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Cross-adaptation and Molecular Modeling Study of Receptor Mechanisms Common to Four Taste Stimuli in Humans

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Abstract

Psychophysical cross-adaptation experiments were performed with two carbohydrates, sucrose (SUC) and fructose (FRU), and two sweeteners, acesulfame-K (MOD) and dulcin (DUL). Seven subjects were asked to match concentrations that elicited the same intensity as a sucrose reference (30 g/l). Cross-adaptation levels were calculated as the ratio of isointense concentrations measured for a given stimulus before and under adaptation. On average, cross-adaptation between SUC and FRU is low and apparently reciprocal. By contrast, cross-adaptation between SUC and MOD is clearly non-reciprocal: SUC adapts MOD significantly (24%, $P < 0.005$), but MOD fails to adapt SUC (2%, $P < 0.79$). Significant and reciprocal cross-enhancement is observed between DUL and MOD (~–20%, $P < 0.03$), and also between SUC and DUL (~–15%, $P < 0.08$). In parallel, molecular modeling of the four tastants was performed in order to look for the 12 common binding motifs that were isolated on 14 other tastants in a previous study. SUC and FRU each display 10 out of the 12 binding motifs, whereas DUL and MOD only display four and five distinct motifs respectively and do not have any motif in common. Experimental cross-adaptation levels seem to correlate well with the number of motifs that molecules have in common. FRU and SUC share a majority of binding motifs and correlatively show mutual cross-adaptation. Four motifs of MOD are found among the 10 motifs of SUC, which may explain why SUC cross-adapts MOD but not vice versa. By contrast, DUL and MOD do not share any motif and do not cross-adapt. The various molecular mechanisms that may be responsible for cross-adaptation and/or cross-enhancement are discussed in light of our results.

Introduction

Psychophysical studies (Faurion *et al.*, 1980; Schiffman *et al.*, 1981; Lawless and Stevens, 1983) together with electrophysiological recordings of receptor cells (Tonosaki and Funakoshi, 1984, 1989; Bernhardt *et al.*, 1996) or primary gustatory nerve fibers (Faurion and Vayssettes-Courchay, 1990; Hellekant and Ninomiya, 1991) suggest the existence of multiple and independent binding sites for organic tastants. However, the characteristics of these different receptor sites, particularly the way in which they probe distinct submolecular motifs displayed on the surface of sapid molecules, should be more precisely addressed.

In 1972, Kier suggested a trifunctional binding motif comprising two adjacent H-bonding groups, AH and B, and a hydrophobic patch, X, which could explain the similarly sweet tastes of cyclamate, saccharin, perillaldehyde oxime and some nitroanilines. Recently, we developed a molecular modeling approach to elucidate the binding process for organic tastants (Froloff, 1994; Froloff *et al.*, 1996). Using an original multistep pattern-matching procedure, we generalized Kier's proposal by characterizing 12 common motifs on the molecular surfaces of 14 tastants, each motif containing 2–5 binding moieties (H-bond donors or acceptors as well as hydrophobic patches). Seven of these 12 motifs were shown to significantly and optimally account for the similarities in stimulatory effects of the 14 molecules as measured on 58 human subjects (Faurion, 1993). We therefore presented these seven binding motifs as good candidates to be recognized by seven distinct taste receptor sites.

In the present study, two carbohydrates—sucrose (SUC) and fructose (FRU)—and two sweeteners—acesulfame (MOD) and dulcin (DUL)—were modeled using the same methodology in order to look for the same 12 binding motifs on their respective molecular surfaces. DUL is the only molecule common to both previous and present studies, while SUC, FRU and MOD are newly modeled molecules.

In parallel, we measured the similarity between paired molecules using psychophysical cross-adaptation, namely the variation of perceived intensity for a given stimulus after stimulation with another stimulus. We then examined whether these effects could be explained by the presence of common binding motifs on the surface of the stimulating molecules.

Materials and methods

Psychophysics

Each subject (three males and four females, aged 21–36 years) participated in a 2 h experimental session/day, 4–5 days/week for 8 weeks. They were asked not to smoke, eat, chew gum or wash their teeth half an hour prior to each session. They were paid for their contribution.

Subjects were trained to determine, for each stimulus, the concentration C_1 which matched the intensity of a 88 mM (30 g/l) SUC reference. An elementary test combined a forced-choice paired comparison and the up-and-down Dixon staircase procedure (UD test: Dixon and Mood, 1948; Dixon and Massey, 1960). During one elementary UD test, stimuli were presented pairwise (5 ml of test stimulus and 5 ml of reference) by a computer-driven automaton, with a 1 min delay between pairs. The subject tasted the stimulus for 5 s, memorized the peak intensity, expectorated and rinsed with tap water. Then, the subject tasted the reference for 5 s and indicated which solution was stronger. Subjects were preliminary trained with various complex taste stimuli to rate total perceived intensities without any reference to semantic descriptors such as 'sweet' or 'bitter'. The concentration of the stimulus in the next pair was increased or decreased in a geometric progression depending on the subject's response. The isointense concentration C_1 was calculated from Dixon's formula as $C_1 = R^k \times C_f$, where R is the constant dilution ratio (here $R = 1.4$), k is a coefficient found in the statistical table of Dixon that depends on the subject's response pattern on five pairs presented after the first change of response (e.g. 'stimulus B stronger', instead of 'stimulus A stronger'), and C_f is the final concentration presented. One UD test was completed within ~7 min (~7 presented pairs). Each subject performed several UD tests for a given stimulus in a 2 h session. Long-term reproducibility was tested for each stimulus and each subject, and the mean of at least six UD tests gave the stimulus concentration perceived as intense as the reference across several sessions for the subject. A training period was allowed for each subject, such that data of the first sessions were often discarded for each stimulus.

In order to assess cross-adaptation, subjects were also trained to determine the isointense concentration C_2 of a stimulus in adapting conditions during modified UD tests. In such a test, the subject first tasted the adapting stimulus for 20 s and expectorated. The subject then immediately tasted the test stimulus for 5 s and memorized the peak intensity. Last, the subject rinsed thoroughly, waited for 5 s, tasted the SUC reference for 5 s and indicated whether the test stimulus or the reference was stronger. The next series of stimulations were done after a 1 min delay; a modified UD test was hence completed within ~10 min.

We checked that under such conditions the perceived intensity of the SUC reference was not significantly altered by the initial adaptation. Indeed, when a carbohydrate solution (FRU or SUC) was used as the adapting stimulus, it had to be in contact with the tongue for at least 20 s and immediately followed by the test stimulus in order to yield significant adaptation. No adapting effect could be observed when a short delay (~5 s) was allowed between adapting and test stimulations. Moreover, stimuli other than SUC and FRU were shown not to adapt SUC. It is thus very unlikely that the SUC reference was modified in intensity when tasted as the third solution in a row after a thorough rinse and a 5 s delay.

For each subject, the same experimental conditions were repeated at least twice over successive sessions and data from several UD tests were averaged. For some subjects the first session was discarded as a learning session. Data were collected for each molecule and each subject as: (i) isointense concentrations (C_1) to SUC 88 mM (30 g/l) without adaptation; (ii) isointense concentrations (C_2) to SUC 88 mM after 20 s stimulation with the same or another adapting stimulus.

Stimuli were two carbohydrates, SUC and FRU, and two sweeteners, MOD (potassium 6-methyl-1,2,3-oxathiazin-4-one-2,2-dioxide) and DUL. Stock solutions were made in sterile water of known and constant composition on the day of the experiment and maintained in a thermostat-controlled bath (±0.1°C). Glassware was sterilized with boiling water before each experiment. The concentration of each adapting solution was chosen as the mean of the individual isointense concentrations (SUC, 88 mM; FRU, 160 mM; MOD, 0.65 mM; DUL, 0.55 mM). These values were similar to those previously observed on a larger (40–60) group of subjects (Faurion *et al.*, 1993). Each subject tasted all possible pairs (adapting stimulus/test stimulus) with SUC, MOD and DUL (six cross-adaptation pairs and three self-adaptation pairs); FRU reciprocal cross-adaptation with SUC was also measured (two additional pairs).

Molecular modeling

The crystal structure of MOD (Paulus, 1975) was used for modeling. The atomic coordinates were retrieved from the Cambridge Structural Database (CSD) (Allen *et al.*, 1979, 1983, 1991) under the reference code KMOTZD. The predominant form at pH 7.0 is the anionic form since the pK_A of acesulfame is ~2.0 at 25°C as determined from titration in our laboratory.

A low-energy conformation for DUL was generated from standard bond lengths and bond angles, and energy-minimized by thorough Monte Carlo Metropolis

Table 1 Self-adaptation and cross-adaptation between the four tastants

	SUC/SUC	MOD/MOD	DUL/DUL	SUC/MOD	MOD/SUC	SUC/DUL	DUL/SUC	SUC/FRU	FRU/SUC	MOD/DUL	DUL/MOD
C_1 (mM)	87 ± 4	0.63 ± 0.10	0.52 ± 0.19	0.66 ± 0.13	86 ± 7	0.54 ± 0.15	87 ± 7	158 ± 29	91 ± 11	0.66 ± 0.22	0.62 ± 0.11
C_2 (mM)	107 ± 13	1.12 ± 0.23	0.91 ± 0.06	0.82 ± 0.13	88 ± 15	0.45 ± 0.11	75 ± 6	174 ± 30	98 ± 7	0.50 ± 0.12	0.52 ± 0.08
$100 \times (C_2 - C_1)/C_1$	24%	77%	76%	24%	2%	−16%	−13%	10%	8%	−25%	−17%
P	0.006	0.0002	0.002	0.005	0.79	0.08	0.04	0.23	0.17	0.01	0.03

First tastant in title row is adapting, second tastant in title row is adapted. C_1 is concentration of second tastant eliciting isointense perception in normal conditions with respect to 88 mM sucrose. C_2 is concentration of second tastant eliciting isointense perception in adapting conditions. C_1 and C_2 shown here are averaged over the individual values given by the seven subjects (±SD). P is the Student's t-test double-sided significance level on paired individual C_1 and C_2 values.

(Metropolis *et al.*, 1953) conformational search in the MM2 force field (Allinger, 1977). A further energy refinement was performed in the Austin Model 1 semiempirical Hamiltonian (AM1) (Dewar *et al.*, 1985) available in MOPAC (Quantum Chemistry Program Exchange, Indiana University, Bloomington, IN).

The three major tautomers of FRU in water (Cockman *et al.*, 1987) were modeled, namely β-D-fructopyranose, β-D-fructofuranose and α-D-fructofuranose. FRU is indeed known to have several tautomeric forms in aqueous solution due to mutarotation. Using a combination of GC and GC–MS with a buffered 0.50 M fructose solution (pH 4.4) over the temperature range 10–55°C, Cockman *et al.* found that, at 35°C, the three major components of FRU are β-D-fructopyranose (69.4%), β-D-fructofuranose (23.6%) and α-D-fructofuranose (5.7%). There are also small amounts of the α-D-fructopyranose form (1.0%) and the open-chain ketone form (0.3%), which we ignored in the present study. The atomic coordinates of β-D-fructopyranose were retrieved from the CSD (reference code FRUCTO02: Takagi and Jeffrey, 1977). The atomic coordinates of β-D-fructofuranose and α-D-fructofuranose were both retrieved from the Klotho database (Dunford-Shore *et al.*, 1994). These three-dimensional coordinates were calculated from stereochemical configuration information by the CONCORD program (Tripos, Inc., St Louis, MO) (Rusinko *et al.*, 1986, 1989; Pearlman, 1987), using a proprietary pseudo-energy minimizing algorithm.

SUC has several metastable conformational states in aqueous solution due to an almost free rotation around the glycosidic linkage between the α-glucopyranosyl and the β-fructofuranosyl rings, and free rotations of the hydroxymethyl groups. We thus decided to model six different conformers of SUC: the crystal structure (Brown and Levy, 1973; CSD ref. code SUCROS04) together with five other low-energy conformers determined by NMR spectroscopy of aqueous sucrose and molecular modeling (Hervé du Penhoat *et al.*, 1991; Meyer, 1994; C. Meyer and S. Pérez, personal communication).

The oxygen and nitrogen atoms bearing electron lone pairs were classified as H-bond acceptors, those bearing hydrogens as H-bond donors, and those bearing both (namely the oxygens belonging to hydroxyl groups) as H-bond acceptor/donor centers. The molecular surfaces ('smoothed' van der Waals surfaces: Richards, 1977) of the various molecular structures were generated with the program GEPOL93 (Pascual-Ahuir *et al.*, 1987, 1994; Pascual-Ahuir and Silla, 1990; Silla *et al.*, 1991) with a surface density of ~5 dots/Å^2 and a water-probe radius of 1.4 Å. The hydrophobic surface of a molecule was defined as the set of molecular surface dots closest to the molecule's non-polar atoms.

The molecular structures thus modeled were cut into fragments using the automatic procedure as described previously (Froloff *et al.*, 1996). Fragments were cut such that they redundantly sampled all possible binding faces of the molecular surfaces. All fragments were then compared pairwise to 12 fragments (see Figure 6 in Froloff *et al.*, 1996) representing the 12 binding motifs previously isolated. These motifs either comprise one hydrogen-bonding heteroatom (HBA) (the H-bonding donor of fragment type 1), two HBAs (fragment types 2–6), three HBAs (fragment types 7–11) or four HBAs (fragment type 12). A (4 × 12) binary table was constructed from the result of this comparison. For each of the four molecules, the occurrence of each of the 12 predefined motifs was noted as 1 and its absence as 0.

Results

Psychophysics

Table 1 gives the molecular concentrations which are perceived as intense as a constant reference sucrose solution (88 mM), prior to adaptation (C_1) and under adaptation (C_2), averaged for the group of seven subjects. Figure 1 summarizes the results of self- and cross-adaptation between the four stimuli.

Without adaptation, individual results were stable among successive sessions for a given stimulus. As in a number of previous psychophysical studies, large and significant interindividual differences of sensitivity were observed for the four tastants. For example, one subject perceived 0.36 ± 0.03 mM DUL to be as intense as the reference, whereas another subject needed 0.84 ± 0.13 mM DUL to perceive

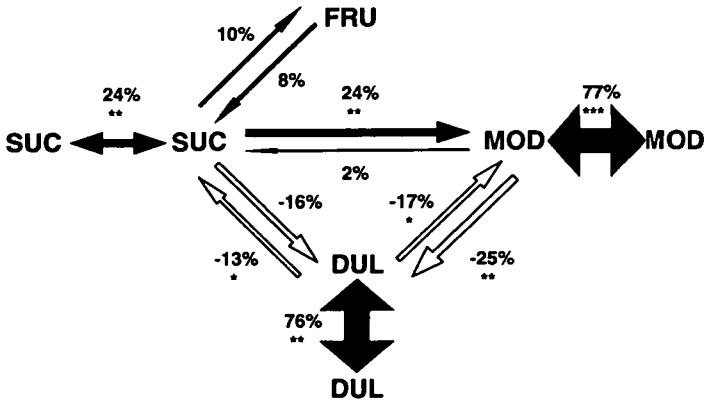

Figure 1 Summary of self- and cross-adaptation results. Single-headed arrows denote cross-adaptation (black) or cross-enhancement (white) between adapting and test stimuli. Double-headed arrows denote self-adaptation of stimuli. Next to each arrow is indicated the percentage change of perceived isointense concentration for the test stimulus before and under adaptation (positive: adaptation; negative: enhancement), averaged over the seven subjects (see Table 1). Arrow width is proportional to the percentage of cross-effect. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

isointensity ($P < 0.05$). Considering the whole set of subjects, the average concentrations giving the same perceived intensity as the reference (88 mM SUC) were: FRU, 160 mM; MOD, 0.65 mM; DUL, 0.55 mM. There was thus a factor of ~100 between the average isointense concentrations of carbohydrates and those of sweeteners (C_1 in Table 1). The order of potency (on a molar basis) to elicit the same perceived intensity as 88 mM SUC was DUL (×160), MOD (×135), SUC (1) and FRU (×0.55).

Large, stable and significant interindividual differences in self- and cross-adaptation between stimuli were also observed. For SUC, the level of self-adaptation (percentage change between C_1 and C_2) varied from 38 to 0% depending on subject, and was significant ($P < 0.05$) for six out of seven subjects. For SUC/FRU, the individual levels of cross-adaptation varied from 50% (adaptation) down to –24% (enhancement) depending on subject. Large interindividual differences in taste intensity perception have been observed in previous psychophysical studies (e.g. Faurion, 1993). Such differences are consistent with the hypothesis of multiple independent taste receptor sites (Faurion *et al.*, 1980) that are present in various proportions on each subject's tongue. They should be kept in mind when considering the averaged data that are reported in Table 1 and Figure 1.

As seen from Table 1 on data averaged across subjects, molecules show strikingly different levels of self-adaptation. Self-adaptation is strong for both intense sweeteners MOD and DUL with 77% ($P < 0.0002$) and 76% ($P < 0.002$) concentration increase needed to match the reference after adaptation as compared to normal conditions. On average, the seven subjects perceive a concentration of 0.63 mM MOD with the same intensity as the reference, as compared to 1.12 mM MOD in self-adapted conditions (MOD after MOD). Comparatively, SUC shows a moderate yet significant level of self adaptation (24%, $P < 0.006$). The individual percentage levels of self-adaptation were significant ($P < 0.05$) for 18 out of 21 cases regardless of the subject or the molecule tested.

As also seen from Table 1, it seems that the level to which a first molecule is able to cross-adapt a second molecule is always lower than the self-adaptation level of the second molecule. There is no exception to this rule for the four molecules tested. Adaptation between FRU and SUC is not significant, yet is quantitatively half of SUC self-adaptation (~10%, $P < 0.23$).

Moreover, cross-adaptation is clearly non-reciprocal. This is most striking for SUC and MOD: SUC adapts MOD significantly (24%, $P < 0.005$), but MOD fails to adapt SUC (2%, $P < 0.79$).

Finally, large and significant cross-enhancement is observed instead of cross-adaptation for all cases which involve DUL both as the adapting and the test stimulus. For example, a significantly lower concentration of MOD after DUL (–17%, $P < 0.03$) was sufficient to obtain isointensity to the reference, as compared to the isointense concentration of MOD without adaptation.

Molecular modeling

A single structure for MOD and a single structure for DUL were considered for molecular modeling, whereas three different structures (i.e. three different tautomers) were considered for FRU and six different structures (i.e. six different conformers) for SUC. Twelve fragments were dissected from MOD, 24 from DUL, 80 from FRU and 220 from SUC.

We looked for the presence of the 12 binding motifs that were defined previously (Froloff *et al.*, 1996) on the four molecules by comparing all 336 fragments to 12 representatives. Table 2 summarizes the results of this search with a binary code. Four distinct binding motifs were found on DUL, which all differ from those found on MOD (five other motifs). Of note, the motifs found on MOD are quite similar to those previously found on saccharin (SAC) (Froloff *et al.*, 1996). This is because MOD and SAC share the SO_2-$N^{(-)}$-CO moiety and mostly differ by virtue of the shape and size of their respective hydrophobic surfaces (a methyl and a benzene ring respectively). As a consequence, MOD fragments have smaller hydrophobic patches (15 Å^2 on average as compared to 21 Å^2 on average respectively).

As could be expected from their multiple H-bonding possibilities (Faurion and Mac Leod, 1982), both SUC and FRU were found to display 10 out of the 12 binding motifs. Having nine binding motifs in common, SUC and FRU are very similar: FRU displays all motifs except 9 and 12, whereas SUC displays all motifs except 8 and 12 (Table 2). Both these carbohydrates appear to be able to display any binding motif made of zero, one or two H-bonding atoms

Table 2 Occurrence of the 12 fragment types on the four molecules

	Fragment no.											
	1	**2**	3	**4**	**5**	6	**7**	8	9	10	**11**	**12**
DUL	**1**	**1**	1	**0**	**0**	1	**0**	0	0	0	**0**	**0**
MOD	**0**	**0**	0	**1**	**1**	0	**0**	1	0	1	**1**	**0**
FRU	**1**	**1**	1	**1**	**1**	1	**1**	1	0	1	**1**	**0**
SUC	**1**	**1**	1	**1**	**1**	1	**1**	0	1	1	**1**	**0**

'1' indicates at least one fragment of this type was depicted on the molecular surface; '0' indicates no such fragment was found on the molecular surface. The 12 fragment types are defined and shown in Froloff *et al.* (1996). Bold characters denote the fragment selection matching best the taste similarities between 14 molecules studied in Froloff *et al.* (1996).

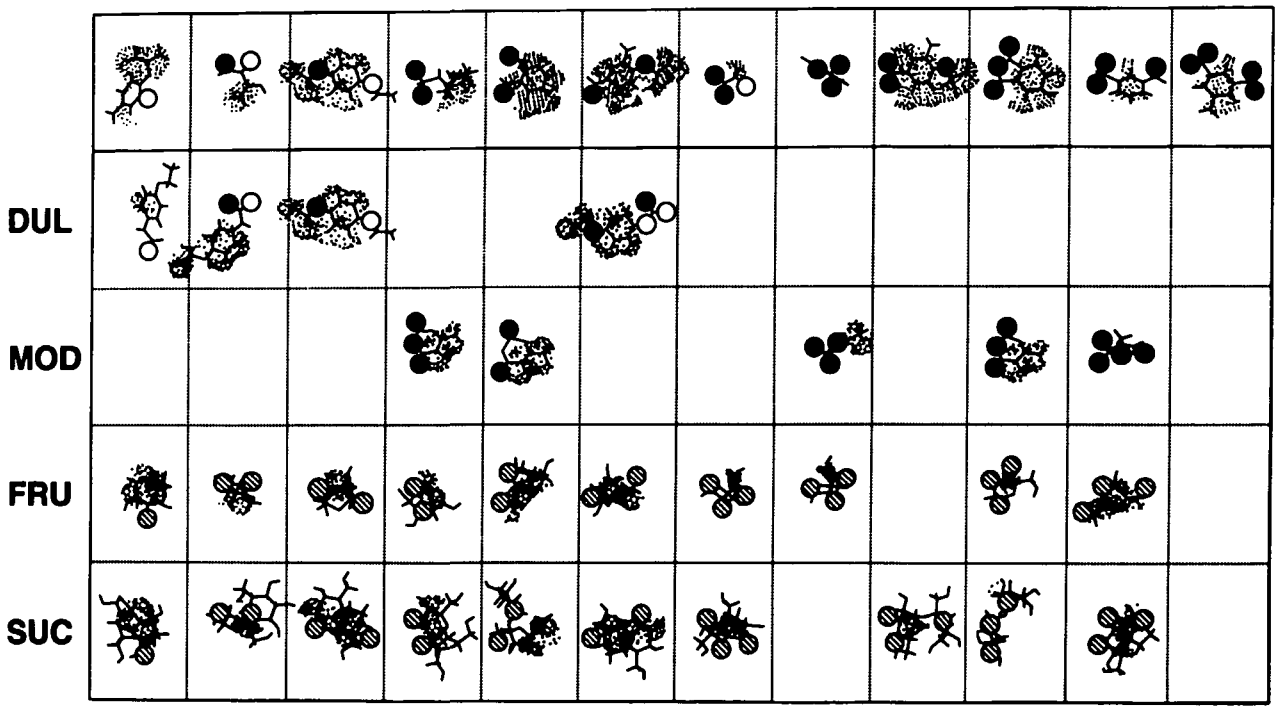

Figure 2 Examples of the fragments found on the four molecules, arranged as in Table 2. Top row: exploratory fragments 1–12 taken from Froloff *et al.* (1996). Other rows: corresponding fragments identified on DUL, MOD, FRU and SUC respectively. A cell is blank whenever the relevant fragment type was not found on the molecule. Fragments are made of H-acceptors (black circles), H-donors (white circles) or acceptor–donor atoms (hatched circles), and hydrophobic patches (gray areas). The underlying molecular skeletons (sticks) are also shown.

(motifs 1–6) and a great variety of motifs with more than two H-bonding atoms (motifs 7–12). Interestingly, the flexibility of both FRU and SUC did not significantly enhance their binding possibilities. The 10 binding motifs of FRU were found on both α-D-fructofuranose and β-D-fructofuranose, and only motif 8 was not found on β-D-fructopyranose. All individual conformers of SUC displayed the 10 binding motifs indicated in Table 2, except for one conformer which happened to lack motif 9. Hence, both SUC and FRU are able to display many distinct binding motifs mostly because of their intrinsic polymorphism (namely their respective multiple H-bonding possibilities). Their high flexibility may, however, further enhance their adhesive properties by facilitating protein–ligand induced fit (Jorgensen, 1991).

Figure 2 shows examples of the fragments that were found on the four molecules. Their respective H-bonding patterns (or subpatterns) are compatible within 1 Å with those of the exploratory fragments (top row in Figure 2, and Figure 6 in Froloff *et al.*, 1996). Exploratory fragments belong to 1-propoxy-2-amino-4-nitrobenzene (fragments 1, 6 and 9), L-threonine (fragment 2), dulcin (fragment 3), cyclamate (fragment 4), 2-nitrobenzoic acid (fragment 5), glycine (fragment 7), 3-nitrobenzenesulfonic acid (fragments 8 and 11), saccharin (fragment 10) and picric acid (fragment 12). Only one fragment of each type is represented in Figure 2

for each of the four molecules, but a fragment may have more than one analog on the same molecule. Moreover, some fragments in Figure 2 are repeated because they are compatible with more than one exploratory fragment in the top row.

Discussion

Structure/adaptation relationships

Adaptation is a general phenomenon in sensory systems. For the chemical senses, it can be observed as two related phenomena (O'Mahony, 1986): (i) the intensity elicited by a long-lasting constant stimulus rapidly reaches a peak and then gradually vanishes; (ii) after an initial prolonged stimulation, the sensitivity to that stimulus is also decreased (the measured threshold value is higher). Both these phenomena occur for any taste stimulus (Hahn, 1934; Abrahams *et al.*, 1937; Krakauer and Dallenbach, 1937) including organics which are believed to be detected by receptor proteins located at the apical surface of taste receptor cells (Abe *et al.*, 1993a,b; Matsuoka *et al.*, 1993; Chaudhari *et al.*, 1996; Kusakabe *et al.*, 1996). Hence, cross-adaptation between two organics has usually been interpreted by the fact that both molecules bind to common taste receptors, whereas failure to cross-adapt indicates independent receptor mechanisms (McBurney, 1972; McBurney *et al.*, 1972; Schiffman *et al.*, 1981; Caprio, 1982; Lawless and Stevens, 1983; Faurion, 1987; Tonosaki and Funakoshi, 1989; Wegert and Caprio, 1991; Michel *et al.*, 1993).

We used here the levels of cross-adaptation between four compounds in order to further validate the 12 common binding motifs that we previously determined on 14 other tastants (Froloff *et al.*, 1996). In that previous study, we tested whether some of these motifs could relate to taste binding sites using a taste similarity index between molecules. This index was equal to the correlation coefficients between paired profiles of isointense concentrations characterizing each individual tastant, across 58 subjects (Faurion, 1993). In the present study, we used another measure of taste similarity based on cross-adaptation experiments. Such experiments allow us to probe more directly the common taste receptor mechanisms that are triggered by two distinct tastants, as opposed to intensity rating experiments where molecules are tested individually on the taste system. Furthermore, the asymmetry of cross-adaptation between two tastants yields additional information that is not provided by a correlation coefficient calculated on columns of data representing subjects' sensitivities. Nevertheless, our present results show that both these measures of taste similarity are quite consistent. Faurion found a high correlation between the psychophysical profiles for SUC and MOD ($r = 0.8$, $n = 47$ subjects; unpublished data), and both these molecules also showed the highest level of cross-adaptation (24%, $P < 0.005$; Table 1). SUC and FRU were found to have a lower yet still significant correlation ($r = 0.7$, $n = 55$ subjects; Faurion, 1993) and they also show cross-adaptation, yet at a lower level than SUC and MOD (~10%, $P < 0.23$). By contrast, SUC and DUL as well as MOD and DUL were not significantly correlated ($r < 0.6$; A. Faurion, unpublished data) and also failed to cross-adapt in any order of presentation.

In our present results, striking relationships can be seen between the cross-adaptation levels and the amount of binding motifs shared by the molecules. For example, FRU and SUC both display 10 binding motifs among the 12 that were searched for on their respective molecular surfaces, and thus share a majority of motifs (nine motifs, see Table 2). Even if we to consider only the subset of seven motifs that were validated in our previous study (bold figures in Table 2), FRU and SUC would still share six motifs out of seven. Correlatively, SUC and FRU showed a 10% level of mutual cross-adaptation, which is half the level of self-adaptation for SUC (24%). MOD displays five distinct binding motifs out of 12 (Table 2), four of which are also displayed by SUC. Moreover, the three taste motifs of MOD (bold figures in Table 2) are also displayed by SUC. The fact that most taste receptors that bind MOD are also likely to bind SUC but not vice versa may explain why SUC cross-adapted MOD but not vice versa (Figure 1). Finally, four distinct motifs were found on the surface of DUL but none of them was found on MOD (Table 2). Correlatively, DUL and MOD failed to cross-adapt but showed mutual cross-enhancement of stimulatory effects. Similarly, Schiffman *et al.* (1981) studied cross-adaptation between seven artificial sweeteners and seven carbohydrates and reported several significant cases of cross-enhancement. Overall, this wealth of structure/adaptation relationships shows that the previously determined binding motifs might also prove useful to predict cross-adaptation between organic tastants. Furthermore, if the motifs are useful to account for cross-adaptation, it brings a confirmation that at least a part of the cross-adaptation data can be related to peripheral receptor events.

Quite interestingly, Lawless and Stevens (1983) found that SUC cross-adapts saccharin (SAC) but not vice versa, a result that parallels the non-reciprocal cross-adaptation that we found between SUC and MOD. Moreover, Schiffman *et al.* (1981) found that MOD and SAC show quite similar cross-adaptation behaviors with 12 other molecules. Finally, Faurion found a high correlation between MOD and SAC in psychophysics ($r = 0.8$, $n = 46$ subjects; unpublished data). All these experimental results relate well with the fact that quite similar binding motifs were disclosed on both MOD and SAC with our modeling approach (see Results).

The only lack of obvious structure/adaptation relationship is seen for the DUL–SUC pair. Indeed, the four binding motifs of DUL are also found on SUC (Table 2). We might have thus expected, as for the MOD–SUC pair, that SUC would cross-adapt DUL but not vice versa. However, not

only did DUL fail to cross-adapt SUC, but SUC also failed to cross-adapt DUL. Instead, a cross-enhancing effect of SUC was found on the perceived intensity for DUL. This effect was significant ($P < 0.05$) for only three subjects out of seven, whereas cross-enhancement of DUL over SUC was significant for five subjects out of seven (results not shown).

The lack of structure/adaptation relationship between SUC and DUL may further indicate that our set of motifs is relevant yet incomplete, as has been discussed in detail (Froloff *et al.*, 1996), and that other common motifs still have to be found on the molecular surfaces of organics in order to fully account for their taste.

Mechanistic hypotheses

The fact that molecules tend to cross-adapt when they are recognized by common taste receptors leaves quite open the question as to which molecular mechanisms are responsible for cross-adaptation and/or cross-enhancement. These mechanisms are likely to take place at the taste system periphery (Diamant *et al.*, 1965; Zotterman, 1971), though central processes cannot be ruled out (Gillan, 1984; Bujas *et al.*, 1995). Diamant *et al.* (1965) demonstrated that perceived intensities are directly proportional to taste nerve response amplitudes. We can reasonably assume that the taste nerve activity cannot adapt by itself, and that this activity is directly related to the amount of neurotransmitters that are released by exocytosis at the basolateral membranes of taste receptor cells. Hence, cross-adaptation and/or cross-enhancement mechanisms are likely to occur inside taste receptor cells at any step that leads from stimulus binding to neurotransmitter exocytosis.

It is interesting to note that the four stimuli tested show quite different efficient concentrations: SUC and FRU are isointense at ~100 mM (as compared to the reference intensity), while MOD and DUL are isointense in the millimolar range (Table 1). If intensities perceived by the subjects directly relate to their taste receptor cell activity, two mutually non-exclusive hypotheses are likely: (i) either SUC and FRU bind more weakly to the taste receptors than MOD and DUL (the K_Ds of FRU and SUC are on average 100 times larger than the K_Ds of MOD and DUL). In this case, given our molecular modeling results (Table 2), SUC and FRU bind loosely to numerous taste receptor sites, whereas MOD and DUL bind more tightly to smaller populations of receptor sites. Or (ii) the taste transduction amplification mechanisms are much less efficient for both SUC and FRU.

Why are sweeteners much more potent stimuli than carbohydrates? Unfortunately, our molecular modeling approach alone cannot strengthen hypothesis (i). Indeed one cannot reasonably quantitate the affinities of a given molecule for putative receptor sites 1–12, basically because such affinities strongly depend on the as yet unknown atomic details of those putative receptor sites (see discussion in Froloff *et al.*, 1996). Such a limitation is inherent to any indirect molecular modeling approach where the three-dimensional structures of the receptors are unknown. However, flexibility of SUC and FRU may partly explain a weaker overall affinity of these molecules for receptors, since they may lose more entropy due to freezing of single bonds upon binding. The entropic cost for the restriction of a free single bond rotation that has two to three equienergetic states to only one accessible state is 0.42–0.66 kcal/mol at 300K (R ln 2 and R ln 3 respectively: Finkelstein and Janin, 1989; Novotny *et al.*, 1989). Should the binding of SUC with a given receptor result in immobilization of the glycosidic linkage between the α-glucopyranosyl and the β-fructofuranosyl rings, one would expect an entropic cost of ~1 kcal/mol and hence a K_D value ~5 times greater than that of a rigid analog.

Hypothesis (ii) may be substantiated by recent data on taste transduction. According to the present knowledge of the taste transduction process, organic molecules are thought to bind to receptor proteins located in the chemosensory membrane and activate second-messenger cascades via coupling G proteins (Striem *et al.*, 1989; McLaughlin *et al.*, 1992; Ruiz-Avila *et al.*, 1995; for reviews, see Kinnamon and Margolskee, 1996; Lindemann, 1996). Bernhardt *et al.* (1996) recently demonstrated that the non-sugar sweeteners SC-45647 (Tinti and Nofre, 1991) and SAC evoke intracellular calcium responses in rat circumvallate taste receptor cells which are due to the release of calcium ions from intracellular stores (IP_3-dependent pathway). By contrast, they showed that SUC induces an intracellular calcium increase in the same cells that is due to permeation of calcium ions through membrane ionic channels activated by depolarization (cAMP-dependent pathway). These differences in the way in which increases in the intracellular calcium concentration occur could have important consequences on the efficiency of the transduction process. The ultimate step of the transduction process in taste cells is the exocytosis of synaptic vesicles and the activation of primary nerve fibers. The exact process of synaptic activation in these cells is not known but, as in other cells, it must strongly depend on the level of intracellular calcium concentration. It is possible that the release of calcium ions from internal stores by sweeteners is very efficient at triggering synaptic exocytosis. By contrast, the calcium rise following carbohydrate stimulation would be less efficient as stimulus-evoked depolarizations are limited in amplitude and only a few action potentials are elicited (Roper, 1990).

Also, from the experimental results presented here, one can see that the various levels of self-adaptation seem to correlate with the values for the efficient concentrations: SUC is efficient at ~100 mM and at the same time shows a weak level of self-adaptation (24%, Table 1), while MOD and DUL are efficient in the millimolar range and both show high levels of self-adaptation (77 and 76%

respectively). Similarly, Schiffman *et al.* (1994) found that MOD shows much greater adaptation than FRU and SUC in sweetness intensity ratings experiments.

If hypothesis (i) is true (see above), this means that adaptation is more efficient for molecules that bind strongly to the taste receptors. Strong binding would indeed correlate with large life half-times for the stimulus/receptor complexes. Significant competition for the same taste receptor sites could then occur between the adapting molecules that are still bound to the receptors and the test molecules that flow over the tongue. Moreover, if the receptors remain in their active conformation for a longer time, receptor and/or transduction cascade desensitization mechanisms (e.g. phosphorylation, methylation) will have longer to take place and thus will be more efficient.

If hypothesis (ii) is true, this means that adaptation levels depend on the transduction pathways that are activated by tastants. Adaptation may be stronger for tastants that predominantly activate the IP_3-dependent pathway because the calcium pool that is available from internal stores is limited. By contrast, adaptation may be lower for tastants that activate the cAMP pathway because much larger quantities of calcium are available from the extracellular medium.

Finally, our experimental results as well as available literature data (Schiffman *et al.*, 1981) show that self-enhancement does not occur but some cases of cross-enhancement can be observed between tastants. Cross-enhancement may occur if two tastants elicit distinct transduction pathways which nevertheless coexist in the same taste cells (synergy). It may also simply result from subadditive effects if taste receptor cells activated by tastant 1 are distinct from cells activated by tastant 2, and if they still show residual activity when tastant 2 is applied on the tongue. Further experimental data on taste reception and taste transduction are, however, needed to understand in detail the various cross-effects that we observed between the four stimuli of the present study.

Concerning the large interindividual differences in self- and cross-adaptation that were observed in our experiments, these may relate to the various levels of expression of each individual taste receptor on each subject's tongue. Two molecules may show large and significant cross-adaptation on average for all subjects, but may not cross-adapt for a particular subject because this subject expresses lower amounts of the taste receptors that recognize both molecules.

Conclusions

The results of the present study are compatible with the hypothesis that taste cross-adaptation occurs between two molecules if they are recognized by common taste receptor sites. Using a set of common binding motifs that were extracted from 14 other molecules (Froloff *et al.*, 1996), we found clear relationships between the amount of common binding motifs that are shared by two molecules and their levels of mutual cross-adaptation. In addition, we found that non-reciprocal cross-adaptation may occur when one molecule only displays a subset of the motifs that are displayed by the other molecule. Finally, absence of cross-adaptation, or cross-enhancement, seems to occur when both molecules bear distinct motifs. The set of binding motifs is consistent with all cross-adaptation data, except for the enhancement that is seen between SUC and DUL. The latter is, however, non-significant ($P < 0.08$). This confirms that at least some of these motifs are good candidates to be recognized by distinct taste receptor sites. They may thus prove useful in predicting cross—adaptation between other tastants. Besides, the fact that SUC and FRU are likely to be bound by many more distinct taste receptors than MOD and DUL, and yet are weaker stimuli, may either be due to the fact that both SUC and FRU bind more weakly to their cognate receptors, or that the taste transduction processes are less efficient for both these stimuli.

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