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Comparison of differences between PWD/PhJ and C57BL/6J mice in calcium solution preferences and chorda tympani nerve responses

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ABSTRACT

We used the C57BL/6J (B6) and PWD/PhJ (PWD) mouse strains to investigate the controls of calcium intake. Relative to the B6 strain, the PWD strain had higher preferences in two-bottle choice tests for $CaCl_2$, calcium lactate (CaLa), $MgCl_2$, citric acid and quinine hydrochloride, but not for sucrose, KCl or NaCl. We also measured taste-evoked chorda tympani (CT) nerve activity in response to oral application of these compounds. Electrophysiological results paralleled the preference test results, with larger responses in PWD than in B6 mice for those compounds that were more highly preferred for the former strain. The strain differences were especially large for tonic, rather than phasic, chorda tympani activity. These data establish the PWD strain as a "calcium-preferring" strain and suggest that differences between B6 and PWD mice in taste transduction or a related peripheral event contributes to the differences between the strains in preferences for calcium solutions.

1. Introduction

Calcium is an essential nutrient required by the body for diverse physiological activities, and it is therefore not surprising that animals consume calcium when they need it [32]. The sense of taste plays an important role in this behavior. For example, calcium-deprived rats have elevated intakes of $CaCl_2$, even if there is minimal absorption of the solution [7,20,23]. Calcium deprivation also changes taste-evoked responses and measures of taste reactivity, so the taste of calcium appears to change based on the need for the mineral [14,21,23].

In human subjects, the acceptance of green vegetables is inversely related to their calcium content [35], presumably due to calcium ions contributing a bitter taste to the foods. However, calcium, does not always taste purely bitter or unpleasant. Some mouse strains avidly consume solutions or vegetables with a high calcium content [2,36,37], and people with disturbances of calcium metabolism prefer foods high in calcium [18,33]. Human subjects attribute bitter, salty, sour, and sweet tastes to $CaCl_2$ and calcium lactate when asked to make ratings based on these primary taste qualities [17,31], but there is also evidence that calcium-containing compounds have a unique taste quality that is distinct from bitterness, saltiness, sourness, sweetness, or umami taste [28]. This psychophysical evidence implies that there may be a specific gustatory transduction mechanism for calcium that is distinct from those for other taste qualities. This view is also supported by work using inbred mouse strains and their segregating generations, which show low correlations between preferences for $CaCl_2$ and for representatives of primary taste qualities [2,34,36,37] and by the presence of a population of chorda tympani nerve fibers in mice that respond more vigorously to calcium and magnesium than to exemplars of bitter, salty, sour, or sweet taste qualities [24].

One possible taste transduction mechanism for calcium involves the calcium-sensing receptor (CaSR). CaSR is functional in amphibian taste [26] and is expressed in rodent taste bud cells [9,37]. Binding of Ca^{2+} ions to CaSR in taste bud cells could be responsible for the aspects of calcium's taste that are shared with magnesium but not other minerals, because the receptor is selective for Ca^{2+} and Mg^{2+} ions [4]. The gene, *Tas1r3*, which codes for the protein T1R3, is also hypothesized to be important for calcium taste. Recent work suggests that the pronounced preference for calcium by the PWK/PhJ (PWK) strain is due to polymorphisms of *Tas1r3*, and mice that lack *Tas1r3* show less avoidance of $CaCl_2$ than do B6 mice with an intact gene, possibly because binding of Ca^{2+} to T1R3 imparts a negative taste [38]. There is also a possibility that CaSR and T1R3 dimerize to form a gustatory receptor for calcium, given that T1R3 is known to dimerize with the T1R1 and T1R2 proteins to form receptors for umami and sweet taste, respectively [39].

Nonetheless, there remain unanswered questions related to calcium taste transduction and appetite. For example, although T1R3 may be involved in the unpleasant aspect of calcium's taste, *Tas1r3*-knockouts prefer some concentrations of $CaCl_2$, implying there is an alternate mechanism involved in the positive taste of calcium

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[38]. In addition, QTL mapping using the B6 and PWK strains has identified several additional markers involved in preferences for calcium [37]. Moreover, the involvement of taste sensation in guiding calcium intake does not rule out additional contributions from non-gustatory mechanisms, such as postingestive feedback.

In previous work, we have demonstrated that the PWK/PhJ strain has a strong preference for calcium-containing solutions [2,37]. In the current work, we use PWD/PhJ (PWD) mice which, like the PWK/PhJ strain, are of *Mus m. musculus* origin as opposed to more commonly used laboratory mouse strains derived from *Mus m. domesticus* origin [11]. However, the PWD mice offer an advantage over PWK/PhJ mice in having available consomic strains on a B6 background [12], which will facilitate future genetic analyses. Here, we characterize preferences for taste solutions in PWD mice for the first time. We also measure taste-evoked responses in the chorda tympani (CT) nerve in these strains, in order to examine the contribution of peripheral gustatory mechanisms in isolation.

2. Methods

2.1. Experiment 1: Behavioral testing

2.1.1. Subjects

Two-bottle tests were performed in 18 C57BL/6J (B6) and 17 PWD/PhJ (PWD) mice (8 males for each strain and the rest females). All animals were purchased from The Jackson Laboratory (Bar Harbor, ME). Procedures were approved by the Animal Care and Use Committee of the Monell Chemical Senses Center. Mice were kept at 23–26 °C with a 12:12 h light:dark cycle (lights off at 7 PM) and were maintained on AIN-76A diet (Dyets, Bethlehem, PA, cat. no. 100000) and tap water *ad libitum*.

2.1.2. Preference tests

Details of drinking tube construction, cage layout, and the position of drinking tubes during tests are described elsewhere (http://www.monell.org/MMTPP/). The mice were given 48-h access to two drinking tubes, one of which contained water and the other a taste solution. The positions of the tubes were switched at 24 h. Preference scores were defined as the total solution consumed in 48 h divided by the total fluid (solution plus water) consumed. Although our main interest in this experiment was calcium-containing stimuli, we used a broader array of compounds in order to examine the specificity of any observed strain differences, and we chose concentrations that would be unlikely to cause extremes of avoidance or preference, but that would be likely to reveal differences between mouse strains. Solutions used were 50 mM $CaCl_2$, 50 mM calcium lactate (CaLa), 50 mM $MgCl_2$, 50 mM KCl, 100 mM NH_4Cl, 100 mM NaCl, 5 mM citric acid, 30 μM quinine hydrochloride (QHCl), and 8% sucrose. A day with access to a single bottle of water was interposed between each 48-h test.

2.1.3. Analysis

A two-way mixed ANOVA was performed to assess whether the strains differed in general in their taste preferences (effect of strain) and whether they differed on preferences for certain solutions (strain × concentration interaction). Preliminary analyses also included Sex as a factor, but there were no differences between males and females so the results presented here are for both sexes combined. Post-hoc *t*-tests were used to assess strain differences in preferences for individual compounds. For all tests $p<0.05$ was considered to be significant.

Within each strain, preference scores for each compound were also assessed relative to 50% (i.e., indifference relative to water) by calculating the 95% confidence interval around the mean. Overlap between the confidence interval and 50% indicated indifference, whereas intervals that were higher or lower than 50% indicated significant preference or avoidance of the compounds, respectively.

2.2. Experiment 2: Electrophysiology

2.2.1. Subjects

Measurements of CT activity were made in 6 male B6 and 6 male PWD mice. All animals were purchased from The Jackson Laboratory (Bar Harbor, ME). Procedures were approved by the Animal Care and Use Committee of Ball State University. Mice were kept at 23–26 °C with a 12:12 h light:dark cycle (lights off at 7 PM) and were maintained on AIN-76A diet (Dyets, Bethlehem, PA, cat. no. 100000) and tap water *ad libitum*. Mice did not receive stimulus solutions prior to electrophysiology to ensure that they had similar gustatory backgrounds.

2.2.2. Surgery

Each animal was anesthetized with a mixture of ketamine, xylazine and acepromazine (90, 20, and 3 mg/kg, respectively; i.p., with further doses as necessary). A tracheotomy was performed to prevent suffocation, and the animal was placed supine with the head secured in a non-traumatic head holder. In all animals, the CT nerve was accessed through the right ear by puncturing the tympanic membrane and exposing the right CT nerve adjacent to the malleus [6]. An electrode made of platinum/iridium wire was placed on the nerve, and the multiunit signal was amplified, filtered, rectified and integrated with a time constant of 1.0 s. A few drops of mineral oil were placed in the wound site at the vicinity of contact of the nerve with the electrode to prevent desiccation of the nerve. An indifferent electrode was positioned in nearby muscle tissue.

In previous experiments we used a different surgical approach to access the CT ventrally through the neck. We were interested in whether type of surgery might affect the size of the taste-evoked responses, and so we compared the results for B6 mice in the current experiment with those of B6 mice from a prior study [38]. Identical taste compounds and stimulation procedures were used in the two experiments. We performed a two-way ANOVA and did not observe a significant difference in the size of responses (effect of surgical method and method × chemical interaction, n.s.).

2.2.3. Stimuli and delivery

The following taste stimuli (mixed in distilled water) were applied on the tongue with deionized water as background rinse: $CaCl_2$ at 0.1, 1, 10, 100 mM; CaLa, $MgCl_2$, NaCl and KCl, all at 10 and 100 mM; 500 mM sucrose; 10 mM citric acid; 20 mM QHCl. Concentrations were not matched exactly with those in experiment 1, but rather were chosen to be more comprehensive and so that they would include at least one for each compound that would evoke a robust multiunit CT response. The order of application of compounds was random, except that different concentrations of a given compound were applied in ascending order. In addition, 100 mM NH_4Cl mixed in distilled water was applied at regular intervals throughout the entire process to serve as a reference stimulus. The anterior tongue was placed in a flow chamber, and deionized water rinse and stimulus solutions were applied by continuous flow with a rate of 0.5 ml/s. The rinse and all stimulus solutions were presented at room temperature. Each stimulus presentation lasted for 20 s and was followed by at least 60 s of rinse.

When possible, stimuli were reapplied for a given CT preparation, and relative response sizes were averaged across all applications. Across the entire experiment, the Pearson product moment correlation between the responses sizes of the first and second applications was +0.92, indicating that there was a high degree of stability in our preparations.

2.2.4. Analysis

Three response types were calculated for each stimulus application: 10-s net, peak, and tonic. Ten-s net values were based on the area-under-the-curve of the integrated voltage for 10 s after stimulus