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The genetics of tasting in mice

VI. Saccharin, acesulfame, dulcin and sucrose

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Summary

Twenty-six strains of mice were tested for their reaction to four different sweet substances; saccharin, acesulfame, dulcin and sucrose. There was considerable strain variation in the degree to which they found the sweet substances preferable to water. The variation in preference for any one sweet substance is very highly correlated with the variation in preference for the other sweet substances. This is interpreted to mean that there is only one sweetness receptor, although an alternative explanation in terms of variation in psychological motivation is not discounted. The difference between C57BL/6Ty and DBA/2Ty is largely due to a single gene, *Sac*.

1. Introduction

In the previous paper in this series (Lush & Holland, 1988) it was noted that strains of mice differ in the degree to which they can detect the sweet taste of glycine. To some strains glycine also has a bitter taste and this interferes with the sweetness and makes glycine a rather unsuitable substance with which to investigate the genetics of sweetness. It seemed more sensible to use intensely sweet substances which are sweet at concentrations well below those at which any bitterness might be detectable. This paper describes work using three synthetic sweeteners, saccharin, acesulfame and dulcin, and also sucrose.

It has been known for some years that strains C57BL/6 and DBA/2 differ in their reaction to solutions of saccharin (Capretta, 1970; Pelz *et al.* 1973). Fuller (1974) found that C57BL/6J mice strongly prefer saccharin solutions to water over quite a wide range of saccharin concentrations and DBA/2J mice have a much weaker preference in the same direction. This strain difference in behaviour appeared to be due to a single gene which Fuller (1974) called *Sac*. However, Fuller was not able to show that *Sac* alleles segregate in the progeny of a cross to give phenotypically distinct classes. To some extent, therefore, the existence of the *Sac* gene itself has remained uncertain. One aim of the following work was to confirm Fuller's results and to extend them by using more sweet substances and a greater variety of mouse strains.

2. Materials and methods

The strains of mice are those which were used previously (Lush 1981; Lush & Holland, 1988). The taste testing technique has been described in detail (Lush 1984). Distilled water was used for dissolving the chemicals and also for the control burettes. Sucrose, from BDH, was of Analar grade. Saccharin (sodium salt of *o*-sulfobenzimide) and dulcin (4-ethoxyphenylurea) both came from Sigma. Acesulfame (potassium salt of 6-methyl-1,2,3-oxathiazine-4(3H)-one-2,2-dioxide) was a gift from Hoechst U.K. Ltd. The structural formulae of the three synthetic chemicals are shown in Fig. 1.

3. Results

Five strains, DBA/2Ty, C57BL/6Ty, SWR, 129/Sv and STS were each tested with six concentrations of saccharin from 0·2 up to 5·0 mM. The resulting

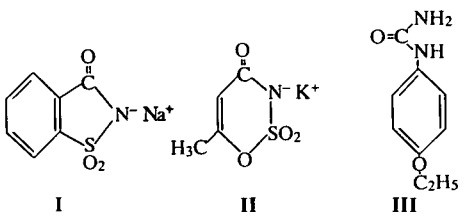

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Fig. 1. Structural formulae of (I) saccharin, sodium salt (II) acesulfame, potassium salt (III) dulcin.

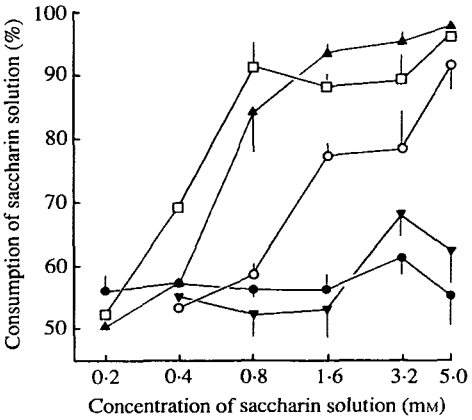

Fig. 2. Concentration-response curves of five strains with saccharin. ▲, C57BL/6Ty; □, STS; ○, SWR; ▼, 129/Sv; ●, DBA/2Ty. Each point is the mean of between two and eight experiments. Vertical bars are S.E.M.S.

concentration-response curves are shown in Fig. 2. It is clear that there are large strain differences. DBA/2 shows only a slight preference for saccharin and this does not increase with increasing saccharin concentration. On the other hand the SWR, C57BL/6 and STS curves show that for these strains the higher concentrations are increasingly attractive.

In order to obtain a more complete picture of the strain variation, all 26 available strains were tested with 1·6 mM saccharin. The data from this survey are given in Table 1 and show a continuous range of responses. Since all these strains are kept in the same mouse room and have the same environment one must conclude that the strain variation is largely genetically determined. Before attempting to analyse the genetics, the same strains were tested with three other sweet substances; acesulfame (3·2 mM), dulcin (3·2 mM) and sucrose (50 mM). The concentration of each was chosen on the basis of preliminary tests with the same five strains as were used for Fig. 2. The results of all these surveys are given in Table 1 and are displayed in Fig. 3 where the saccharin result for each strain is plotted against the results got with acesulfame, dulcin and sucrose. The results with the different tastants are highly correlated, showing that the physiological variable which is subject to genetic variation in these mice is one which responds to a number of different sweet substances. The correlation coefficients are as follows: saccharin *vs.* acesulfame, $r = 0{\cdot}958$; saccharin *vs.* dulcin, $r = 0{\cdot}885$; saccharin *vs.* sucrose, $r = 0{\cdot}952$.

The strains as a whole show fairly continuous

Table 1. *Consumption of four sweet substances by 26 strains of mice. Each cage contained up to four mice*

	Tastant							
	Saccharin (1·6 mM)		Acesulfame (3·2 mM)		Dulcin (3·2 mM)		Sucrose (50 mM)	
Strain	Cages tested	Mean tastant consumed (%)	Cages tested	Mean tastant consumed (%)	Cages tested	Mean tastant consumed (%)	Cages tested	Mean tastant consumed (%)
C57BL/6Ty	3	93	4	95	2	86	2	97
ST/bJ	4	91	2	90	2	85	5	93
STS	8	88	3	94	2	85	2	89
C57BL/10	3	87	2	95	4	77	2	95
C57BL/6By	4	86	2	92	2	81	2	94
IS	3	83	5	95	4	96	2	91
C57L	4	80	2	87	4	73	2	87
TO	4	77	4	77	2	79	2	85
SWR	4	77	3	80	2	75	2	84
Schneider	4	74	4	74	2	67	2	79
A2G	4	73	2	73	2	72	2	74
SEA	4	68	4	67	4	66	2	65
A/J	4	67	2	56	4	58	3	66
SM/J	4	64	2	60	6	72	2	69
BALB/cA	4	62	3	58	6	50	2	54
129/Rr	4	60	2	64	2	55	2	57
AU	4	59	2	61	4	52	3	51
C3H/He	3	59	2	59	1	61	2	61
BALB/cBy	3	59	2	57	5	56	3	60
DBA/2Ty	3	56	3	50	3	61	2	53
CBA/Ca	5	56	2	65	2	62	2	67
CE	8	55	2	53	8	65	2	50
DBA/1Lac	3	55	2	54	2	52	1	54
AKR	3	53	4	45	2	49	2	50
129/Sv	6	53	3	57	6	54	3	51
NMRI	4	50	2	48	2	55	4	61

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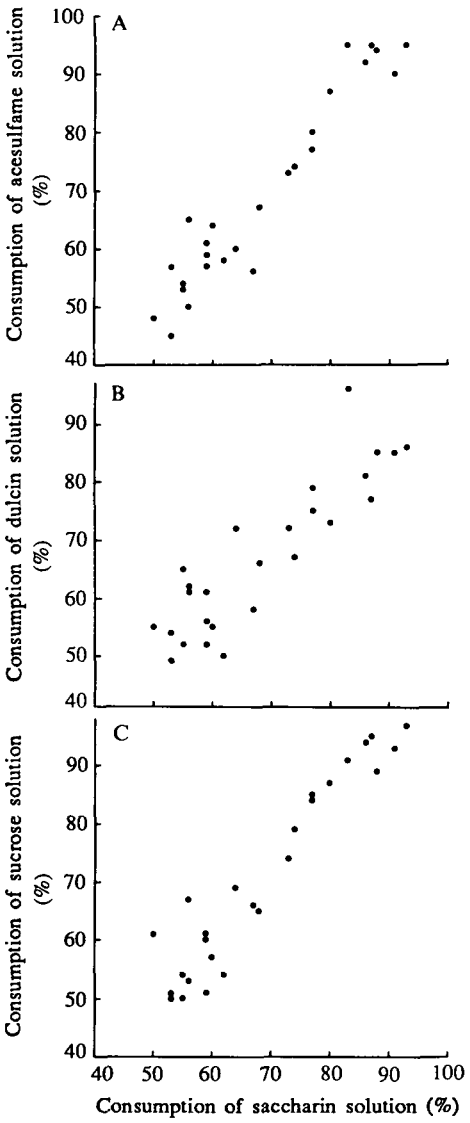

Fig. 3. Scatterplots of the data in Table 1 to show the high correlations between the twenty-six strains in their consumption of four sweet substances. A, saccharin *vs.* acesulfame; B, saccharin *vs.* dulcin; C, saccharin *vs.* sucrose.

variation between those which have a strong preference for 1·6 mM saccharin and those which are more or less indifferent to it. In spite of this continuous variation it was considered that there might be one or two genes which have a relatively major effect on the phenotype. Since C57BL/6Ty and DBA/2Ty are almost at opposite ends of the phenotypic range, twenty BXD RI strains were tested with saccharin and with acesulfame. The results are given in Table 2 and displayed in Fig. 4 and show clearly that the RI strains fall into two groups, one group of nine 'tasters' and another group of eleven 'non-tasters'. This indicates that one gene accounts for a large part of the difference between C57BL/6Ty and DBA/2Ty.

The existence of this gene was confirmed by means of a backcross. DBA/2Ty males were mated with C57BL/6Ty females and four F_1 progeny were tested with 5 mM saccharin and with 5 mM acesulfame. This concentration was used because experience with other tasting genes has shown that dominance is more evident at high concentrations. All the F_1 mice were found to be tasters of saccharin and of acesulfame. Male and female F_1 mice were therefore backcrossed to DBA/2Ty and a total of 32 progeny were tested with both tastants. The results are given in Fig. 5 and show that the progeny fall into 2 groups, with 15 in the taster group and 17 in the non-taster group. This is not significantly different from a 1:1 ratio. Inspection of Fig. 5 shows that although the results with the two tastants are in complete agreement with each other, the actual separation of the mice into 2 groups is achieved by the acesulfame test and not by the saccharin. If they had been tested only with saccharin it would have been barely possible to distinguish the 2 groups, which was what Fuller (1974) found. Nevertheless, it seems sensible to continue to use Fuller's symbol *Sac* for this gene, with alleles Sac^b and Sac^d in strains C57BL/6 and DBA/2 respectively.

The chromosomal position of *Sac* is not yet known. In the backcross progeny the coat colour genes brown (*b*, on chromosome 4) and dilute (*d*, on chromosome 9) showed no linkage with *Sac*. The strain distribution pattern of *Sac* alleles in the BXD RI strains is discordant with that of the quinine-tasting gene *Qui* in 15 out of the 20 RI strains (Lush & Holland, 1988), therefore *Sac* is clearly not linked to the bitterness gene cluster on chromosome 6. In the backcross progeny of the F_1 male the taster and non-taster phenotypes occurred equally frequently in both sexes, which shows that the *Sac* is not sex-linked.

4. Discussion

The simplest interpretation of the data is that there is only one type of sweetness receptor in the mouse. The genetic variation affects the functioning of this receptor and therefore affects its sensitivity to all four sweet substances. This would explain the absence of any strain which deviates from the correlations shown in Fig. 3. Saccharin and acesulfame have some similarity in their structures as can be seen in Fig. 1, but dulcin is quite different and so also is sucrose. Nevertheless, if they all act on the same receptor they must have some common feature which is recognised by the receptor.

The way in which *Sac* affects the functioning of the sweetness receptor is not known. Nor is it known if *Sac* is the only gene which has such an effect. The strains which are near the middle of the range, for example SWR, must either have an *Sac* allele different from those present in C57BL/6Ty and DBA/2Ty or else be different at one or more other genetic loci which affect the sweetness receptor.

The genetics of sweetness tasting in the mouse is

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Table 2. *Consumption of saccharin and acesulfame by the BXD RI strains*

RI Strain	Tastant: Saccharin (3·2 mM) Mean tastant consumed (% ±S.E.M.)	Tastant: Acesulfame (3·2 mM) Mean tastant consumed (% ±S.E.M.)	Strain distribution pattern[a]
1	62±3·4	53±1·7	D
2	66±8·1	57±4·1	D
5	95±2·2	93±2·7	B
6	88±1·5	82±2·2	B
8	57±2·0	58±2·0	D
11	92±1·1	89±3·8	B
12	65±2·6	57±4·9	D
15	80±4·3	81±2·6	B
16	68±2·2	57±1·7	D
18	64±3·5	60±1·3	D
19	86±4·0	77±2·8	B
22	64±3·0	56±2·6	D
24	84±6·1	79±1·0	B
25	81±5·6	77±2·7	B
27	62±4·6	54±3·8	D
28	55±4·9	51±3·2	D
29	93±1·5	85±1·9	B
30	55[b]	53[b]	D
31	84±2·3	88±2·0	B
32	55±2·3	52±2·6	D

[a] D, like DBA/2Ty; B, like C57BL/6Ty.
[b] Only two BXD 30 cages were tested.

very different from the genetics of bitterness tasting. With bitterness there are many genes, but each determines a receptor which is restricted in the chemicals it responds to (Lush & Holland 1988). This is understandable in evolutionary terms since bitterness has presumably evolved as a warning system to ensure that harmful chemicals are avoided, and for that purpose a high degree of specificity and sensitivity would be advantageous. A sense of sweetness, on the other hand, might seem to be rather an unnecessary luxury for a rodent which, in the wild state, feeds largely on insects and other small invertebrates (Berry *et al.* 1973). Sweetness in the mouse does not need to be such a discriminating taste and can manage with only one type of receptor.

If it is true that the mouse has only one type of

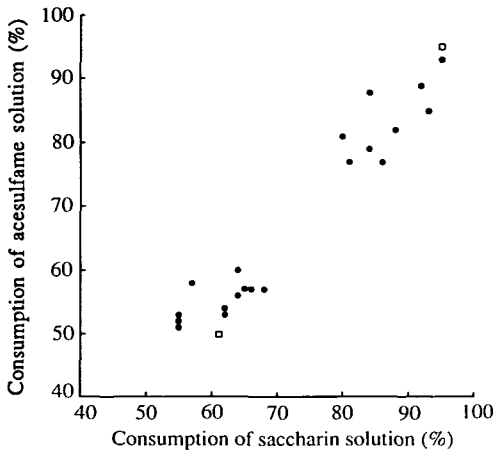

Fig. 4. Consumption of saccharin (3·2 mM) and acesulfame (3·2 mM) by 20 BXD RI strains; ●, RI strains; □, founder strains C57BL/6Ty (upper) and DBA/2Ty (lower).

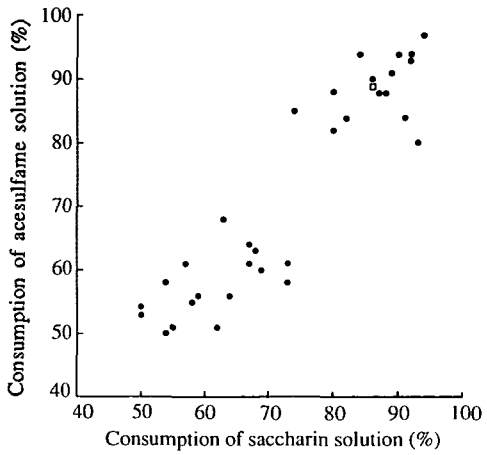

Fig. 5. Consumption of saccharin (5 mM) by the 32 progeny of the backcross (C57BL/6Ty × DBA/2Ty) × DBA/2Ty and its reciprocal. □, mean of four F_1 mice; ●, the 32 progeny.

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sweetness receptor, what does this imply for other mammals which can taste sweetness, for example Man? Since saccharin, acesulfame and dulcin are synthetic chemicals not found in the natural environment, the ability of the mouse to taste them as sweet can only have evolved as an accidental property of the sugar receptor. If the sugar receptor in Man shares a common evolutionary origin with that of the mouse, it seems probable that humans also taste all four substances by means of the same receptor. This conclusion is not in agreement with the views of some other workers on human taste. For example, Faurion (1987) measured variation in the recognition thresholds for seven sweeteners (including saccharin and sucrose) between 91 subjects and found no correlations. Faurion considers that there are at least 5 different types of sweetness receptor in humans. Genetical studies have shown that *Drosophila melanogaster* has three different types of sweetness receptor, each of which responds to a different kind of sugar (Arora *et al.* 1987; Tanimura *et al.* 1988). This is also understandable in evolutionary terms because there are several different kinds of sugar present in the rotting fruit and vegetation which is the source of food for *Drosophila* in its natural habitat.

It must be conceded that the variation described in this paper could be of a psychological rather than a physiological nature. In other words, the strains may all perceive the same degree of sweetness but they may react to it differently, some strains being more highly motivated than others to go back to the sweet solution to drink. The question could be settled by making neurophysiological measurements of the neural impulses coming from the tongue in mice of different strains when stimulated with a sweet solution. In this way Shingai and Beidler (1985) showed that variation in behaviour towards the bitter substance sucrose octaacetate is due to variation of the peripheral receptors in the tongue and need not involve any variation in the central nervous system.

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References

Arora, K., Rodrigues, V., Joshi, S., Shanbhag, S. & Siddiqi, O. (1987). A gene affecting the specificity of the chemosensory neurons of *Drosophila*. *Nature* (*London*) **330**, 62–63.

Berry, R. J., Jakobson, M. E. & Triggs, G. S. (1973). Survival in wild-living mice. *Mammal Reviews* **3**, 46–57.

Capretta, P. J. (1970). Saccharin and saccharin-glucose ingestion in two inbred strains of *Mus musculus*. *Psychonomic Science* **21**, 133–135.

Faurion, A. (1987). Physiology of the sweet taste. *Progress in Sensory Physiology* **8**, 130–201.

Fuller, J. M. (1974). Single locus control of saccharin preference in mice. *Journal of Heredity* **65**, 33–36.

Lush, I. E. (1981). The genetics of tasting in mice I. Sucrose octaacetate. *Genetical Research* **38**, 93–95.

Lush, I. E. (1984). The genetics of tasting in mice IV. Quinine. *Genetical Research* **4**, 151–160.

Lush, I. E. & Holland, G. (1988). The genetics of tasting in mice V. Glycine and cycloheximide. *Genetical Research* **52**, 207–212

Pelz, W., Whitney, G. & Smith, J. C. (1973). Genetic influences on saccharin preference in mice. *Physiology and Behaviour* **10**, 263–265.

Shingai, T. & Beidler, L. M. (1985). Interstrain differences in bitter taste responses in mice. *Chemical Senses* **10**, 51–56.

Tanimura, T. Isono, K. & Yamamoto, M. T. (1988). Taste sensitivity to trehalose and its alteration by gene dosage in *Drosophila melanogaster*. *Genetics* **119**, 399–406.