《Research Note》

Behavioral Responses of Chicks to a Saccharin-Quinine Mixture

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To date, few reports have been published on the sensitivity of birds to sweet tastes. Therefore, in this study, the behavioral responses of White Leghorn chicks to the sweet taste of saccharin and the bitter taste of quinine were assessed. Three chicks were provided with a solution of 3.0 mM quinine and a mixture of 3.0 mM quinine mixed with 0.1, 0.5, 1.0, or 10.0 mM saccharin in a two-bottle choice test for 48 h. It was found that the chicks consumed more of the 0.5 mM saccharin/3.0 mM quinine mixture but significantly less of the 10.0 mM saccharin/3.0 mM quinine mixtures than the quinine solution alone ($P < 0.05$). The aversive behavior of 3.0 mM quinine solution was eased when mixed with 0.5 mM saccharin, indicating that chicks are detecting the sweetness associated with the 0.5 mM saccharin. The aversion to the 1.0 and 10.0 mM saccharin solutions might be stronger than to the 3.0 mM quinine solution alone. These findings suggest that chicks are able to detect this artificial sweetener.

Key words: chick, leghorn, quinine, saccharin, taste

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Introduction

Taste is an important sense for recognizing nutritional value and discriminating between safe and dangerous foods. Taste receptors are able to detect five different tastes: salty, acidic, sweet, umami, and bitter. Among these, a bitter taste is related to harmful and toxic substances (Go *et al.*, 2005). The sensitivity of mice to sweet and umami tastes has previously been investigated using mixtures of sweet or umami substances and quinine hydrochloride (QHCl), which is an aversive, bitter-tasting substance, and have demonstrated that sweet or umami flavors can mask the bitter taste (Hsiao and Fan, 1993; Contreras *et al.*, 1995; Murata *et al.*, 2003). However, large differences in sensitivity to sweet and umami substances have been observed among mouse strains. It has been reported that G protein-coupled receptors in the taste receptor type 1 (T1R) family are related to sweet and umami tastes. Located on the 4th chromosome in mice and on the 1st chromosome in humans, T1R3 is a SAC receptor gene that confers a sensitivity to saccharine (Hoon *et al.*, 1999; Kitagawa *et al.*, 2001; Max *et al.*, 2001; Montmayeur *et al.*, 2001; Sainz, *et al.*, 2001). However, it has been proposed that, though the T1R2/T1R3 heterodimer detects sweetness, the T1R1/T1R3 heterodimer responds to umami taste in humans, suggesting that T1Rs are critical for both sweet and

umami tastes (Max *et al.*, 2001; Nelson *et al.*, 2001).

In chicks, the gustatory system appears to function before hatching, and newly-hatched chicks are able to sense taste. Chickens have three bitter taste receptors that can be activated in a cell-based assay: T2R1, T2R2, and T2R7 (Behrens *et al.*, 2014; Hirose *et al.*, 2015). It has previously been shown that chicks consume lower amounts of a quinine solution than they do water, indicating that they show an aversion to bitter-tasting substances (Gentle, 1975). Although it has been considered that birds are also able to taste sweetness, there have been few reports of chicks sensing the taste of artificial sweeteners. Therefore, the present study examined the behavioral responses of White Leghorn chicks to an artificial sweetener by offering them a choice between a solution of quinine mixed with saccharine and quinine alone in a two-bottle choice test.

Materials and Methods

Behavioral Responses of Chicks to Saccharin

Three male White Leghorn chicks (Japan Layer, Gifu, Japan) aged 2–20 d were placed in a cage (20 cm × 18 cm × 16 cm) in a windowless room under 24-h lighting and a temperature of 30°C. The food was free intake. From days 2–4, the chicks were provided water from a bottle (CLEA Japan, Tokyo, Japan) with a nipple drinker for chicks used in the poultry industry. From days 5–20, chicks were provided with solutions of 3.0 mM quinine (Quinine Hydrochloride Dihydrate: Tokyo Chemical Industry, Tokyo, Japan) and a solution of 3.0 mM quinine mixed with either 0.1, 0.5, 1.0, or 10.0 mM saccharin (Saccharin Sodium Salt Hydrate: Sigma-

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Fig. 1. Mean fluid intakes (g) of a 0.1 mM saccharin/3.0 mM quinine mixture and a 3.0 mM quinine solution by chicks in a two-bottle choice test. Data were obtained from three chicks and are expressed as means $\pm$ SE. Means with different letters signify significant differences ($P < 0.05$). A: 0.1 mM saccharin/3.0 mM quinine. B: 0.5 mM saccharin/3.0 mM quinine. C: 1.0 mM saccharin/3.0 mM quinine. D: 10.0 mM saccharin/3.0 mM quinine.

Aldrich, Tokyo, Japan) for 48 h in bottles with nipple drinkers. The behavioral responses of the chicks to the drinking solutions were then compared using a two-bottle choice test by recording the amount of solution that was consumed by each chick. To prevent their position from affecting choice, the bottles were switched after 24 h. The examination was repeated five times, and the amount of intake solution was measured every 24 h.

Statistical Analysis

The data were analyzed using Student's t-test to determine whether there was a significant difference between treatments. Results are presented as means $\pm$ SE.

Results and Discussion

The amounts of the saccharin/quinine mixtures and quinine

solution alone that were consumed in each choice test were: 48.2 ± 7.5 g vs. 50.9 ± 9.4 g, respectively, for the 0.1 mM saccharin mixture; 73.0 ± 7.9 g vs. 48.3 ± 10.6 g, respectively, for the 0.5 mM saccharin mixture; 35.5 ± 9.8 g vs. 60.15 ± 9.4 g, respectively, for the 1.0 mM saccharin mixture, and 34.0 ± 6.8 g vs. 64.5 ± 10.3 g, respectively, for the 10.0 mM saccharin mixture (Fig. 1). The chicks consumed significantly less of the 1.0 and 10.0 mM saccharin mixtures than the 3.0 mM quinine solution ($P < 0.05$). This result indicates that taste may affect food intake, which would subsequently affect the health of the chicks. Compared to the 3.0 mM quinine, the chicks consumed twice as much of the 0.5 mM saccharine mixture ($P < 0.05$) but half as much as the 10.0 mM saccharine mixture ($P < 0.05$) (Fig. 2).

It has been previously shown that mice exhibit a weak

Fig. 2. **The relative change of fluid intakes of a saccharin/quinine mixture and a 3.0 mM quinine solution that designates the quinine as standard.** Means with different letters signify significant differences ($P < 0.05$) by comparison with quinine.

aversion to the bitter taste of quinine, which is alleviated by the addition of sweeteners (Bachmanov *et al.*, 2001). Mixtures of sweet and bitter compounds have also been used in various other behavioral experiments, demonstrating that beagles are able to taste saccharin when it is mixed with quinine (Furuta *et al.*, 2010). Although it has been found that chickens avoid bitter tastes (Gentle, 1975; Furuta *et al.*, 2008), there have been few reports on their ability to detect sweetness when artificial sweeteners are mixed with quinine, as observed in mice.

In this experiment, no significant changes were observed amount of the mix solution and the QHCl to compare with the 1st day and the 2nd day. Bottle positioning had no influence on the results of this experiment. It was clear that 0.1 mM saccharin was not sufficient for chicks to detect a difference from quinine alone. By contrast, there was a tendency for chicks to drink more of the 0.5 mM saccharin mixture than quinine alone ($P > 0.05$), indicating that they could detect the sweetness of the saccharin, which weakened their aversion to the quinine. However, the chicks consumed significantly less of the 1.0 and 10.0 mM saccharin mixtures ($P < 0.05$), possibly due to the combination of sweet and bitter tastes also being aversive to them. Similarly, it has been reported that beagles are more averse to the taste of a mixture of saccharin and quinine than to the bitterness of quinine alone (Furuta *et al.*, 2010).

These findings indicate that White Leghorn chicks are able to detect the artificial sweetener saccharin. Since different strains of mice have different sensitivities to sweetness, it is conceivable that different breeds of chickens would also exhibit such differences. This experimental method should be used to investigate the sweetness sensitivities of chicken breeds, as such information would be useful in improving chicken feed by catering to the preferences of the different breeds.

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