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Aversive Effect of Tannic Acid on Drinking Behavior in Mice of an Inbred Strain: Potential Animal Model for Assessing Astringency

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ABSTRACT: Astringency, an orosensory sensation associated with dietary tannins, contributes to food appetitiveness/aversiveness. However, astringency perception varies greatly among individuals. This study examined whether genetically homogeneous naïve mice display appetitiveness/aversiveness when provided with tannin-containing drink solutions. Ingestion of serial dilutions of tannic acid (TA) by inbred mice (A/Snell) was assessed by a one-bottle preference test. Drink intake was far predominant at night (circadian rhythm). TA concentration-dependently inhibited daily drink consumption. Overnight consumption of TA solutions (range = 0.5−8 g/L) decreased linearly to zero during the first night and was recovered significantly during subsequent nights. TA also inhibited drink consumption in another two inbred mouse strains. The protein fraction of saliva collected from naive mice was markedly reactive with TA at the concentrations shown to affect drink consumption. Thus, testing for drink ingestion in inbred mice during short-term (overnight) exposure to tannin-containing liquid foods represents an advantageous animal model for assessing astringency.

KEYWORDS: tannic acid, gallotannin, astringency, *Mus musculus*, inbred strain

INTRODUCTION

Over the past 20 years the use of mice has provided insight into the molecular, genetic, cellular, physiological, and behavioral bases of taste perception. A large number of those studies have been based on the use of murine models for bitterness,[1] sweetness,[2] saltiness,[3] umami,[4] and sourness.[5] In addition to those basic tastes, astringency also contributes significantly to the appetitive or aversive character of a food for both human and animals.[6,7] However, animal models for astringency have not been reported yet. Astringency is a complex oral tactile and diffuse sensation.[8,9] Astringency perception is subject to bias and varies greatly among individuals.[10,11] Accordingly, astringency of a given food sample must be rated by a panel of trained sensory judges. In addition, training of sensory panelists involves some degree of interindividual variability, and so it also contributes to subjectivity in astringency assessment. Accordingly, more objective procedures to rate astringency are necessary.[12] In this regard, a number of studies have documented an association between astringency, as perceived by human trained sensory panels, and physicochemical interactions of salivary proteins, or proteins in general, with food tannins, as measured by a variety of in vitro experimental assays.[9,13,14] Furthermore, high-affinity interactions of tannins, particularly with salivary proline-rich proteins and histatins, have been proposed to be involved in astringency.[15,16] In addition, a close correspondence between in vitro interaction intensity of the protein fraction of human saliva with different tannins (gallotannins and proanthocyanidins) and the intensity of astringency elicited by the latter ones has been recently reported.[17] Thus, such widely documented association between astringency and tannin−protein interactions has lent strong support to the use of physicochemical indices that may represent that kind of intermolecular interactions and hence astringency. The best known of these indices is the Glories gelatin index, which represents the ability of tannins to precipitate a highly diverse family of collagen-derived proteins or gelatins.[18] However, that method is only an estimation of astringency; its inaccuracies derive from variations in both the extent of tannin hydrolysis and the variable composition of gelatins. Accordingly, disagreements between gelatin index and astringency scores have been frequently reported.[12] In that regard, animal models may offer the possibility of a more objective assessment of astringency.

Studies in animals have also lent direct or indirect support to an association between tannin interactions with salivary proteins and astringency perception. Thus, in livestock animals, those interactions have also been associated with increased astringency and decreased palatability.[7] Thus, it has been also a recurrent observation that species feeding on high-tannin diets display higher contents of salivary proline-rich proteins than coexisting species sharing a single ecosystem but feeding on vegetables with low tannin contents.[19] In addition, diets containing high-tannin sorghum in contrast to diets containing low-tannin sorghum have been shown to be powerful inducers of salivary proline-rich proteins in rats and mice.[20] Also, forced brush painting of the mouse mouth with solutions of tannic acid, a highly astringent and protein-precipitant gallotannin,[21,22] has resulted in parotid gland hypertrophy and massive appearance of proline-rich proteins in saliva.[23] As a whole, animal studies would suggest that tannins represent astringent stimuli that can be neutralized by their complexation with some macromolecules occurring in saliva, such as the proline-rich proteins. In our view, animals displaying an absence or paucity of salivary proline-rich proteins,

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such as rodents, would represent an advantageous experimental model to assess quantitatively the aversive astringent effect of tannins. There is a lot of evidence showing that orosensory (taste) perception depends on a strong genetic component.[24] Accordingly, genetic uniformity of the experimental animals might be also advantageous for setting up a quantitative assay. This study aims to examine the influence of tannic acid, a common astringent tannin, on the drink intake of an endogamic mouse strain as an indicator of astringency perception.

MATERIALS AND METHODS

Animals. Male mice weighing 24 ± 3 g were used when 2−3 months old. Mice of the A/Snell strain, which have been inbred for over 50 years in our laboratory, were used in most of the experiments.[25] Inbred mouse strains C3H/HeJ and C57BL/6J were originally obtained from Jackson Laboratories (Bar Harbor, ME) and have been inbred for over 5 years in our colony. The animals were maintained at 21 ± 1 °C on a 12 h light and 12 h dark schedule (7 a.m.−7 p.m.) and fed ad libitum on a standard commercial mouse pelleted diet (Champion SA, Santiago, Chile) comprising by weight 20.5% crude protein, 5% fat (ether extract), 5% crude fiber, 50% non-nitrogenated extract, 7% ash (minerals), 11% moisture, and vitamins. All of the animal maintenance methods are under the guidelines of the University of Chile−Faculty of Medicine (ICBM)'s Animal Care and Utilization Committee.

Tannic Acid (TA). Tannic acid from nut galls of *Quercus infectoria* (Sigma Chemical Co., St. Louis, MO), a yellowish-brown powder comprising 7% water, 0.3% ash, <0.004% metal impurities (as Pb), and <3 ppm arsenic, was further characterized by reversed-phase HPLC-DAD and spectral analysis. Briefly, 100 mg of TA was dissolved in 10 mL of 20% v/v ethanol, stirred at room temperature in the dark for 2 h, and concentrated in vacuo to a fourth of its original volume. The concentrate was extracted three times with 2.5 mL of diethyl ether and three times with 2.5 mL of ethyl acetate, and the organic fractions were combined. The organic extract was evaporated to dryness in vacuo, the residue was dissolved in 200 μL of 1:1 v/v methanol/water, and 5 μL aliquots were subjected to HPLC. Reversed-phase separations of TA were carried out in triplicate at 25 °C using a 300 mm × 3.9 mm i.d., 4 μm particle size, Nova Pack C18 column. A photodiode array detector (Waters model 991, Milford, MA) was set at 280 nm. Two mobile phases were used: A, water/acetic acid (98:2 v/v); and B, water/acetonitrile/acetic acid (78:20:2 v/v/v). A two-step gradient was carried out at a constant flow rate of 1.0 mL/min: 0−55 min, 100−20% A; and 55−70 min, 20−10% A. Equilibration times of 15 min were allowed between injections. Each major peak in the HPLC chromatogram of the TA extract was characterized both by retention time in the 0−90 min range and absorption spectrum in the 250−400 nm wavelength range.[26] To perform the animal assays, stock solutions of tannic acid (10 or 20 g/L, pH 3.1) were prepared by dissolving the tannin in deionized water (0.062 μSiemens/cm) at room temperature (15−25 °C) with the assistance of mechanical stirring for 2 h. Serial dilutions were prepared with deionized water.

Consumption Pattern of A/Snell Mice in a Two-Bottle Preference Test. Two mice were placed in 14 × 26 × 20 cm plastic cages containing woodchip bedding and fitted with steel wire lids. The cages were provided with two 50 mL graduated Falcon centrifuge tubes with silicone stoppers and borosilicate glass spouts. The spouts protruded through the wire mesh about 4 cm above the cage floor with the spouts nearly 6 cm apart during tests. Both tubes contained aliquots of a single sample of deionized water. After 1 day of acclimation, tubes were weighed to the nearest 0.1 g and replaced on the corresponding cage at 9 a.m. Consumption from each individual tube was assessed at various times of the day. Preference ratios (Pr) were calculated using the following algorithm: Pr = consumption of water from tube 1/consumption of water from (tube 1 + tube 2), where tube 1 is the one displaying the lower consumption and tube 2 is the one displaying the higher consumption. A Pr below 0.15 was taken as discriminant (bottle-biased consumption).[1]

Assessment of Fluid Consumption Using a Single-Bottle Test. Groups of two mice per cage, as normalized by age, sex, and weight, were offered an ascending concentration series of tannic acid. A control group consisted of two mice that were offered deionized water. A single tube was placed in each cage. Mice were placed in the cages 24 h before the start of the experiment. During this period, the tube corresponding to each cage contained deionized water. To start the test, water from each tube was replaced (with no replacement of the tube) by either fresh deionized water (control) or by an experimental tannic acid solution. The initial content of fluid in each tube was recorded gravimetrically to the nearest 0.1 g, as indicated above. Fluid contents were then recorded at various times of the day.

Interaction of the Mouse Salivary Protein Fraction with TA. Saliva from A/Snell mice was collected after pilocarpine stimulation, as described elsewhere.[25] Aliquots of saliva were mixed in Eppendorf tubes with series of growing concentrations of TA solutions (water served as control) and incubated for 5 min at room temperature. Microliter aliquots of the saliva−TA mixtures were placed on a cellulose membrane, allowed to dry spontaneously, fixed in 5% trichloroacetic acid, washed in 80% ethanol, stained for protein in 0.25% Coomassie blue for 20 min, and washed exhaustively with 7% acetic acid. After a final wash in distilled water, the cellulose membrane was dried under a light lamp and photographed for morphometric analysis of the blue spots using Image J v.1.32 software (National Institutes of Health, Bethesda, MD). A decrease in the diffusion area of the salivary protein spot was interpreted as interaction between salivary protein and tannic acid.

Statistical Analysis. All values reported are the mean ± standard deviation of at least three independent experiments unless otherwise noted. Statistical differences between groups were determined using a two-tailed Student *t* test, with $p < 0.05$ as the statistical rejection criterion (α).

RESULTS

Characterization of Tannic Acid. TA is a commercially available extract obtained from a number of plant species. In this study, an extract of *Q. infectoria* galls was used. The extract was highly soluble in water at room temperature. The absorption spectrum of freshly prepared aqueous solutions of TA showed a single peak at 275 nm. At this wavelength, light absorption was found to be directly proportional to the concentration of TA in the range from 1 to 5 mg/mL. Under those conditions, the percent solution extinction coefficient for a water solution of TA was 2544 $(g/100\ mL)^{-1}\ cm^{-1}$. HPLC fractionation of TA and UV detection (280 nm) showed about 40 peaks eluting from 3 to 70 min. The most prominent peaks displayed retention times around 5.0 (gallic acid), 27, 29, 38, 44, 51, 61, and 62 min. Spectral analysis (wavelength range 250−400 nm) of individual peaks of the chromatogram showed, with no exception, a single prominent broad absorption peak having a maximum at about 275−278 nm, a feature that is distinctive of gallotannins (Figure 1). Absorption spectra corresponding to either ellagitannins or condensed tannins were not observed. Thus, the TA extract would consist entirely of gallotannins.

Bottle-Biased Water Consumption of A/Snell Mice in Two-Bottle Tests. Consumption patterns in two-bottle preference tests seem to vary for mice that do not taste a compound. Thus, mice may display either a roughly equal consumption from both bottles or a bottle-biased consumption.[1] To test which of both consumption patterns the experimental mice display in the present study, a two-bottle preference test was performed with

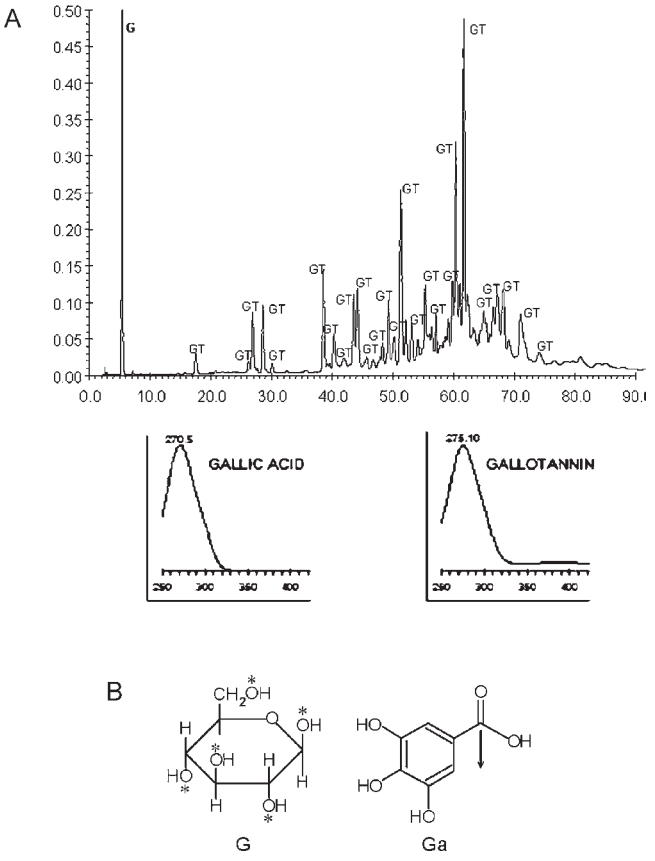

Figure 1. Tannic acid composition. (A) Tannic acid extract was fractionated by HPLC-DAD, and each individual peak with a retention time shorter than 90 min was characterized by spectral analysis (250−400 nm). With no exception, the TA components of the chromatogram were found to correspond to gallotannins (GT) (peak at 275−278 nm) and free gallic acid (G) (peak at 270 nm). Typical spectra of gallic acid and gallotannins are shown at the bottom of the panel. (B) Gallotannins are complex esters between the carboxyl group of gallic acid (arrow, Ga) and one or several OH groups of a core glucose (asterisks). Further esterification between the carboxyl group of additional molecules of gallic acid and one or more OH groups on some or all of the galloyl moieties of the polygalloyl glucose underlies the great structural diversity of gallotannins that becomes evident after HPLC fractionation.

both bottles containing deionized water. In this preliminary study, total water consumption by two A/Snell mice was 10.2 ± 1.2 mL, $N = 20$, every 24 h. No significant differences were observed from day to day (paired t test: $t_3 = 1.67$, $P = 0.1937$) (Table 1). However, in each cage water was mostly consumed from one of the two bottles, a situation represented by a Pr below 0.15 at day 1 of the experiment, or, equivalently, by a water consumption from one of the bottles over 5-fold the one from the other bottle. Reversion in bottle position was not found to affect the biased water consumption (data not shown).

Identification of the Range of A/Snell Mouse Sensitivity to Tannic Acid. Because A/Snell mice drink predominantly from just one bottle during a two-bottle test, a preference test for any substance may lead to pseudoavoidance or pseudopreference observations and so to inconsistent data. When water consumption by two A/Snell mice was measured in parallel using a single-bottle test, that is, one bottle per cage, total water consumption was 10.2 ± 1.1 mL, $N = 8$, per two mice every 24 h. This consumption was indistinguishable from the one observed using

Table 1. Water-Containing Bottle Preference Ratios for A/Snell Mice[a]

		water consumption[b]		preference ratio[c]	
		day 1	day 2	day 1	day 2
cage 1	bottle 1	0.7	0.2		
	bottle 2	8.7	8.9		
	cage	9.4	9.1	0.074	0.022
cage 2	bottle 1	1.4	0.5		
	bottle 2	9.3	9.6		
	cage	10.7	10.1	0.135	0.050
cage 3	bottle 1	10.9	11.4		
	bottle 2	0.4	0.0		
	cage	11.3	11.4	0.035	0.000
cage 4	bottle 1	0.2	3.5		
	bottle 2	12.4	7.5		
	cage	12.6	11.0	0.020	0.318

[a] Representative of three independent experiments. [b] Daily consumption of water expressed in mL/two mice. [c] Preference ratio = bottle with lower consumption/total consumption per cage. Two-bottle test, 24 h.

the two-bottle test (unpaired t test: $t_{14} = 0.922$, $P = 0.372$). Given the consistency of these observations and the absence of biased-water consumption, we decided to test mice for their intake of water versus their intake of a range of TA concentrations by using a single-bottle test. Serial 100-fold dilutions of the stock solution of TA showed that intake of TA solutions at concentrations below 0.2 g/L was indistinguishable from that of water. By contrast, 20 g/L tannic acid resulted in full suppression of drinking during a 24 h period (Figure 2A). To get a more accurate titration of that aversive effect, mice were tested for their intake of TA solutions in the range of 0−10 g/L. This study showed a minor but significant inhibition of drinking over a 24 h period at a concentration of 1 g/L tannic acid and a full suppression of drinking over the same period in mice that were offered 10 g/L tannic acid (Figure 2B). Thus, concentrations over the range from 1 to 10 g/L tannic acid seem to produce a dose-dependent aversive effect that can be appreciated by a corresponding decrease in fluid intake.

Circadian Variations of Water Drinking Behavior in A/Snell Mice. In natural environments animals typically alternate between two major discrete distinct states, active and inactive. Drinking is part of the active state.[27] However, genetic heterogeneity seems to underlie phenotypic variations in a variety of circadian rhythms in mice, including drinking behavior.[28] In this study we assessed the daily profile of water consumption in inbred A/Snell mice. To this end, three major periods within a day were arbitrarily identified, namely, morning (from 9 a.m. to 1 p.m.), afternoon (from 1 p.m. to 6 p.m.), and overnight (o'nite) (from 6 p.m. to 9 a.m.). To compare drinking behavior during these periods differing in length, we estimated the average rate of water consumption on a per hour basis using a single-bottle assay. As shown in Figure 3, the average rate of water intake at night is about 5-fold the one in the afternoon and about 2-fold the one in the morning. These circadian variations were highly consistent and invariable.

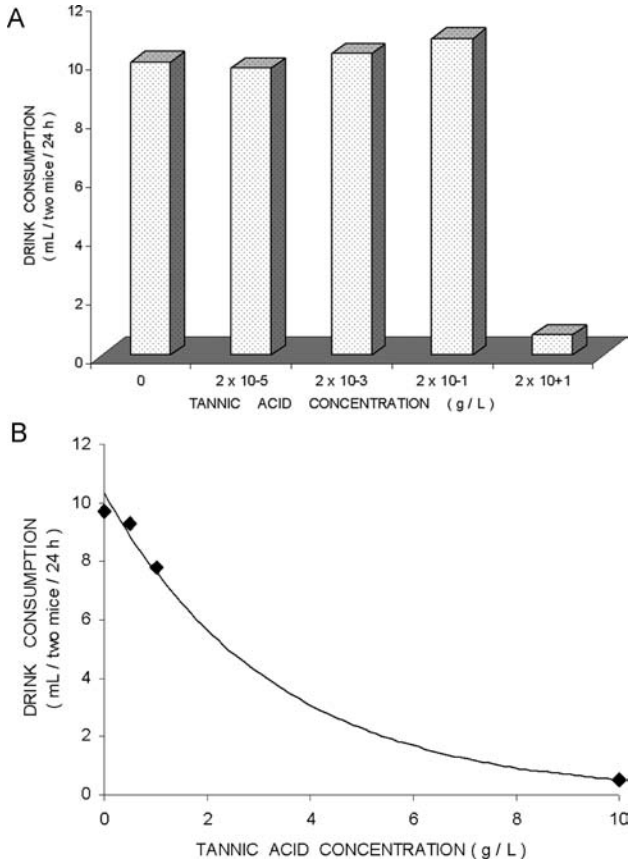

Figure 2. Tannic acid consumption of A/Snell inbred mice provided with a wide range of TA solutions. (A) Pairs of animals of the same sex and age were tested for their preference for a range of TA-containing solutions from $2 \times 10^{+1}$ to 2×10^{-5} g/L using a one-bottle test as described under Materials and Methods. The figure presents the mean volume (triplicates) of TA solution consumed by two mice at each TA concentration in 24 h. (B) Independent experiments using the same design as in panel A were conducted except that the range of tested TA-containing solutions was narrowed to 0.5−10 g/L. Standard deviations were <5% of the corresponding means.

Sensitivity of A/Snell Mice to Tannic Acid in Water. Considering the relevance of drinking during the active state of A/Snell mice, we conducted experiments to assess the aversive effect of TA concentrations in water specifically during the first night period (from 6 p.m. to 9 a.m.) after the start of the experiment. The day before the start of the experiment pairs of experimental animals were distributed in single cages with a bottle containing deionized water. At the start of this experiment (6 p.m.), water from each bottle was replaced by either fresh deionized water (control) or by one of a series of TA concentrations within a narrow range (from 0.5 to 10 g/L). The content of fluid in each bottle was recorded gravimetrically to the nearest 0.1 g, both at the start of the experiment and at 9 a.m. the following day. Fluid intake during the first night of the experiment was calculated as the difference between both weights. As shown in Figure 4, intake was found to decrease linearly over almost all the range of TA concentrations. At a TA concentration of 8 g/L the intake was negligible.

Interaction of Tannic Acid with the Mouse Salivary Protein Fraction. Drinking of TA solutions implies a direct physical contact between TA and mouse saliva. When an aliquot of mouse saliva is dotted on a cellulose membrane, the salivary protein fraction together with the salivary water diffuses radially. Protein staining reveals a roughly circular homogeneous distribution of the mouse salivary protein. When saliva from A/Snell mice was mixed with growing concentrations of TA before placement of an aliquot of the mix on the cellulose membrane, the pattern of salivary protein diffusion on the cellulose membrane was markedly altered. In effect, TA provoked in a concentration-dependent manner over the range from 10 to 30 g/L both a decrease in the whole area of protein diffusion and the appearance of nondiffusible complexes between TA (yellowish brown) and the

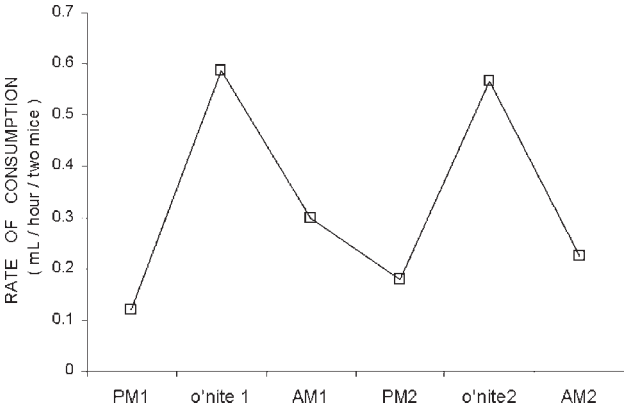

Figure 3. Circadian drinking behavior in A/Snell inbred mice. Pairs of animals of the same sex and age were tested for water consumption during two consecutive days using a one-bottle test. Three major periods within a day were arbitrarily identified, namely, morning (from 9 a.m. to 1 p.m.), afternoon (from 1 p.m. to 6 p.m.), and overnight (o'nite) (from 6 p.m. to 9 a.m.). Mean rates of water consumption on a per hour basis were calculated for each period. The figure presents mean rates of water consumption (triplicates) by two mice at each period of the day during two consecutive days. Standard deviation at each point was <5% of the corresponding mean.

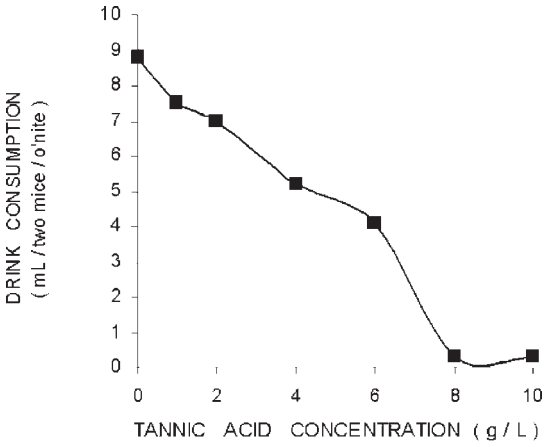

Figure 4. Night-time consumption of a narrow range of tannic acid solutions by A/Snell inbred mice. Pairs of animals were tested for their preference for a range of TA-containing solutions from 0.5 to 10 g/L using a one-bottle test. The animals were provided with one of the tested solutions at 6.00 p.m., and consumption was assessed at 9 a.m. the following day. Details of the experiment are given under Materials and Methods. The figure presents the mean volume (triplicates) of TA solutions consumed overnight (o'nite) by two mice at each TA concentration. Standard deviation at each point was <5% of the corresponding mean.

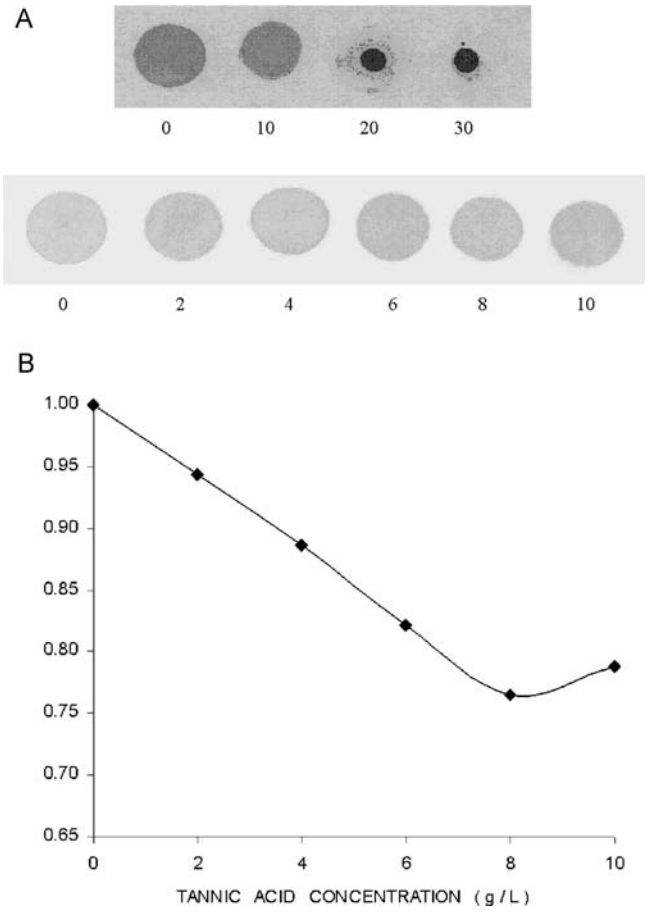

Figure 5. Effect of tannic acid on the mode of diffusion of the mouse salivary protein fraction on cellulose membranes. (A) When mouse saliva is diluted 1:1 volume ratio with water (0) and then an aliquot is dotted onto an absorbing cellulose membrane, radial diffusion occurs. Upon fixing and staining with Coomassie blue, a homogeneous distribution of the salivary protein over all of the originally moistened circular surface is revealed. When saliva is diluted 1:1 volume ratio with TA solutions (concentrations in g/L are indicated correspondingly below each protein spot), protein diffusion is altered in a concentration-dependent manner. Over the range from 10 to 30 g/L (top row), TA produces a partial (10 g/L) or complete (30 g/L) inhibition of protein diffusion. Over the range of TA concentrations at which a progressive decrease in drink consumption had been observed (from 2 to 10 g/L), decrease in protein diffusion was apparently also progressive (bottom row). (B) Image analysis measurement of the protein diffusion area on the cellulose membrane showed a continuous concentration-dependent inhibition of protein diffusion by tannic acid. Data shown are representative of three independent experiments. Standard deviation at each point on the curve was <5% of the corresponding mean.

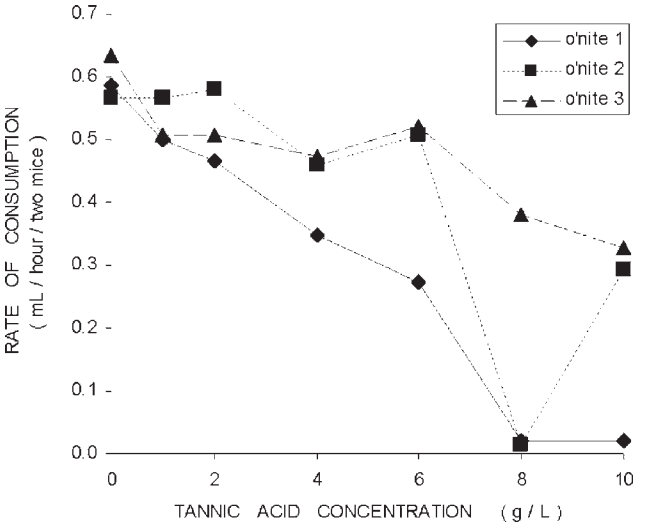

Figure 6. Resumption of tannic acid consumption in A/Snell mice initially expressing aversion to the tannin. Pairs of animals were tested for their preference for a range of TA-containing solutions from 0.5 to 10 g/L using a one-bottle test. Each pair of mice was exposed continuously to the corresponding TA solution, and fluid intake was assessed at various times of the day during 72 h. The figure, which is representative of three independent experiments, presents the mean volume (triplicates) of TA solutions consumed by two mice at each TA concentration over three consecutive nights. Standard deviation at each point was <5% of the corresponding mean.

salivary protein (Coomassie Blue positive) (Figure 5A). When the experiment was repeated with TA concentrations at which mice experienced a progressive decrease in drink consumption (range from 2 to 10 g/L), we also observed a concentration-dependent progressive decrease in the whole area of protein diffusion. At the highest TA concentration in this experiment, at which drink consumption during the first night was negligible, the whole area of protein diffusion was decreased by as much as 30% (Figure 5B). This antidiffusive effect produced by lower concentrations of TA on the salivary protein represents the formation of complexes between TA and the protein fraction of mouse saliva. In humans, this latter in vitro physicochemical phenomenon has been closely correlated with the ability of tannins to provoke astringency.[17]

Resumption of Tannic Acid Intake in Mice Expressing Aversion to the Tannin. The presence of tannin in the drinking water produces aversion, which is expressed by a partial or full concentration-dependent decrease in fluid intake over the range from 0.5 to 10 g/L tannic acid. Such an effect is clearly identified during the first active stage at night time. Continuous exposure of mice to TA and assessment of fluid intake during the following 72 h showed a significant but partial recovery in nighttime consumption all over the experimental range of TA concentrations. Even TA concentrations that produced a full cessation of fluid intake during the first night were found to be only partially inhibitory of TA consumption during nights 2 and 3 of the experiment (Figure 6). Those changes were found to mirror the ones occurring during the whole second and third days of the experiment (unshown).

Comparative Effect of Tannic Acid on Fluid Intake in Three Inbred Mouse Strains. A number of taste phenotypes have been found to differ among inbred mouse strains.[29,30] Because the present study has been conducted using a specific mouse strain (A/Snell), additional comparative experiments were performed with two independent inbred mouse strains, C3H/HeJ and C57BL/6J. Animals of the three inbred mouse strains, paired by sex and age, were examined for their ability to drink a TA solution at a concentration shown to be partially aversive in A/Snell mice. To do so, two pairs of mice from each of the three strains were placed in single cages and were offered deionized water in single bottles at 9 a.m. the day before the start of the experiment. At 6 p.m. of the day of start of the experiment the content of the bottle of one of the two cages corresponding to each strain was replaced by fresh deionized water, whereas the

Table 2. Nighttime Consumption[a] of a Tannic Acid Solution by Three Inbred Mouse Strains

strain	water	tannic acid[b]
A/Snell	10.3 ± 0.4	5.6 ± 0.4
C3H/HeJ	13.5 ± 0.5	6.8 ± 0.5
C57BL/6J	10.5 ± 0.3	4.9 ± 0.3

[a] Consumption from 6 p.m. to 9 a.m. expressed in mL/two mice (mean ± SD of triplicates), one-bottle test. [b] 0.5% tannic acid in water.

content of the bottle of the second cage was replaced by 5 g/L tannic acid. The content of fluid in each bottle was recorded gravimetrically to the nearest 0.1 g. At 9 a.m. the following day, the content of fluid in all of the bottles was recorded again. Fluid intake during the night was calculated as the difference between both weights. As shown in Table 2, intake of deionized water was significantly higher in the C3H/HeJ mice as compared to C57BL/6J and A/Snell mice (unpaired *t* test: $t_4 = 7.205$, $P = 0.0020$, and $t_4 = 7.473$, $P = 0.0017$, respectively). However, with no exception, fluid intake was reduced by half in each of the three inbred mouse strains when water was replaced by 5 g/L tannic acid.

DISCUSSION

An inbred mouse strain, A/Snell, has been used to assess mouth sensitivity of mouse to water solutions of tannic acid, a mostly hydrolyzable and astringent gallotannin complex extract from *Q. infectoria*.[21,22] Altered drinking behavior, as characterized by a reduced intake of solutions containing TA during a relatively short period of time, was considered as an indicator of mouse sensitivity. Using a two-bottle preference assay we first observed that A/Snell mice display a significant bottle-biased consumption, a feature that may mask true avoidance or true acceptability assessments.[1] On this background we set a one-bottle assay in which serial dilutions of TA were assayed by measuring fluid intake by pairs of mice over a period of 24 h. Under these experimental conditions we found that TA produces significant aversive effects at concentrations of ≥1 g/L and that such aversive effect becomes fully suppressive of fluid intake at concentrations of ≥8 g/L. The assay was highly reproducible.

A number of studies using the mouse model for taste perception assessment have measured fluid intake during 1- or 2-day terms.[1,29−32] Such long-term exposure times open the possibility for postingestive effects. Thus, previous studies have shown that mice of several strains do not avoid certain bitter substances, but after a few days develop a strong aversion to them. Contrarily, other studies have shown that some mouse strains displaying aversion to a given stimulus will start to prefer the tastant within a few days.[33] To minimize postingestive confounding factors, in the past few years brief-access assays have been designed for examining taste phenotypes among mice.[29] These tests used to be conducted in automated multistimulus lickometers in which the access to taste solutions is controlled by a computer-operated shutter. As part of the method, water is temporarily removed to motivate stimulus sampling and the animals should experience a training period during which they learn to lick in the apparatus. It is not known whether eventual strain differences of mice in some of these complex methodological aspects may bias the orosensory assay. Anyhow, differences between intake tests and brief-access tests have been reported from time to time. On the other hand, it is well-known that animal species display alternate active and inactive states in which a number of activities, including drinking and eating behaviors, are preferentially associated with one of these states.[27] In our study we arbitrarily fractionated the 24 h day into morning (9 a.m.−1 p.m.), afternoon (1−6 p.m.), and overnight (6 p.m.−9 a.m.) periods and found a highly reproducible circadian rhythm in which the rate of water intake on a per hour basis was much higher during night than during morning or afternoon hours. On that basis, in this study determinations of TA-containing fluid intake were conducted by assessing overnight fluid consumption. Considering that a number of factors, other than orosensoriality, may also influence drink consumption, we opted for monitoring overnight intake after short-term exposure of the animals to the tested solutions. Accordingly, intake was monitored during the first night following the exposure to TA. Under these experimental conditions, a roughly linear decrease of fluid intake was observed when TA was offered over the range from 0.5 to 8 g/L tannic acid. Such a range of TA concentrations would then be representative of the oral sensitivity in mouse to TA.

In this study we also evaluated whether aversion to tannic acid was a persistent feature among A/Snell mice. To this end, we assessed consumption of TA solutions during a continuous follow-up of 3 days. Interestingly, even those concentrations of TA that resulted in full suppression of drinking during the first night were found to be just partially aversive from the second night onward. Whether physiological or behavioral postingestive mechanisms following the acute challenge with TA may account for this observation has not been resolved yet. Anyhow, our observations highly suggest that to test acceptance or aversion for TA using the mouse model, the assay should comprise just the first night. This short-term experimental design results in accurate and reliable observations.

Another consideration in setting the mouse model for assessing tannic acid effects deals with the type of animal. A variety of gene-coded taste receptors have been found to mediate food acceptance and rejection in mammals.[1,30,34] Mice of an inbred strain are homozygous at virtually all of their loci. The present study was performed using in-bred mice from a definite strain (A/Snell) instead of heterogeneous stock mice. Such an aspect may well account for the highly reproducible and repeatable data on daily consumption on a per mouse per hour basis. However, this aspect was also highlighted by significant interstrain differences in the oral fluid intake. In effect, water consumption by C3H/HeJ mice was consistently 30−40% higher than those displayed by animals of the A/Snell and C57BL/6J mouse strains. Despite these differences, aversion to TA during the first night of the experiment was percentually similar in mice of the three in-bred strains.

Tannic acid is part of a chemically complex family of polyphenols known as tannins. Most tannins exhibit a high potential to produce astringency, a tactile perception that seems to influence to a major extent either aversiveness or appetitiveness of a food.[7] Astringency in foods and drinks is usually assessed by trained human sensory panels.[12] Certainly, it is difficult to prove whether astringency or whether other sensory effects explain the aversive behavior of mice to TA, at least during the first night of the experiment. Interestingly, the narrow range of concentrations at which TA has been found to produce a measurable effect in A/Snell mice is of the same order of magnitude as the TA concentration producing changes in drinking behavior in a number of species, including man, primates, and marsupials, particularly when other diet components are simultaneously provided.[22,35,36] Given its

putative relationship with astringency, it would be expected that TA may interact with salivary proteins in the mouse mouth and produce an astringent sensation. In this study we have unequivocally shown that concentrations of aqueous solutions of TA producing an aversive drinking behavior among mice are also highly interactive with the protein fraction of mouse saliva in vitro.[37] By the same token, local applications (paintbrushing) in the mouse mouth with TA have been found to induce the synthesis of salivary proline-rich proteins, a family of proteins displaying high affinity for tannins.[23] In humans, interactions of constitutively expressed salivary proline-rich proteins with tannins have been frequently invoked as part of the mechanism producing astringency.[13−17] Tannin−salivary protein interactions have also been associated with increased astringency and decreased palatability in livestock animals.[7] In accordance with the present results, the aversive behavior of mice to tannic acid, most likely in association with an astringent sensation, would occur immediately after the exposure of the animals to the tannin and would precede the synthesis of salivary proline-rich proteins occurring several hours later.[23] Thus, the synthesis of these proteins in mouse may well represent an adaptive response to neutralize aversive effects of the tannin. In this regard, we also showed that continuous presence of TA in drinking water is somewhat less aversive to mice during the second and third nights compared to the first one. Whether learning or adaptive processes, or both, may explain these experimental observations is now part of current research. Altogether, this study has demonstrated that under the present experimental conditions, mice may be advantageously used as an untrained animal model to compare the astringent power of complex and diverse tannin mixtures either extracted from solid foods or present in tannin-containing drinks. At least three conditions must be fulfilled by the animals to be used in the assessment of astringency: (a) their sensitivity for the tested substance should be comparable to that of humans, (b) the animals should be genetically homogeneous, and (c) they must respond according to a behavioral paradigm (drink consumption in our study) that can be associated with an objective phenomenon linked to astringency (interaction of the salivary protein fraction with TA in our study). As shown or referenced in this paper, the mouse model for astringency assessment we are describing satisfies all three conditions.

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