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Construction of Assay System for Salt Preference and Analyses of Regulatory Mechanism of Salt Homeostasis in Model Fish Species

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Summary

Although both human and fresh water fish belong to vertebrate class, their osmotic circumstances are different. Human lose salts through perspiration and obtain them from food. Fresh water fish are under the threat of salt shortage because of low osmotic potential of the circumstance and always trying to obtain salts via gill and digestive tract. However, mechanism involved in the behavioral regulation of salt uptake remains to be elucidated. To analyze the regulation of preference toward salt in vertebrates, we aimed to develop behavioral assay system for salt preference of fresh water fish, medaka. Citric acid was used for standard tastant because of its physical property similar to that of sodium chloride and its easy detection by monitoring pH. Two kinds of foods were produced taking retaining ability of water soluble tastants into consideration. One is the water in oil (W / O) type food where citric acid and molten agar was dispersed into palm oil by detergent. This emulsion was sprayed and the resulting particles were fed to one month old medaka larvae together with those containing no tastant. The larvae showed no interest to the food after pecking it several times. Next we examined oil in water (O / W) type food labeled with two kinds of fluorescent dye (DiI and DiO) each of which corresponds to the presence and the absence of tastant. As the control experiment, Denatonium (DN), which has been proved to be avoided by medaka, was included into the food. Three groups of larvae were fed with 1) DN food labeled with DiI plus no tastant food labeled with DiO, 2) DN food labeled with DiO plus no tastant food labeled with DiI and 3) no tastant food labeled with DiI plus no tastant food labeled with DiO, respectively. Then the ratio of ingested food containing tastant were analyzed in each larvae. As a result, aversion to DN was detected as the smaller ratio of DN food ingestion in group 1 than that of no tastant food in group 3. The same behavioral assay were performed using citric acid resulting in the detection of no significant difference of ingestion ration of citric acid food. This may results from higher efflux rate of citric acid than expected. We will conduct this two color labeled assay using water insoluble acid such as alginic acid and polygalacturonic acid and their salts.