dissertation

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学位論文題名
The title of the doctoral dissertation

Evaluation of the Functional Expression of Otopetrin 1 (OTOP1), a Proton Channel, in Brown Adipocytes
（プロトンチャネルである Otopetrin 1（OTOP1）の褐色脂肪細胞での機能的な発現の評価）

Otopetrin 1 (OTOP1) is a proton (H^+) channel that mediates acidic taste perception in type III taste receptor cells. According to data from an mRNA expression database, *Otop1* shows high expression in mouse brown adipose tissue (BAT). BAT is a thermogenic organ composed of brown adipocytes (BAs), which play a pivotal role in non-shivering thermogenesis. A study using *Otop1* knockout (KO) mice suggested OTOP1 could influence BAT function in certain conditions. However, critically, it has not been investigated whether OTOP1 is functionally expressed in BAs. Demonstrating that OTOP1 is functionally active in BAs is essential for interpreting the *Otop1* KO results and for elucidating its role in BAs. Therefore, in this research, the functional expression of OTOP1 was evaluated in BAs using *Otop1* KO mice.

In Chapter 1, a library of pharmacologically active compounds, LOPAC^{1280}, was screened to identify potential inhibitors of OTOP1 using whole-cell patch-clamp electrophysiology and a heterologous overexpression system. Although zinc (Zn^{2+}) has been used previously to block OTOP1 H^+ currents, its inhibitory effect requires millimolar concentrations and is influenced by experimental conditions such as anion composition, membrane potential, and extracellular pH. In addition, cellular toxicity at high Zn^{2+} concentrations led to the search for more suitable inhibitors. Through this screening, Cibacron Blue 3G-A was identified as a novel and potent

inhibitor of OTOP1. Unlike Zn^{2+}, Cibacron Blue 3G-A inhibited OTOP1 currents at micromolar concentrations, and its inhibition was not affected by the aforementioned experimental factors. Despite the limitation that Cibacron Blue 3G-A may have off-target effects, its discovery will facilitate the evaluation of OTOP1's functional expression.

In Chapter 2, the functional expression of OTOP1 in BAs and its potential physiological role in BAT were evaluated using *Otop1* knockout (KO) mice. Whole-cell patch-clamp recordings revealed that wild-type (WT) BAs displayed H^+ currents, which were absent in *Otop1* KO BAs. The H^+ currents in WT BAs were inhibited by the OTOP1 inhibitors, Cibacron Blue 3G-A and Zn^{2+}. Cytosolic pH measurements revealed *Otop1*-dependent pH reduction in response to application of acidic solutions. These results indicate that OTOP1 is active on the plasma membrane of BAs. Additionally, the oxygen consumption rates were compared between WT BAs and *Otop1* KO BAs, but no clear involvement of OTOP1 was observed. Although the roles of OTOP1 in BAs were not clarified, this study provides evidence of endogenous activity of OTOP1 in BAs. The present findings allow future studies of OTOP1's function in BAs to proceed under the assumption of its endogenous activity. Moreover, BAs can serve as a useful cellular model for investigating the function of endogenously expressed OTOP1, alongside taste receptor cells.

In conclusion, this study identified Cibacron Blue 3G-A as a novel inhibitor of OTOP1 and demonstrated that OTOP1 is functionally expressed at the plasma membrane of BAs. Although the physiological role of OTOP1 in BAs remains unclear, the findings presented here are expected to advance future research into both OTOP1 and BAs.