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Effects of Mother's Dietary Exposure to Acesulfame-K in Pregnancy or Lactation on the Adult Offspring's Sweet Preference

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Abstract

This study investigates whether mother's exposure to the artificial sweetener acesulfame-K (AK) during pregnancy or lactation affected her adult offspring's sweet preference. It was found that mother's dietary exposure to AK in pregnancy or lactation decreased the preference thresholds for AK and sucrose solutions in the adult offspring, whereas the preference pattern and the most preferred concentration for AK or sucrose solution were unchanged. Furthermore, the preference scores in the exposure groups were increased significantly when compared with the control group at a range of concentrations for AK or sucrose solution. The existence of AK and its dynamic changes within 24 h in amniotic fluid during pregnancy or in mother's milk during lactation after a single oral infusion of AK solution were revealed by the methods of reversed-phase high-performance liquid chromatography and mass spectrometry. Our data suggest that AK can be ingested by the prenatal or postnatal mice through their mother's amniotic fluid or breast milk, producing a long-dated function on the adult's sweet preference.

Key words: acesulfame-K, amniotic fluid, mouse, taste

Introduction

The individual difference in food preferences and eating habits, as one of the most mysterious phenomena in human behaviors, has not been fully understood yet (Ganchrow et al. 2003; Mennella et al. 2004). Lines of evidence have demonstrated that both genetic differences (Duffy and Bartoshuk 2000;Drewnowski et al. 2001) and experiences (Elston and Timms 1992; Schaal et al. 2000; Smriga et al. 2002) are involved in the formation of food preferences. Specifically, the early experience of taste changes (e.g., exposure to different flavors in amniotic fluid and mother's milk) may underlie the individual differences in food acceptability throughout the life span (Mennella and Beauchamp 1999; Mennella et al. 2001; Smriga et al. 2002; Mennella and Beauchamp 2005; Beauchamp and Mennella 2009).

From an evolutionary perspective, heightened preferences for sweet-tasting food is the most important biological function because sweetness is associated with readily available calories from carbohydrates in nature (Glendinning 1994). Acesulfame-K, as a high-intensity and noncaloric sweetener, is currently used in food, beverage, oral hygiene, and pharmaceutical products in nearly 90 countries (Armenta et al. 2004). In this study, we hypothesized that mother's dietary exposure to the artificial sweetener AK might affect the adult offspring's sweet preference if AK could be transferred to the amniotic fluid or breast milk and transported to the pups. We established a particular mouse model system with early exposure to AK-heightened sweet diets during pregnancy or lactation. The sweet preference patterns and preference

scores of the mice offspring were then detected by the 2-bottle preference (TBP) tests in their adulthood. Moreover, reversed-phase high-performance liquid chromatography (RP-HPLC) and mass spectrometry were used to monitor the existence of AK and its dynamic changes within 24 h in amniotic fluid or in mother's milk after a single intragastric administration of AK solution.

Materials and methods

Animals

Male and female ICR mice, purchased from Zhejiang Academy of Medical Sciences (China), were used in this study. The National Research Council's Guide for the Care and Use of Laboratory Animals was followed. All mice were housed in the animal quarters of the Biology Department at the Institute of Technology and had free access to deionized water and normal formula (Purina Lab Chow) (24.5% protein, 50.3% carbohydrate, and 4.4% fat; 3.93 kcal/g gross energy; 0.31% sodium, 0.99% potassium, and 1.46% calcium). Room temperature was approximately 23 °C, with 50% humidity. The schedule of lighting was maintained on a 12-h light–dark cycle, with the light phase beginning at 8:00 AM. Three females and one male, 8-week old, were placed in one cage at approximately 5:00 PM. Mating was indicated by the presence of sperm in a vaginal smear the following morning, defined as gestational day 0. The pregnant mice were single caged from gestational day 6. All pups were weaned from their mothers on postnatal day (PD) 21. Male pups were chosen from 6 different mothers and then housed in cages (without mother, every 5–6 males in a cage), with free access to deionized water and normal formula (without AK) from weaning until the time of testing.

Solution preparation

Sweetener solutions were prepared in deionized water. Two sweeteners AK and sucrose (Beijing Yuan Ye Food Chemistry Co., Ltd) were used. The range of the solution concentrations was chosen according to the previous reports (Schiffman and Gatlin 1993; Bachmanov et al. 2001). We prepared the following 2 series of solutions in half-log steps (10 concentration gradients): one series of AK solutions containing 0.01, 0.04, 0.13, 0.42, 1.33, 4.21, 13.28, 41.97, 132.63, and 419.12 mM and the other series of sucrose solutions containing 0.03, 0.10, 0.32, 1.20, 3.80, 12.00, 38.00, 125.00, 400.00, and 1400.00 mM.

Experimental design

Three experimental groups were designed for the behavioral tests. There were 6 pregnancy mice in each group (Table 1). 1) The control group: the pregnant mice and their pups were given the normal formula (Purina Lab Chow) and water; 11 male pups were chosen from 6 mothers after weaning. 2) The pregnancy exposure group: the pregnancy mice were given the normal formula and water until single caged on gestational day 6 and then fed with the sweet formula (5 g AK/kg normal formula, Purina Lab Chow) manipulated by Zhejiang Academy of Medical Sciences and water till birth. We calculated the dosage of AK consumed based on their 24-h intake. This dosage of AK used in mice referred to the previous report (Bandyopadhyay et al. 2008). Eleven male pups were chosen from 6 mothers after weaning and fed with normal formula and water until testing. 3) The lactation exposure group: the pregnancy mice were given the normal formula and water until birth and then fed with the sweet formula till PD21; 11 male pups were chosen from 6 different mothers after weaning and fed with normal formula and water until testing. Each pregnant or lactating mouse consumed approximately 400–600 mg/kg body weight everyday (Table 2). In all the 3 groups, male pups were housed in cages (without mother, every 5–6 males in a cage) since weaning. The body weights of mothers and offspring in each group were monitored. No significant difference was observed among the 3 groups at birth, weaning, or 8 weeks either for mothers or the pups (Table 3).

Two-bottle preference test

The TBP behavioral test was referred to the recommended ways of Monell Chemical Senses Center (Bachmanov et al. 2001). After adaptation to the deionized water of double-tube for 1 week, every 8-week-old adult male mouse was single caged and presented with 2 tubes, with one tube containing a certain concentration of sweetener solution (AK or

Table 1 The litter size and sex ratio in the each group

Group (6 mothers per group)	Litter size (mean ± SD)	Litter sex ratio (male/female, mean ± SD)
Control	12.2 ± 2.0	1.11 ± 0.29
Pregnancy exposure	11.8 ± 2.0	1.05 ± 0.31
Lactation exposure	11.5 ± 2.2	1.15 ± 0.18

SD, standard deviation.

Table 2 The body weights and 24-h intakes of chow of mothers in each group

Group (n = 6 per group)	24-h intake of chow (g, mean ± SD)	Body weight (g, mean ± SD)
Control (weaning)	5.27 ± 0.79	43.74 ± 2.95
Pregnancy exposure (gestational day 18)	6.08 ± 0.91	67.56 ± 3.42
Lactation exposure (weaning)	5.11 ± 0.92	43.46 ± 3.17

SD, standard deviation.

Table 3 The body weights of mothers and offspring at birth, weaning, and the testing day

	Control (g)		Pregnancy (g)		Lactation (g)	
	Mother	Offspring	Mother	Offspring	Mother	Offspring
Birth[a]	45.41 ± 2.14 (n = 6)	1.63 ± 0.16 (n = 73)	45.91 ± 3.01 (n = 6)	1.61 ± 0.11 (n = 71)	45.29 ± 2.07 (n = 6)	1.62 ± 0.13 (n = 69)
Weaning	43.74 ± 2.95 (n = 6)	18.29 ± 2.66 (n = 73)	43.41 ± 3.05 (n = 6)	18.13 ± 2.58 (n = 71)	43.46 ± 3.17 (n = 6)	18.24 ± 2.60 (n = 69)
8 Weeks (only male)	—	38.40 ± 2.17 (n = 22)	—	38.33 ± 2.37 (n = 22)	—	38.09 ± 2.28 (n = 22)

Body weight was expressed as mean ± standard deviation.
[a]Mothers just finished the delivery and offspring were on postnatal day 0.

sucrose) and the other tube containing deionized water. The tubes used here for TBP test were modified serological transfer pipettes (COSTAR; 25 ml, nonpyrogenic) (Bachmanov et al. 1996, 2001): double-barreled placed side-by-side with rubber band in a fixed cage at the right side, double-apex apart from 15 mm, tube tips into the cage 25 mm. Each tube had a stainless steel tip with a 3.2-mm diameter hole so that mice could easily lick the liquid. The sweetener concentrations tested were increased by half-log steps as mentioned earlier. Each concentration was tested for 48 h. The positions of the tubes were switched every 24 h to control side preference. Daily measurements were made in the middle of the light period by reading the fluid volume accurate to 0.2 ml. There were no breaks between testing different concentrations. The sweet preference score was defined as: (24-h intake of sweetener solution/(24-h intake of sweetener solution + 24-h intake of deionized water)) × 100.

Sampling

A separate group of pregnant mice were prepared for amniotic fluid or mother's milk sampling. These mice were fed with the normal formula and water until sampling. For amniotic fluid sampling, the intragastric administration of deionized water (2 ml) or AK solution (10 mg/ml, 2 ml) was performed on the pregnant mice on gestational day 18 (9:00–9:30 AM). For milk sampling, the intragastric administration was performed on PD6 (9:00–9:30 AM.). The dosage of intragastric infusion of AK (10 mg/ml, 2 ml) was according to the average daily consumption of AK-sweetened chow (5 g AK/kg of the diet) for each mother mouse, which was around 400 mg/kg body weight per day. The milk samples were collected by milking the lactating mice by hands. A sample of about 30-μl milk for each mouse was obtained. Oxytocin (0.1 ml) was intraperitoneally administrated before the milk sampling. The amniotic fluid or milk sample was first collected 1 h after the intragastric administration and then collected for another 5 times at 5, 9, 13, 17, and 21 h, respectively. The intragastric administration and sampling were referred to the recommend ways of the United States National Institutes of Health (http://mammary.nih.gov/tools/mousework/milking-mice/index.html).

RP-HPLC and mass spectrometry

The standard methods of RP-HPLC and mass spectrometry (Agilent 1100, liquid chromatography-mass spectrometry device) were applied to detect AK in the amniotic fluid or mother's milk of mouse. The sample of amniotic fluid or mother's milk was purified in advance. The purifying procedure was as follows: a centrifuge tube (1.5 ml) was added with amniotic fluid 100 μl (or mother's milk 10 μl), bidistilled water 300 μl (or 390 μl), and methyl cyanides 800 μl, vortexed, and centrifuged at 10,000 r·min^{-1} for 10 min. The supernatant was carefully removed to a new tube, added with dichloromethane 800 μl, votexed, and centrifuged at 10,000 r·min^{-1} for 10 min. The supernatant was reserved and filtered through a filter membrane (0.45 μm). Thus, the purified sample was obtained and ready for RP-HPLC or mass spectrometry analysis.

The chromatographic conditions: Lichrospher C18 column (4.6 mm × 250 mm, 5 μm); T = 30 °C; flow rate was 0.80 ml/min; λ = 227 nm. The mobile phase composition was methanol–NH_4Ac (5:95), and the volume injected was 20 μl. The mass spectrometric conditions: electrospray ionization (ESI) (-), drying gas flow -rate was 10 l/min, nebulizer pressure was 30 Psi, drying gas temperature was 350 °C, capillary voltage was 3000, mass range was 100–300 amu, and the fragmentor was 100.

Data analyses

Data were expressed as mean ± standard error (or mean ± standard deviation). Average daily (24 h) fluid intakes were calculated for each mouse for each solution concentration in the TBP tests. As the relationship between body weight and fluid intake was indicated previously (Bachmanov et al. 1998), body weights of individual mice were measured before and after each test series, averaged, and used to calculate fluid intakes per 30 g body weight (the approximate weight of an adult mouse). The significance of preference or avoidance of a taste solution in the TBP tests was determined by comparing the solution and water intakes with paired t-tests. Because this comparison was made for 10 sweetener concentrations for each group, Bonferroni correction was applied to avoid potential false-positives owing to multiple

comparisons. The level of statistical significance was defined as $P < 0.05/10$ or 0.005 (Bachmanov et al. 2001). Preference or avoidance thresholds were defined as the lowest solution concentrations that were consumed in significantly larger or smaller amounts than water, respectively. Two-way analysis of variance with treatment as between-group factor and concentration as the within-group factor was used to analyze the sweet preference scores. Scheffé post hoc tests were used to evaluate differences between individual means. $P < 0.05$ was considered statistically significant.

For quantitative analysis of AK contents, 18 pregnant mice for amniotic fluid sampling and 18 lactating mice for milk sampling were used, 3 mice for each time point (1, 5, 9, 13, 17, and 21 h). A sample of about 30-µl milk for each mouse was obtained. And the amniotic fluid sample for each lactating mouse was about 300 µl. Samples (amniotic fluid or milk) from 3 different mice was mixed together and analyzed by the standard methods of RP-HPLC in triplicate. Student's *t*-tests were used to evaluate differences between individual means. The level of statistical significance was defined as $P < 0.05$.

Results

Sweet preference patterns

The sweet preference patterns of adult male mice at the age of 8 weeks were detected by the TBP tests to explore the influence of early maternal exposure to the AK-sweetened diet during the embryonic or lactation periods. A typical indifference–preference–avoidance pattern was observed in the control, pregnancy exposure, or lactation exposure group as a function of increasing AK concentration (Figure 1A,C,E). Mice preferred 1.33–132.63 mM AK solutions relative to water in all the groups mentioned above, and the most preferred

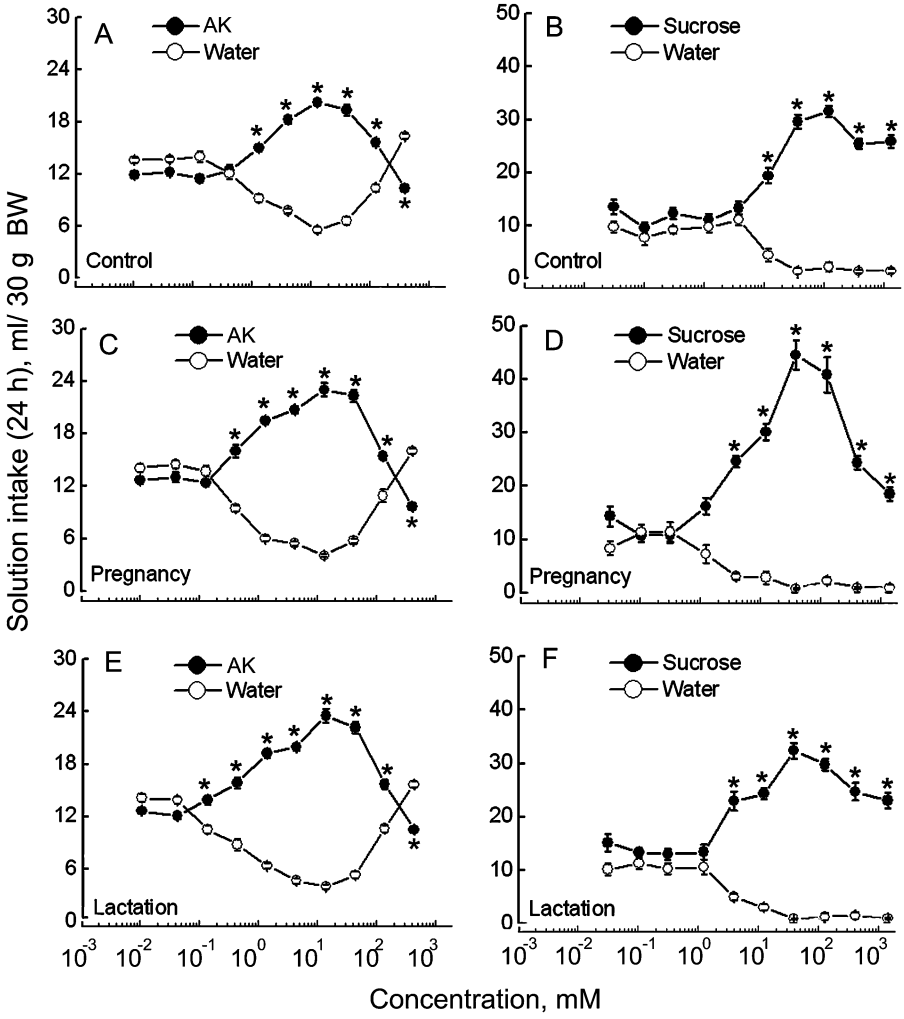

Figure 1 Two-bottle tests for AK and sucrose solutions. Adult male mice offspring at the age of 8 weeks were tested to explore the effects of early maternal exposure to AK-sweetened diet during the gestation or lactation period. Twenty-four hours' fluid intake expressed as mean ± standard error. Paired *t*-test and Bonferroni correction were applied and significance was set at $\alpha = 0.005$ (0.05/10 *t*-test). *$P < 0.0001$; $n = 11$. BW, body weight.

concentration was 13.28 mM (Figure 1A,C,E). However, the preference thresholds in the pregnancy and lactation exposure groups were 0.42 and 0.13 mM, respectively, lower than the threshold of 1.33 mM in the control group. The avoidance threshold was the same 419.12 mM in all the 3 groups (Figure 1A,C,E). As for the sucrose solutions, the typical indifference–preference pattern was demonstrated and the most preferred concentration was about 125.00 mM in each group, whereas the preference thresholds in both the pregnancy and lactation exposure groups were 0.38 mM, lower than 12.00 mM in the control group (Figure 1B,D,F).

Sweet preference scores

The preference scores in both the pregnancy and lactation exposure groups were significantly higher than that of the control group at a range of AK concentrations, and the highest preference score of each group occurred at the same concentration of 13.28 mM (Figure 2A). Mice in the pregnancy exposure group had higher preferences for 0.42, 1.33, 4.21, and 13.28 mM of AK solutions when compared with those in the control group ($P < 0.001$), and the increases in the preference percentage were 23.33%, 23.21%, 12.95%, and 7.94%, respectively. Thus, the average increase was 16.83% (Figure 2A). Meanwhile, the preference scores in the lactation exposure group were significantly higher at 0.13, 0.42, 1.33, 4.21, and 13.28 mM of AK solutions than those in the control group ($P < 0.001$). The increases in the preference percentage at these concentrations were 20.11%, 26.93%, 20.88%, 15.53%, and 8.91%, respectively, and the average increase was 18.47% (Figure 2A). Notably, mice in the lactation exposure group had a higher preference percentage at 0.13 mM of AK, when compared with those in the pregnancy group ($P < 0.001$) (Figure 2A).

As for the sucrose solutions, the highest preference score in each group occurred at the same concentration of 125.00 mM (Figure 2B). The preference scores in the pregnancy and lactation exposure groups were significantly higher than that of the control group at 3.80 mM ($P < 0.05$ and $P < 0.001$, respectively), and the increases in the preference percentage were 48.13% and 49.79%, respectively (Figure 2B).

Existence of AK in amniotic fluid and mother's milk

The behavioral tests indicated that mother's oral exposure to AK during the pregnancy or lactation stage did lead to a significant increase in the preference scores and a broader range of the preference concentrations, but there was no change in the pattern of preference in the adult mice offspring. By applying RP-HPLC and mass spectrometry, the presence of AK in the amniotic fluid or mother's milk was detected at 4 h post a single intragastric administration of AK solution (10 mg/ml, 2 ml). Results from RP-HPLC showed that a presumed specific absorption curve for AK in the standard sample occurred at about 9 min (Figure 3A), and such an absorption curve was also detected at 9 min approximately in either the amniotic fluid (Figure 3D) or mother's milk sample (Figure 3E) after the oral infusion of AK solution. However, there was no specific curve at 8–10 min in the amniotic fluid or mother's milk from the control group mice treated with deionized water intragastrically (Figure 3B,C). Furthermore, the existence of AK was confirmed by mass spectrometry. The *m/z* number of the main peak was approximately 162 either in the standard sample or in the sample of amniotic fluid or mother's milk from the AK solution intragastrically treated mice.

Dynamic changes of AK in amniotic fluid and mother's milk

The calibration curves of RP-HPLC were linear over the range of 4.060–500.5 μg/ml for AK. The relative standard deviations of the intra- and inter-day precisions for the analysis of AK were between 0.70% and 3.52% with accuracies (relative error) below 1.57%. The average extraction recoveries of amniotic fluid and milk samples were 95.22 ± 3.87 and 98.02 ± 2.08, respectively. The HPLC method herein was fully validated and successfully applied to the dynamic studies of AK.

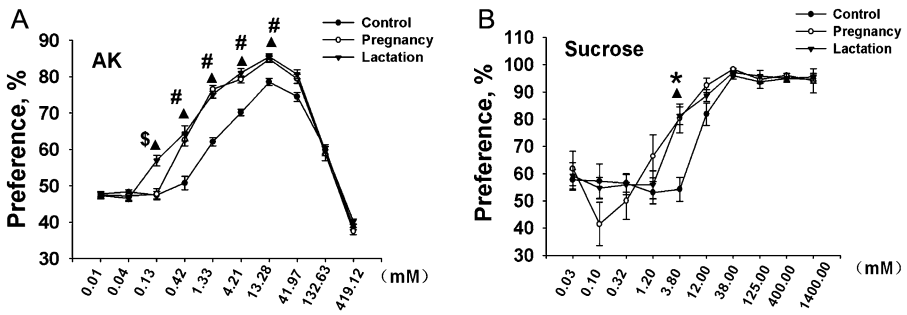

Figure 2 Preferences of the control and the exposure groups for AK and sucrose solutions. Adult male mice offspring of 8 weeks, with early maternal exposure to AK-sweetened diet in the gestation or lactation, were tested. Vertical bars represent standard error. $^{*}P < 0.05$, $^{\#}P < 0.001$, pregnancy versus control; $^{\blacktriangle}P < 0.001$, lactation versus control; $^{\$}P < 0.001$, pregnancy versus lactation; Scheffé post hoc tests; $n = 11$.

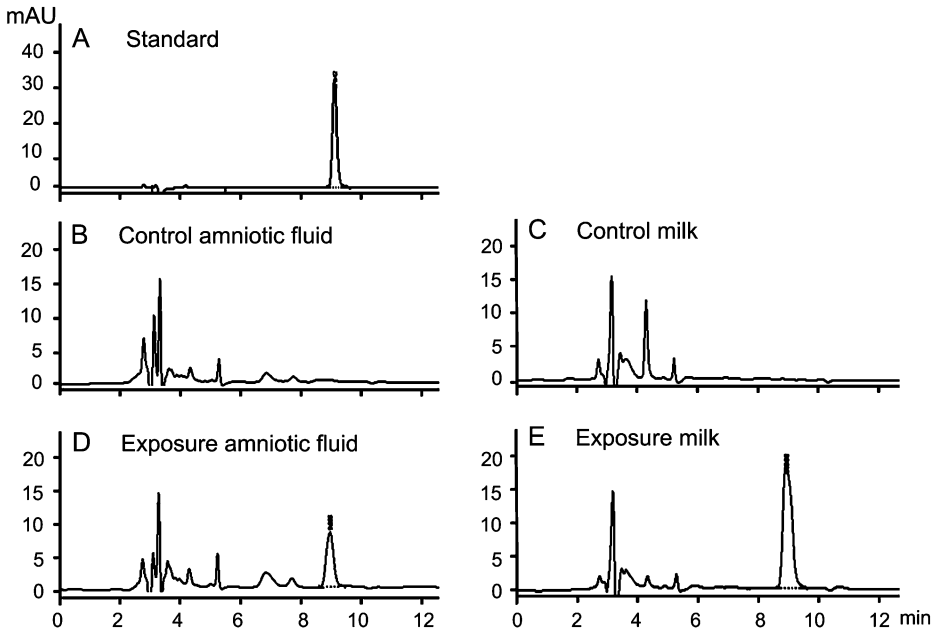

Figure 3 The RP-HPLC chromatograms. Samples were from standard AK solution **(A)**, amniotic fluid **(B)**, or mother's milk **(C)** of the control group treated with the deionized water intragastrically and amniotic fluid **(D)** or mother's milk **(E)** of the AK-treated (intragastrically) group. The specific absorption curve of AK emerged around 9 min. Wavelength = 227 nm.

The changes of AK content within 24 h in the amniotic fluid during pregnancy or in the mother's milk sample during lactation after a single oral infusion of AK solution were further studied by RP-HPLC. The content of AK was gradually increased and then decreased after the intragastric administration, with a peak at 5 h in the amniotic fluid (44.73 ± 4.94 μg/ml) or at 9 h in the mother's milk (361.96 ± 13.71 μg/ml) (Figure 4). At 21 h post the oral infusion, there was still a considerable content of AK in the mother's milk sample (125.85 ± 10.79 μg/ml), with a detectable level in the amniotic fluid (6.70 ± 1.14 μg/ml) (Figure 4). Moreover, the concentration of AK in mother's milk was constantly higher than that in the amniotic fluid ($P < 0.001$) (Figure 4), which may underlie the basis of enhanced preference for AK solution at 0.13 mM.

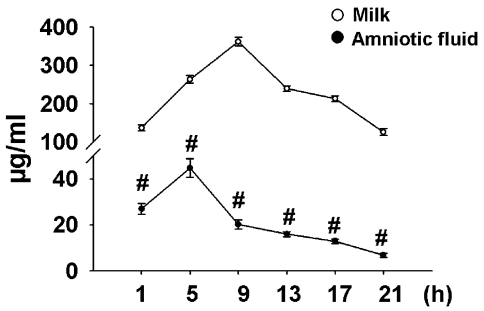

Figure 4 Quantitative analysis of AK contents by RP-HPLC. Samples were from amniotic fluid or mother's milk of the AK-treated group at 1, 5, 9, 13, 17, and 21 h post the intragastric administration. Vertical bars represent standard error. $^{\#}P < 0.001$, compared with the milk content, *t*-tests; $n = 3$.

Discussion

In this study, we found that the artificial sweetener AK, except for the elimination largely in the urine (Renwick 1986), was able to be transferred to amniotic fluid or mother's milk after oral exposure, which influenced the offspring's sweet preference in the adulthood. As shown in our results, mother's dietary exposure to AK in pregnancy or lactation decreased the preference thresholds for AK and sucrose solutions in the adult mice offspring, without changing the preference pattern or the most preferred concentration. Nevertheless, the exposure groups had enhanced preferences for AK or sucrose solutions.

Flavor is defined as the perceptual combination of 3 anatomically distinct chemical senses: taste, smell, and chemosensory irritation. Taste stimuli, which must be dissolved in saliva, are detected by taste receptor cells located in taste tissue in the tongue, palate, and perhaps even in the gut (Bachmanov and Beauchamp 2007). Taste is generally thought to be composed of 5 primary qualities: sweet, salty, bitter, sour, and umami or savory. Sweet, umami, and salty substances are preferred inborn, whereas bitter and many sour substances are rejected. Both genetic differences and experiences contribute to the taste preferences. For example, B6 and 129 mice differed in their oral but not their postoral response to fat and sugar. The postoral actions of intralipid and sucrose were able to increase the oil and sweetener preferences (Sclafani 2007). However, the experiential and nutritional factors can, to some degree, override genetic differences in peripheral taste sensitivity in determining food appetite (Sclafani 2006).

Prenatal developmental events appear to influence infant and child's taste preferences. For example, several studies suggest that severe maternal emesis can have an enduring influence on the response of offspring to salty taste (Crystal and Bernstein 1998; Leshem 1999). In another experimental study, human infants whose mothers were randomly assigned to drink carrot juice during the last trimester of pregnancy enjoyed carrot-flavored cereals more than infants whose mothers did not drink carrot juice or eat carrots (Mennella et al. 2001). However, whether the prenatal developmental events affect adult taste preferences is unclear. Here, our data showed that mother's dietary exposure to AK changed the sensory environments in which the fetal mice lived, the amniotic sac, as AK was transmitted and flavored the amniotic fluid. Fetuses could swallow the AK-flavored amniotic fluid. And such prenatal experience of oral exposure to AK resulted in heightened preferences for sweet solutions in the adulthood.

Flavor learning continues after birth as a consequence of exposure to the first nutrients in mother's milk or its substitute. The early postnatal experiences for breast-fed infants are influenced by the flavor compounds that the mother has chosen. Previous reports have shown that the exposure to a flavor (e.g., carrots, garlic, fruits) in mother's milk influenced infants' liking and acceptance of that flavor in a food base (Mennella and Beauchamp 1993; Mennella et al. 1995; Mennella et al. 2001; Forestell and Mennella 2007). A recent study also found that breast-fed human infants accepted peaches more than formula-fed infants, as determined by intake, rate of consumption, and facial expressions. This enhanced acceptance of fruit could be due to more exposure to fruit flavors because their mothers ate more fruits during lactation (Forestell and Mennella 2007). However, the influence of postnatal exposure to flavors in mother's milk on adult food preferences remains unknown. This study revealed that mothers' dietary exposure to AK during lactation led to decreased preference thresholds but enhanced preferences for sweet solutions in their breast-fed mice in the adulthood. And the reinforced preferences of sweet taste might be attributed to the early exposure to AK flavors because AK was detectable with a considerable level in the mother's milk samples after a single oral infusion.

As the results show, the preference threshold of AK solution in the pregnancy groups was 0.42 mM, higher than 0.13 mM in the lactation exposure (Figure 1). Moreover, the preference percentage in the pregnancy group was significantly lower than that in the lactation group at 0.13 mM of AK ($P < 0.001$) (Figure 2A). These data suggest that the different early exposure time periods may affect the adult sweet preference. In addition, the RP-HPLC analysis revealed that the concentration of AK in mother's milk was much higher than that in the amniotic fluid after a single oral infusion, which also might be the basis of the differences in the TBP tests mentioned above between the pregnancy and lactation groups.

Mouse strains have large differences in consumption of sweeteners (Capeless and Whitney 1995; Bachmanov et al. 2001; Damak et al. 2003). A previous study compared behavioral responses of C57BL/6ByJ and 129P3/J mice to different sweeteners including AK, and they found that the preferred concentration ranges of AK solution in C57BL/6ByJ and 129P3/J mice were 1.00–100.00 and 10.00–100.00 mM, respectively (Bachmanov et al. 2001). However, the preferred range of AK solution in ICR mice was 1.33–132.63 mM as shown in our results, which was similar to that in the C57BL/6ByJ mice, but different from the 129P3/J mice.

In the behavioral TBP tests, there are 4 typical patterns of preference scores as the concentration of sweetener solution increases: (a) indifference–preference, (b) indifference–preference–avoidance, (c) indifference–avoidance, and (d) only indifference (Bachmanov et al. 2001). Previous work applied a range of 0.01–100 mM AK solutions and found that the preference pattern for the strain C57BL/6ByJ or 129P3/J mice was "indifference–preference" (Bachmanov et al. 2001), whereas we observed a pattern of "indifference–preference–avoidance" for the ICR mice at the concentrations of 0.01–419.12 mM. The discrepancy may be due to the different mouse strains and (or) the expanded range of AK solution tested.

The gustatory system is susceptible to anatomical modification by postnatal taste stimulations (Hendricks et al. 2004; Hill 2004). We previously found that intraoral infusion of AK solution at the early postnatal stage (from PD4 to weaning) increased the number, promoted the maturation, and enlarged the size of fungiform taste bud during the postnatal stages even including the adulthood (9 weeks old), implying that the gustatory system may undergo anatomical and (or) biochemical changes induced by AK stimulation (Zhang et al. 2010). Therefore, it is highly likely that the plasticity of the gustatory system might partially underlie the mechanism of sweet taste behavioral changes induced by early AK exposure (during gestation or lactation).

Several limitations of this study should be considered. First, the pups with their mothers were exposed to the AK-sweetened chow from birth to PD21 in the lactation exposure group. These pups might be predominantly fed with mother's milk until weaning; however, the possibility of eating little solid chow, especially in the late lactation period, could not be eliminated. And this possibility might partially contribute to the changes in the behavioral tests. Second, only male pups were studied here. Whether there is sexual difference in sweet preference by the TBP tests or whether the ICR females exhibit the same change styles requires further research. Third, how AK was transferred into the amniotic fluid or mother's milk and why the concentration of AK in mother's milk was much higher than that in the amniotic fluid remained unclear. We inferred that AK was absorbed by the epithelium of digestive tract and entered into the serum, then transferred to the amniotic fluid or breast milk by some way. Many drugs concentrated in mother's

milk because of the higher fat content, which might be one of the reasons for the higher concentration of AK in the breast milk. However, further studies are needed to elucidate the exact mechanisms.

In summary, we found that the sweetener AK could be transferred to amniotic fluid or breast milk when mothers underwent intragastric infusions of AK solution. And the early developmental events as exposures to AK flavors in amniotic fluid (prenatal) and mother's milk (postnatal) were able to influence adult taste preference.

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