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Sucrose Octaacetate Avoidance in Nontaster Mice is not Enhanced by Two Type-A *Prp* Transgenes from Taster Mice

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Abstract

The *Soa* bitter-sensitivity and *Prp* salivary-protein loci map to distal mouse chromosome six. No recombination has been found between sucrose octaacetate (SOA)-avoidance phenotype and PRP haplotype in any mouse population. *Soa* and *Prp*, therefore, are either very near each other or identical. To assess the latter possibility, two type-A, proline-rich protein genes (MP2 and M14), situated ~30 kb apart at the *Prp* locus, were separately transferred from an SOA-taster inbred strain (SWR) to an SOA-nontaster inbred strain (FVB). Five MP2-transgenic mice and seven M14-transgenic mice were insensitive to 1 mM SOA in two-bottle tests, thus retaining the nontaster FVB phenotype. Each transgenic mouse was mated to control FVB mice. Their transgene-positive F1 and F2 offspring also were insensitive. Transgene expression varied among the founder lines, but SWR-like expression levels, higher than background FVB expression levels, were found in submandibular gland tissue of adult transgenic mice from two MP2 lines and one M14 line. F3 mice from one of these MP2 lines were mated to F2 mice from the M14 line. Nine offspring inherited both transgenes. All nine were insensitive to 1 mM SOA. These findings indicated that expression of mRNAs for both type-A *Prp* genes alone or together did not enhance SOA taste sensitivity in nontaster mice.

Introduction

Sensitivity to the bitter taste of sucrose octaacetate (SOA) varies greatly among inbred strains of mice (*Mus musculus*). Originally, two sensitivity phenotypes, taster and nontaster, were identified. Taster mice avoided 0.1 mM SOA solutions in two-bottle tests, whereas nontaster mice did not (Lush, 1981). More recently a third phenotype, demitaster, was identified. True nontaster mice do not avoid even 1 mM SOA, a near saturated aqueous solution (at 22°C). However, many strains originally classified as nontasters do avoid 1 mM (Harder *et al.*, 1992). Strains exhibiting this intermediate SOA sensitivity were reclassified as demitasters.

The three SOA sensitivity phenotypes are controlled by allelic variation at a single locus, *Soa*, on distal chromosome six (Capeless *et al.*, 1992). A dominant allele Soa^a produces the taster phenotype. Recessive alleles Soa^b and Soa^c produce the nontaster and demitaster phenotypes, respectively. *Prp*, a locus coding for salivary proline-rich proteins (PRPs), maps to the same location (Azen *et al.*, 1989). A correspondence was seen between PRP DNA haplotypes and bitter taste phenotypes in recombinant inbred strains of mice, indicating close genetic linkage between Prp and bitter taste genes (Azen *et al.*, 1986).

Four PRP haplotypes (a, b, c and d, from *Hin*dIII-digested genomic DNA probed with rat PRP33 cDNA) are found among inbred strains (Azen *et al.*, 1989; Azen, 1991). Three PRP-haplotype classes (c, a and b/d) were found to be concordant with the three SOA phenotypes in 14 inbred strains (Harder *et al.*, 1992). Perfect concordance between PRP haplotype and SOA phenotype also was seen in 20 recombinant inbred strains (Capeless *et al.*, 1992) and 24 B6.SW-Soa^a and SW.B6-Soa^b congenic strains (G. Whitney and E.A. Azen, unpublished data). Perfect cosegregation of PRP haplotype and SOA phenotype was found in 42 mice from an outbred strain (Harder *et al.*, 1992). Perfect co-segregation of *Prp* and *Soa* was also found in 67 backcross mice (Lush *et al.*, 1995). The complete lack of recombination between *Soa* and *Prp* observed across these studies indicated the two loci were at least closely linked (<1 cM apart) and perhaps were identical.

The mechanism by which *Soa* allele variation affects bitter sensitivity is not known. The gene product has not been identified. Glossopharyngeal nerve response differences to SOA were concordant with the behavioral differences among inbred strains, suggesting an effect on the peripheral gustatory system (Shingai and Beidler, 1985). A G-protein-mediated, second messenger transduction model for SOA-bitterness has been proposed (Spielman *et al.*, 1994). Rapid transient increases in inositol triphoshate levels, leading to

Ca^{2+} release, were observed in gustatory tissue from taster inbred and congenic strains upon stimulation with SOA + GTP (Spielman *et al.*, 1996). Such increases were not seen in the nontaster congenic partner strain. The hypothesized receptor protein that initiates transduction in this model has not been identified. If *Soa* and *Prp* are separate loci, *Soa* alleles may code for variant forms of this receptor protein. An effect at other points in the transduction pathway is also possible.

If *Soa* and *Prp* are identical, SOA sensitivity differences might instead reflect variation in the amounts and/or SOA-binding affinities of PRPs. PRPs bind bitter tannins in saliva, lowering the free concentration. Increased PRP production was induced in an inbred strain by a series of isoproterenol injections (Glendinning, 1992). These mice did not avoid a tannic acid concentration that saline-injected mice did avoid. Differences in effective SOA concentration reaching taste cell membranes might result from such a mechanism.

The taster phenotype is dominant over the nontaster phenotype in all crosses yet examined (Harder *et al.*, 1992). Identity of *Soa* and *Prp* would be shown if a *Prp* transgene from a taster strain enhanced the SOA sensitivity of a host mouse from a nontaster strain. Non-identity would be indicated if the transgene did not alter the host's SOA sensitivity, provided the transgene was fully expressed.

Only two taster inbred strains have been identified. The taster phenotype was first found in a CFW strain which no longer exists (Warren and Lewis, 1970). SWR mice were later found to be tasters as well (Lush, 1981). Most studies of the genetic basis for SOA sensitivity differences among mice have used SWR/J taster mice. Therefore, this strain provided the *Prp* transgenes used in the present study. The host mice were from strain FVB/NTacfBR (FVB), an inbred strain often used as transgenic hosts. Pilot testing of five male and five female adults with 1 mM SOA indicated FVB mice were nontasters [mean preference ratio in a 48 h, two-bottle test = 0.49, SE = 0.06 (preference ratio = ml solution consumed/ml solution + ml water consumed)]. Two *Prp* transgenes from SWR/J mice were transferred to FVB mice in the present study. The SOA sensitivities of all transgene-positive founder mice, and their descendants across three generations were then assessed.

Materials and methods

Cloning SWR MP2 and M14 genes in lambda phage

Two closely linked and related type-A PRP genes (MP2 upstream of M14) which are at least 30 kb apart in the CD-1 mouse (Ann and Carlson, 1985; Ann *et al.*, 1988) were isolated from the SWR mouse. A partial *Sau*IIIA1 genomic library was made in lambda phage charon 40. The library was screened with an upstream probe (Figure 1a, probe a) from the CD-1 MP2 gene (kindly provided by Don Carlson, University of California, Davis, CA). The identities of the SWR MP2 and M14 genes cloned were characterized by extensive restriction mapping, hybridization with probes from the CD-1 MP2 gene and selective sequencing. The MP2 and M14 inserts were 16.7 and 17.5 kb respectively, and both genes were intact. For MP2 and M14 inserts, regions upstream of exon 1 were 8.2 and 9 kb respectively, and regions downstream from the untranslated final exon 3 were 5.5 and 2.5 kb respectively (Figure 1a).

Transgenic mouse production and detection of transgenes

Transgene fragments containing MP2 and M14 genes were excised from recombinant phage, separated by agarose gel electrophoresis and purified with the Qiaex II gel purification kit (Qiagen, Valencia, CA). Oocytes from the FVB non-taster mouse were microinjected with the fragments, and transgenic mice were generated according to standard procedures (Brinster *et al.*, 1985).

To detect the SWR MP2 transgene, tail genomic DNAs (prepared using the Puregene Kit, Gentra Systems, Minneapolis, MN) were *Xba*I digested, and the Southern blots hybridized to a randomly primed and ^{32}P-labelled fragment from the CD-1 MP2 gene that covers the tandemly repetitive exon 2 (Figure 1a, probe b). The sizes of exon 2 show strain-specific differences, and on Southern blots the ~980 bp *Xba*I fragment from the SWR strain can be distinguished from the 1.1 kb *Xba*I fragment from the background FVB strain (Figure 1b).

To detect the SWR M14 transgene, tail genomic DNAs were XbaI/SalI digested and the Southern blots hybridized to a probe (Figure 1a, probe c) from the CD-1 MP2 gene that covers a portion of intron 2 and exon 3. On Southern blots, the ~2.1 kb *Xba*I–*Sal*I fragment of the SWR strain can be distinguished from the 2.2 kb *Xba*I–*Sal*I fragment of the FVB background strain, again due to strain-specific differences in lengths of the repetitive exon 2 contained in the *Xba*I–*Sal*I fragments (Figure 1c).

Expression studies

A gene-specific RT-PCR test was devised to detect the SWR-type transgene expression in submandibular glands. The submandibular gland was chosen, since earlier transgenic expression studies with MP2 and M14 upstream promotors fused to a *LacZ* reporter gene showed gene expression in granular convoluted tubules of the submandibular gland (Zhuo *et al.,* 1997). The test is based in part on an allele-specific PCR strategy (Wu *et al.,* 1989). In the present case, upstream primers from exon 1 for SWR MP2 and M14 genes are the same, but the downstream primers from exon 3 differ, especially in the most 3′ nucleotide, thus permitting gene-specific amplification. Furthermore, MP2 and M14 cDNAs show gene-specific differences in size due to a different number of tandem repeats in the exon 2 portions. Background amplifications of FBV MP2 or M14 cDNAs were distinguished from those of SWR due to strain-specific size differences arising in the exon 2 portions.

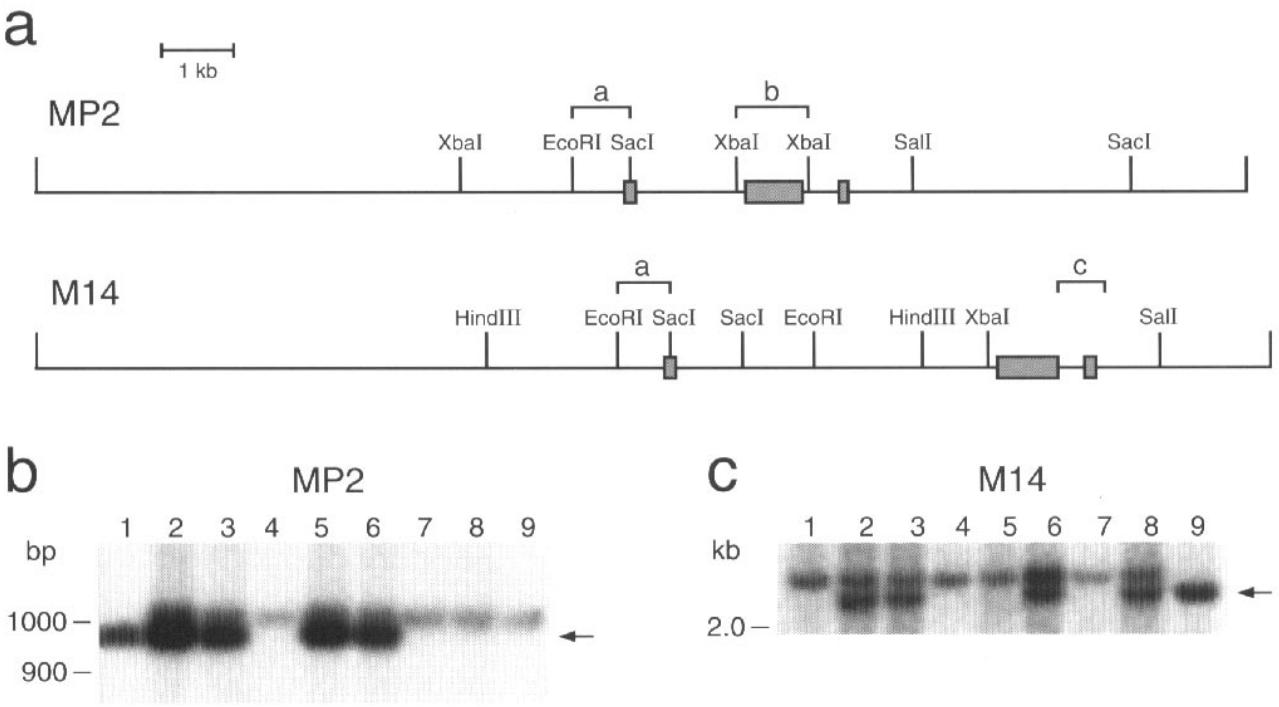

Figure 1 **(a)** Restriction maps and structures of SWR MP2 and M14 genes. Exons are shown as solid boxes, and probes a, b and c that were used are shown above the maps. **(b)** Autoradiogram showing genomic integration of SWR MP2 transgene. The Southern blot was hybridized to probe b shown above. The arrow shows the position of the transgene (*Xba*I digested) at ~980 bp. The FVB background band is at ~1100 bp. Each lane represents results with a different mouse [lane 1—SWR control; lanes 2, 3, 5 and 6—transgene (+); lanes 4, 7, 8—transgene (–); lane 9—FVB control]. **(c)** Autoradiogram showing genomic integration of SWR M14 transgene. The Southern blot was hybridized to probe c shown above. The arrow shows the position of the transgene (*Xba*I–*Sal*I digested) at ~2.1 kb. The FVB background band is at ~2.2 kb. Each lane represents the results with a different mouse [lane 1—FBV control; lanes 2, 3, 6, 8—transgene (+); lanes 4, 5, 7—transgene (–); lane 9—SWR control].

The identities of the amplified cDNAs as MP2 or M14 type were verified by specific restriction patterns, expected sizes and hybridization to an MP2-derived gene probe covering exon 2 of the cDNAs.

The location and identities of the primers are from the CD-1 MP2 gene (GenBank accession no. M12099) and CD-1 M14 gene (GenBank accession nos M23236, J03891) from the CD-1 mouse. MP2 and M14 (forward, exon 1): 5′-AAGATGCTGGTGGTCCTGTTTACAGTG-3′ (734–760 from both MP2 and M14 sequence); MP2 (reverse, exon 3): 5′-ACTTTCTGAGGGAGCCATCGTCCTAC-3′ (3455–3430 from MP2 sequence); M14 (reverse, exon 3): 5′-ACTTTCTGAGGGAGCCATTGTCCTAT-3′ (5970–5945 from M14 sequence).

The primer sequences of the CD-1 and SWR strains are the same as confirmed by sequencing.

Total RNA was prepared from submandibular glands with the RNeasy mini kit (Qiagen, Valencia, CA). The RT-PCR system is based on Tth DNA polymerase amplification, and the reagents were obtained from Epicentre Co. (Madison, WI). Gene-specific primers (1 μM each final concentration) and formamide (2% final concentration) are added as a 'hot start' during the 94°/5 min 'preheat' of the PCR phase. Total RNA (1 μg) and oligo(dT) (12–18) (0.5 μg) are added on ice to a master-mix final volume of 50 μl containing final concentrations of 0.5 mM of each dNTP, 4 × Masteramp enhancer, 3.0 mM $MgCl_2$, 0.1 unit/μl Tth DNA polymerase, 10 mM Tris–HCl, pH 8.3, 50 mM KCl and 0.75 mM Mn SO_4. RT-PCR conditions: 25°/10 min pre-RT, 70°/20 min RT, 94°/5 min 'preheat' (add specific primers and formamide). Reactions were then cycled 40–50 times at 94°/1 min, 67.5°/1 min, 72°/2 min.

SOA sensitivity testing

Five FVB founder mice positive for the MP2 transgene, and seven FVB founder mice positive for the M14 transgene, were given two 48 h, two-bottle tests of 1 mM SOA versus deionized water (see Table 1). A near-saturated solution was used so that even a partial increase in sensitivity would be revealed. Five SWR mice and six transgene-negative FVB mice were tested with these founders as controls. The SWR mice were given two tests with 0.1 mM SOA as well to check for full expression of the taster phenoype. The MP2-positive founders were tested with them.

First and second generation transgene-positive descendants of the founder mice were given two tests with 1 mM SOA (17 F1 and 21 F2 mice with the MP2 transgene; 20 F1 and 15 F2 mice with the M14 transgene). Nine FVB mice, positive for both transgenes, from crosses between

Table 1 SOA preference ratios for FVB founder mice with either the MP2 or M14 *Prp* transgene from the SWR strain (ratio = ml solution consumed/ml solution + ml water consumed). Ratios for SWR and FVB control mice are also given

	No.	Sex	Age (days)	Concentration			
				1 mM	1 mM	0.1 mM	0.1 mM[a]
SWR	1	M	101	0.04	0.04	0.02	0.02
	2	M	101	0.04	0.03	0.04	0.01
	3	M	101	0.02	0.02	0.03	–
	4	M	101	0.03	0.03	0.04	0.05
	5	M	101	0.03	0.03	0.02	0.02
FVB	1	M	105	0.72	0.76		
	2	M	105	0.85	0.67		
	3	M	68	0.25	0.83		
	4	M	68	0.67	0.47		
	5	M	68	0.29	0.37		
	6	F	68	0.94	0.69		
FVB-MP2	7	M	105	0.05	0.95	0.64	0.38
	11	M	105	0.22	0.04	0.30	0.79
	16	M	105	0.44	0.38	0.45	0.42
	17	M	105	0.68	0.54	0.35	0.24
	18	M	105	0.29	0.90	0.53	0.92
FVB-M14	35	M	115	0.53	0.64		
	38	M	115	0.88	0.07		
	40	M	114	0.60	0.94		
	42	M	114	0.27	0.90		
	46	M	114	0.62	0.09		
	33	F	115	0.13	0.90		
	56	F	115	0.11	0.56		

[a]Each column represents one 48 h, two-bottle test.

MP2-positive and M14-positive descendants, were also given two tests with 1 mM SOA.

The descendants were tested without accompanying SWR and FVB controls due to budgetary limitations. However, four groups of naïve C3.SW-*Soa*$^{a/c}$ (C3.SW) linear back-cross mice (total = 81) bred for another project (Boughter and Whitney, 1998) were tested simultaneously, using similar procedures. Each C3.SW mouse was given two 48 h, two-bottle tests with 0.1 mM SOA. The 0.1 mM solutions were diluted directly from the 1 mM solutions used for descendant testing. These C3.SW tests yielded a total of 47 taster mice, slightly more than the 50% expected, with tasters in all four groups. C3.SW mice inheriting the dominant *Soa* allele from SWR/J ancestors exhibit a phenotype indistinguishable from that of SWR/J mice and so constitute comparable taster controls (Boughter and Whitney, 1998).

The SOA (Sigma Chemical Co., St Louis, MO) was dissolved in heated deionized water, then the solution was cooled to room temperature (~22°C). Testing was conducted in a temperature-controlled room on a 14 h light:10 h dark cycle. The mice were individually tested in 10 × 24 × 13 cm suspended stainless steel cages with wire mesh fronts and floors. Food (Purina Mouse Diet 5015) was available *ad libitum* from a hopper at the back of each cage. Fluids were presented to the mice in 25 ml graduated cylinders with neoprene stoppers and curved stainless steel spouts. Inverted cylinders were held by three clips (left, right and center) attached to the front of each cage. The spouts protruded through the wire mesh ~2 cm above the cage floor, with the water and solution spouts ~6 cm apart during tests. The mice were placed in the cages 24 h before the start of the first test to acclimate. A water cylinder was placed in the center clip on each cage during this period. To start the first test, the water cylinder was removed, a fresh water cylinder was placed in the left clip and a solution cylinder in the right clip. The initial fluid levels were recorded to the nearest 0.1 ml. Fluid levels were recorded again 24 h later. The cylinder positions were then reversed to offset any side biases and fluid levels were recorded once more (to account for any spillage during the reversal). Final fluid levels were recorded 24 h later. The second 48 h test followed immediately. For the second test, the initial bottle positions were reversed. A preference ratio (= solution consumed/water consumed + solution consumed) was calculated for each 24 h period of each test. The mean of the two 24 h preference ratios was then calculated for each 48 h test.

Analysis of two-bottle test data

The avoidance criterion for a 48 h test was a mean preference ratio <0.15. This criterion was chosen because it best discriminated taster inbred strains from nontaster and demitaster inbred strains at 1 and 0.1 mM in prior studies (Whitney *et al.*, 1989; Harder *et al.*, 1996; Boughter and Whitney, 1998). In all three studies, a consistent phenotype relative to this criterion in two consecutive 48 h tests was successfully used to select breeders with specific *Soa* genotypes across at least ten sequential segregating generations. Therefore, a minimum of two 48 h tests were conducted at each concentration. Additional tests were given when the initial tests indicated potential SOA avoidance by transgenic mice.

Preference ratios for mice that taste an SOA concentration are consistently below the criterion in multiple tests. Consumption patterns vary for mice that do not taste a concentration. Two patterns are common: (i) roughly equal consumption from both cylinders in each 24 h period and (ii) side-biased consumption. The former yields intermediate preference ratios on each test day, the latter yields one high and one low ratio with cylinder position reversal halfway through the test. The 48 h test mean is intermediate in either case. Occasionally, however, a nontasting mouse drinks predominantly from just one cylinder during a test. Whether purely by chance or with spout tracking involved (via saliva, urine, etc. deposited on the spout the first day), if the water cylinder is chosen the mouse will appear to avoid the SOA solution. Additional tests, with reversed initial cylinder positions, were used to distinguish such pseudo-avoidance from true avoidance. Only true avoidance, reflecting discrimination of the SOA solution from plain water, yields ratios consistently below the criterion across all tests. Inconsistency indicates non-discrimination.

Results

MP2 transgene integration and expression

Figure 1b is an autoradiogram showing genomic integration of the SWR MP2 transgene (*Xba*I digested) in different representative mice (lanes 2, 3, 5 and 6). The Southern blot was hybridized to the probe b (Figure 1a). The arrow shows the position of the trangene around 980 bp. The FBV background band is around 1100 bp. Figure 2a shows the MP2 transgene expression from six representative transgenic mice. Three pairs of mice representing three different lines (18, 11 and 17) are shown in lanes 3 and 4, 5 and 6, and 7 and 8 respectively. Lines 18 and 17 show greater expression of the SWR MP2 transgene (arrow at ~880 bp) than the barely visible FBV background band at ~920 bp. Line 11 shows no transgene expression.

M14 transgene integration and expression.

Figure 1c is an autoradiogram showing genomic integration of the SWR M14 transgene (*Xba*I–*Sal*I digested) in different representative mice (lanes 2, 3, 6 and 8). The Southern blot was hybridized to probe c (Figure 1a). The arrow shows the position of the transgene at ~2.1 kb. The FBV background band is at ~2.2 kb.

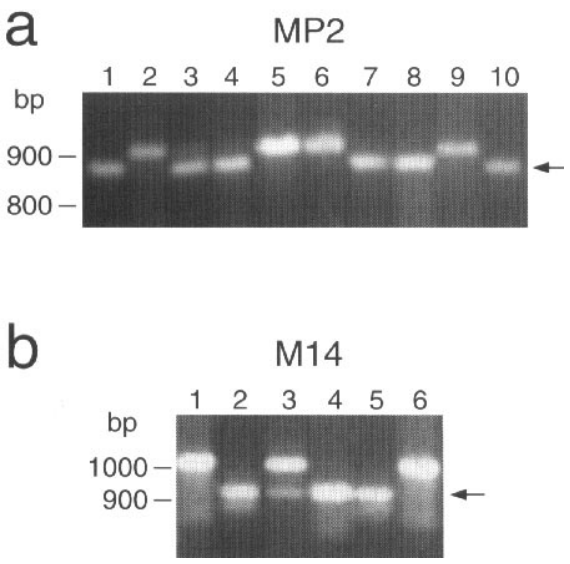

Figure 2 MP2 and M14 transgene expression: submandibular gland RT-PCR products on ethidium bromide stained gels. **(a)** MP2 transgene expression. RT-PCR products from the RNAs of six transgene (+) mice (lanes 3–8) represent results with three lines (two mice for each line). The arrow (at ~880 bp) shows the position of the SWR MP2 transgene product, and the slower migrating band at at ~920 bp represents the background FVB MP2 product [lanes 1 and 10—SWR control; lanes 2 and 9—FVB control; lanes 3 and 4—line 18 (mice 18-4 and 18-3); lanes 5 and 6—line 11 (mice 11-8 and 11-7); lanes 7 and 8—line 17 (mice 17-12 and 17-11)]. **(b)** M14 transgene expression. The RT-PCR products represent the results from one transgene (+) line (no. 56) with two different integration sites A and B (one mouse for each integration site). The arrow at ~920 bp shows the position of the SWR M14 transgene product and the slower migrating band at ~1000 bp represents the background FVB M14 product [lanes 1 and 6—transgene (–) control (FVB band only); lanes 2 and 5—SWR control; lane 3—site A (mouse 1-3-6); lane 4—site B (mouse 2-2-12)].

Figure 2b shows the M14 transgene expression two mice, each representing the same line (no. 56), but a different integration site. Site A (lane 3) shows a weaker expression of the SWR M14 transgene (arrow at ~920 bp) than the prominent FBV background band at ~1000 bp. Site B (lane 4) shows great predominance of the transgene expression compared to that of the absent FVB background expression.

SOA sensitivity of mice with either the MP2 or the M14 transgene

The SWR control mice all had preference ratios well below the 0.15 criterion on both tests at 1 mM (mean = 0.031, SE = 0.002; see Table 1). The FVB control mice all had preference ratios above criterion on both tests (mean = 0.626, SE = 0.066). The MP2-positive FVB mice all had

preference ratios above criterion on at least one test at 1 mM (mean = 0.449, SE = 0.101). The M14-positive FVB mice all had preference ratios above criterion on at least one test as well (mean = 0.517, SE = 0.088). However, one of the MP2-positive mice (no. 11) came close to meeting the avoidance criterion on both tests, so the MP2-positive mice and the SWR mice were tested with 0.1 mM SOA as well. The MP2-positive mice all had ratios above criterion on both tests at the lower concentration (mean = 0.502, SE = 0.069). The SWR ratios all remained well below criterion at 0.1 mM (mean = 0.028, SE = 0.004). Consistent avoidance of 0.1 mM SOA is the taster phenotype. Consistent avoidance of 1, but not 0.1 mM, is the demitaster phenotype. None of the transgenic mice met even the latter standard, and so displayed the nontaster phenotype along with the FVB control mice.

The transgenic mice each were paired with standard FVB mice. Seventeen MP2-positive F1 mice were obtained from founders nos 11, 17 and 18. All had preference ratios above criterion on at least one test at 1 mM (range across both tests = 0.10–0.98). Twenty-one MP2-positive F2 mice were bred by pairing MP2-positive F1 littermates (founder lines nos 11 and 18). All had preference ratios above criterion on at least one test at 1 mM (range across both tests = 0.06–0.97). Eleven MP2-positive F3 mice were bred by pairing MP2-positive F2 littermates from founder line no. 18. These F3 mice were used to produce offspring with both transgenes (see next section).

Twenty M14-positive F1 mice were obtained from founders nos 33, 40 and 56. All had preference ratios above criterion on at least one test at 1 mM (range across both tests = 0.10–0.94). Fifteen M14-positive F2 mice were bred by pairing M14-positive F1 littermates (founder line no. 56). Thirteen of these mice had preference ratios above criterion on at least one test at 1 mM (range across both tests = 0.06–0.97). However, two mice had preference ratios below criterion on both tests (nos 1–2–4 ratios = 0.09 and 0.08; nos 2–2–2 ratios = 0.10 and 0.14). These two mice were retested with 1 mM SOA to confirm their ability to avoid this concentration. Both mice had ratios well above criterion on both retests (nos 1–2–4 retest ratios = 0.25 and 0.64; nos 2–2–2 retest ratios = 0.60 and 0.86). Thus, none of the F2 mice avoided consistently.

SOA sensitivity of mice with both the MP2 and M14 transgenes

Neither transgene alone enhanced SOA sensitivity, but it was possible that both transgenes together might. Eleven MP2-positive F3 mice (founder line no. 18), with SWR-like high expression compared to the FVB baseline (see Figure 2a), were paired with 11 M14-positive F2 mice (founder line no. 56, site B), also with SWR-like high expression levels compared to the FVB baseline (see Figure 2b). A total of 22 offspring were obtained from two M14 female × MP2 male pairs. Nine offspring were positive for both transgenes. All nine had preference ratios above criterion on at least one test at 1 mM (mean = 0.562, SE = 0.061; see Table 2). Even the combination of both transgenes failed to enhance SOA sensitivity.

Table 2 SOA preference ratios for FVB mice inheriting both the MP2 and M14 transgenes (ratio = ml solution consumed/ml solution + ml water consumed)

	No.	Sex	Age (days)	Concentration	
				1 mM	1 mM[a]
FVB-MP2 + M14	1	M	42	0.13	0.93
	2	M	44	0.78	0.59
	3	M	44	0.83	0.53
	4	M	52	0.10	0.43
	5	F	42	0.50	0.54
	6	F	51	0.47	0.80
	7	F	52	0.07	0.77
	8	F	52	0.56	0.70
	9	F	52	0.87	0.52

[a]Each column represents one 48 h, two-bottle test.

Discussion

The sensitivity of nontaster strain mice to the bitterness of SOA was not enhanced by two type-A *Prp* transgenes from a taster strain. The transgenes had no effect on bitter sensitivity, either alone or together. Although these two type-A *Prp* genes have been tested, no type-B *Prp* genes were studied. One type-B gene (MP4) at the *Prp* locus has been sequenced (Roberts *et al.*, 1991).

We tested for the presence of transgene-derived RNA in submandibular glands as an index of expression. In some transgenic lines (both MP2 and M14), relative transgene expression exceeded that of the FVB background. However, we did not test for transgene-specific protein production in the submandibular glands. Therefore, it is possible that protein production may not parallel relative RNA expression levels and may not be under normal regulatory influences. It should be noted that a complete DNA sequence of the coding region for the MP2 gene from the SWR inbred line (the source of the MP2 transgene) showed no mutations (such as a stop codons) that would be likely to seriously alter protein translation (E.A. Azen, unpublished data). With these caveats in mind, it is still possible that *Soa* and type-A *Prp* genes are the same; however, from our data, the weight of evidence would seem to favor the conclusion that they are not the same.

Cosegregation data suggest *Soa* should be within 1 cM of *Prp*. *Prp* currently is mapped at 63.6 cM from the centromere, coincident with microsatellite marker D6Mit13 (in the M14 gene). More precise limits on the possible location of *Soa*, from additional segregation data, might point to other

candidate genes in the 2 cM region around *Prp*. Such limits might also place *Soa* in an area of chromosome six with no currently known candidate genes.

Whatever gene is eventually identified as *Soa* should exhibit several properties. It should have at least three alleles, corresponding to the taster, demitaster and nontaster SOA sensitivity phenotypes (Harder *et al.*, 1992). The allele corresponding to the taster phenotype should exhibit complete dominance over both other alleles (Harder *et al.*, 1992). Gene expression patterns should be consistent with adult-like phenotypes in mice 3–4 weeks old (Harder, unpublished data). Allelic variation at the gene should affect only bitter sensitivity, and only to some bitter compounds, not all (Whitney and Harder, 1994; Harder *et al.*, 1996; Boughter and Whitney, 1998). Compounds affected should include acetylated sugars, strychnine and brucine, denatonium benzoate, picric acid, quinine, isohumulone and propylthiouracil (PROP). Compounds not affected should include, caffeine, thiamine, L-phenylalanine, cycloheximide, humulone, and phenylthiourea (PTC). Finally, the gene should have a site of effect in the peripheral gustatory system (Shingai and Beidler, 1985).

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