

## Development and Application of Evaluation Methodology for Salt Taste Preference

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### Summary

Salt is essential for enhancing the palatability of food. Excessive intake of salt, however, is an indirect cause of lifestyle-related diseases such as high blood pressure, and hence, salt intake must be regulated. Researchers have attempted to develop a substitute for salt for many years but have not yet succeeded in developing a substitute with equivalent palatability. It is said that there are at least 2 types of salty taste: one is palatable salty taste, or the other is not palatable one. Therefore, we tried to evaluate the palatability of saltiness on the basis of its ethology and molecular physiology.

Initially, we evaluated saltiness by using an ethological approach. We developed mice that prefer saltiness, as *Homo sapiens* do, by limiting the intake of sodium chloride and diuretic agents and evaluated the palatability of potassium chloride and lithium chloride by counting the number of times that the mice licked either solution. We found that when potassium chloride, whose palatability is low, was added to sodium chloride, the mice did not lick the solution any more than they licked the pure sodium chloride solution. In contrast, when lithium chloride was added to sodium chloride, the mouse licked the solution more number of times, which is indicative of increased palatability. Similarly, when glycine ethyl ester, which is known to have a saltiness-enhancing effect, was added to sodium chloride, the mice licked the solution more often, which again is indicative of increased palatability.

We also tried to establish a saltiness evaluation method by using cultured cells expressing epithelial sodium channel (ENaC), which is a candidate receptor in saltiness perception. Our aim was to evaluate the taste of food materials easily. We used cultured cells because they are easy to handle, and developed a saltiness evaluation method by using cultured cells and fluorescence imaging technique. We first studied the experimental conditions and found that the response to saltiness was observed when the genes for ENaC  $\alpha$ ,  $\beta$ , and  $\gamma$  subunits were expressed transiently in CHO cells. Subsequently, we measured the response from the ENaC-expressing CHO cells when glycine ethyl ester was added to the sodium chloride solution and found that there was no increase in the intensity of the response to saltiness due to sodium chloride. Our results suggest that ENaC might not be involved in increasing the saltiness when glycine ethyl ester is added and that other molecules could be responsible for it. The evaluation method used in this study however has a limitation: the responses of the culture cells were unstable, which made quantitative and efficient evaluation difficult. We hope to resolve this problem in the future.