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Time–Intensity Evaluation of Acid Taste in Subjects with Saliva High Flow and Low Flow Rates for Acids of Various Chemical Properties

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Abstract

The role of the chemical properties of sour stimuli and the role of the human saliva flow rate on acid perception were investigated in 11 high saliva flow rate (HF) and 11 low saliva flow rate (LF) subjects with a continuous stimulus delivery flow rate of 3.2 ml/min and using the time–intensity technique for perception recording. Continuously measuring the pH on the tongue surface on three HF and three LF subjects showed that HF subjects' saliva decreased the acidity of the acid solution more efficiently than the LF subjects' saliva did. However, HF subjects exhibited higher perceived intensity for acid solutions than LF subjects. At equal pH, the order of the efficiency of acids indicated that HCl was the least efficient acid stimulus and acetic acid the most efficient. At equal concentration, the order of efficiency was the opposite (citric acid > malic acid > lactic acid > acetic acid), indicating that titratable acidity rather than pH has to be considered when comparing weak acids. At high concentrations, the ratio of relative efficiency is more in favor of the hydrophobic than the hydrophilic acid in HF subjects compared with LF subjects, i.e. HF subjects are more sensitive to hydrophobic stimuli. Hydrophobic molecules may diffuse more easily into the epithelium of HF than LF subjects, and reach more efficiently trigeminal nerve endings in addition to taste receptor cells.

Key words: acids, human, lingual pH, saliva, time–intensity

Introduction

At low concentrations, acid stimuli primarily evoke a sour taste, but at strong concentrations a sensation of irritation indicates that they activate the trigeminal lingual system. Acid perception results then from both activating taste cells and the perigemmal trigeminal free nerve endings embedded within the epithelium or intermingled between taste cells in the taste bud (Nagy *et al.*, 1982; Yamasaki *et al.*, 1984).

Sourness is related to the proton concentration, as sourness intensity decreases with increasing pH, but pH is not sufficient to explain the intensity of acid solutions. Weak acids, for example, elicit a greater response than HCl at equal pH, as observed both in human psychophysics experiments (Ganzevles and Kroeze, 1987) and in electrophysiological nerve recordings in animals (Beidler, 1967). Norris *et al.* (1984) demonstrated that sourness depends on titratable acidity. Moreover, CoSeteng *et al.* (1989) established a classification of sourness strength of several weak organic acids of equal titratable acidity and pH "acetic > malic > tartaric = lactic > citric acids" and they observed that the intensity of perception in the mouth decreased as the number of carboxylic groups increased. So it is questionable whether titratable acidity alone is sufficient to explain acid taste intensity. Hence the acid response might rely on both the free, or potentially free protons, and on the non-dissociated molecule (Lyall *et al.*, 1997). Ogiso *et al.* (2000) observed that anions should not contribute to the perception of acids as a relatively high concentration (100 mM) of a weak acid, buffered at pH 7, in conditions where the molecule is totally dissociated, does not elicit any response on the rat chorda tympani nerve.

Protons were shown to enter hamster taste receptor cells (TRCs) via ENaCs, amiloride-sensitive apical epithelial sodium channels (Gilbertson *et al.*, 1992, 1993), to inhibit voltage-sensitive K^+ channels on TRCs (Kinnamon and Roper, 1988), and to activate acid-sensing ion channels (ASICs) located on the apical membrane (Lin *et al.*, 2002) and on the basolateral membrane of taste receptor cells (Ugawa *et al.*, 1998). Finally, H^+ ions may cross the basolateral cell membrane via the paracellular pathway to enter TRCs (Stewart *et al.*, 1998; Lyall *et al.*, 2001). Beidler (1967) and Gardner (1980) suggested that a highly non-dissociated lipophilic acid molecule would easily cross the lipid phase of cell membranes, which was confirmed by studies of Gutknecht and

Tosteson (1973) and Evtodienko *et al.* (1996). Lyall *et al.* (1997, 2001) showed that non-dissociated acids may diffuse into taste receptor cells by passive diffusion across the apical membrane. Furthermore, Bryant and Moore (1995) suggested that non-dissociated acids may reach trigeminal free nerve endings via a transcellular pathway.

The mechanisms of the action of acids on taste perception should not be considered independently from the saliva action. Saliva helps to protect taste receptor cells from mechanical, thermal, bacterial and viral aggression, and transports taste molecules to taste receptors. In human subjects, the basal secretion of saliva constitutes a reference environment to which taste receptor cells are adapted. Thus, stimulus concentrations have to exceed saliva concentration to elicit a response (McBurney and Pfaffmann, 1963; Bartoshuk, 1978).

Acid stimuli are very potent stimulators of salivary secretion compared with organic stimuli such as sucrose (Chauncey *et al.*, 1967; Lagerlöf and Dawes, 1985). Saliva is secreted by the major (parotid, submandibular, and sublingual) and minor (labial, lingual, buccal, and palatal) salivary glands. The proportion of each type of saliva (parotid, submandibular, sublingual etc.) is different in the stimulated whole saliva (Whole saliva is constituted of several saliva coming from different salivary glands.) compared with the unstimulated whole saliva (Young and Schneyer, 1981; for a review, see Humphrey and Williamson, 2001). Saliva acts as a buffering-pH system, which affects the degree to which the sourness can be perceived. Strong variations in human salivary flow rate were shown (Christensen, 1985). They were further related to the pH modification within the mouth (Shannon and Frome, 1973; Christensen, 1986; Christensen *et al.*, 1987). A higher flow rate of saliva suggests a more efficient buffering effect since the buffer volume is more important, as suggested by Christensen *et al.* (1987). Although all these studies, using the sip and spit method, have investigated the relationship between the saliva flow and the perception of acids, none have considered the perception in relation to the pH and its modulation by the quantity of saliva at tongue level.

This study was undertaken in order to investigate the impact of the chemical properties (pK_a, carboxyl group number and hydrophobicity) of the molecule and the saliva flow rate on the temporal development of the sensation evoked by acids. This was accomplished using the time–intensity method, in which the stimulation procedure (continuous application of microquantities) ensured a relatively long-lasting constant stimulation, mimicking to a certain extent the actual effect of acid stimulation during mastication. The pH was also simultaneously measured on the tongue surface for the sake of comparison with the temporal perception.

Materials and methods

Subjects

Subjects were from the local university and the local community. A sample of 63 subjects (51 women and 12 men, 28 ± 10 years old) participated in the saliva flow rate evaluation, of whom 22 were selected for further experiments. They were asked not to eat, drink or smoke for at least 30 min before the session. Subjects were paid for their participation.

Measurements of whole saliva flow rate and selection of subjects participating in the main experiment

Unstimulated and stimulated whole saliva flow rates were measured on 63 subjects using the technique of Dawes *et al.* (2000). For the unstimulated saliva secretion, subjects were asked to rinse their mouth with tap water and to rest for 1 minute. After swallowing their saliva, they were asked to wait 40 s without swallowing and then spit out into a pre-weighed cup. This measure was repeated five times per subject. The flow rate of unstimulated saliva secretion ranged from 0.2 to 1.6 g/min (mean ± SD = 0.6 ± 0.4 g/min; $n = 63$).

For the stimulated saliva flow rate evaluation, subjects were asked to rinse their mouth with tap water and then to rest for 1 min. After swallowing their saliva, they put 5 ml of HCl, pH 2.5, in their mouth. After 40 s, they spat out the entire contents of the mouth (5 ml HCl + stimulated saliva) into a pre-weighed cup. The test was repeated five times after a rinse and a 1 min rest period. In both cases, cups were immediately weighed to prevent evaporation. After two sessions, 10 measurements for each of the unstimulated and stimulated whole saliva flow rates were averaged for each subject. The flow rate of saliva secretion stimulated by HCl, pH 2.5, ranged from 0.2 to 3.0 g/min (mean ± SD = 1.1 ± 0.7 g/min; $n = 63$).

Although the rank of a subject was usually similar in both distributions for stimulated and unstimulated secretions, a few subjects presented contradictory evaluations: i.e. a high flow rate for stimulated saliva and a low flow rate for unstimulated saliva or conversely. Subjects with contradictory saliva flow rates and medium saliva flow rates were discarded from the study.

From the distributions of whole saliva flow rates, 11 subjects (29.0 ± 10.3 years old) with both the lowest unstimulated and stimulated whole saliva flow rates (LF subjects: unstimulated mean saliva flow rate = 0.2 ± 0.1 g/min; stimulated mean saliva flow rate = 0.2 ± 0.2 g/min; $n = 11$) and 11 subjects (27.0 ± 9.5 years old) with both the highest unstimulated and stimulated whole saliva flow rates (HF subjects: unstimulated mean rate = 1.2 ± 0.2 g/min; stimulated mean rate = 2.2 ± 0.4 g/min; $n = 11$) were selected for the experiment (Table 1). Basal saliva secretion and stimulated flow rate were significantly different in the HF group of subjects ($P < 0.001$; paired Student's t-test; df = 10), but remained non significantly different in the LF group. Among these 22 subjects, seven were smokers (15 cigarettes/day, $n = 1$; 8–12 cigarettes/day, $n = 2$; 5–7 cigarettes/day, $n = 2$; <5 cigarettes/day, $n = 2$). None of these subjects received any medication known to modify the saliva flow rates.

Table 1 Individual unstimulated and stimulated saliva flow rates

Subjects	Unstimulated saliva flow rate (g/min)	Stimulated saliva flow rate (g/min)
LF subjects		
L1[a]	0.30 ± 0.14	0.15 ± 0.13
L2	0.37 ± 0.09	0.26 ± 0.18
L3	0.11 ± 0.03	0.60 ± 0.42
L4[a]	0.16 ± 0.11	0.03 ± 0.05
L5	0.23 ± 0.15	0.15 ± 0.22
L6	0.15 ± 0.04	0.05 ± 0.09
L7	0.21 ± 0.08	0.15 ± 0.18
L8	0.24 ± 0.07	0.35 ± 0.28
L9	0.35 ± 0.13	0.08 ± 0.09
L10	0.27 ± 0.08	0.12 ± 0.27
L11[a]	0.17 ± 0.07	0.17 ± 0.15
Mean ± SD	0.23 ± 0.08	0.19 ± 0.16
HF subjects		
H1	1.34 ± 0.33	2.85 ± 0.33
H2	1.05 ± 0.16	2.24 ± 0.45
H3[a]	0.88 ± 0.17	2.56 ± 0.23
H4	1.25 ± 0.20	1.84 ± 0.40
H5	1.31 ± 0.57	1.67 ± 0.25
H6[a]	1.55 ± 0.29	2.28 ± 0.39
H7	1.23 ± 0.45	2.15 ± 0.40
H8	1.27 ± 0.35	1.85 ± 0.24
H9	1.15 ± 0.21	2.29 ± 0.16
H10[a]	0.97 ± 0.30	1.74 ± 0.24
H11	0.84 ± 0.21	2.22 ± 0.31
Mean ± SD	1.17 ± 0.21	2.15 ± 0.36***

Each individual entry is an average of 10 evaluations of spontaneous secretion (unstimulated saliva flow rate) or saliva secretion after 5 ml HCl, pH 2.5, held in the mouth for 30 s.
[a]subjects chosen for in-mouth pH measurements.
***$P < 0.001$ (df = 10) by paired Student's *t*-test between unstimulated and stimulated saliva flow rate.

Training of subjects

The 22 selected subjects were trained in the general procedure using 15 solutions out of 28 for five repetitions (Table 2). Time–intensity profiles were collected for the 22 selected subjects, 15 stimuli, five repetitions first then five repetitions suppressing retronasal olfaction in order to assess information only from taste and trigeminal systems.

The evolution of the training was observed for each subject and statistically evaluated for HF and LF groups on 11 of the 15 stimuli of the learning phase excluding the lower concentrations. Learning was assessed looking at the evolution of the coefficient of variation (CVar) of HF and LF time–intensity profiles and the evolution of the maximum intensities (I_{max}) of time–intensity profiles of both groups across repetitions.

The learning was checked repetition per repetition in the five first repetitions with retronasal olfaction and in the five first repetitions without retronasal olfaction. Individual profiles were first normalized by dividing the amplitude at each point by the mean intensity of the profile (line normalization) to eliminate differences of intensities due to differences of pH. One hundred and twenty-one normalized profiles were thus averaged for each repetition (11 subjects × 11 stimuli) and the standard deviation was calculated at each point (sampling frequency: one point per second). The coefficient of variation (C_{Var}, SD/mean) was then calculated at each point and the evolution of the profile of C_{Var} was observed repetition per repetition. The C_{Var} decreased progressively during the period of stimulation from ~0.50 to 0.20 between repetitions 1 and 4, then remained stable during the following repetitions, confirming both groups of subjects had fulfilled the learning of time–intensity evaluation within four repetitions. Moreover, further suppressing retronasal olfaction did not modify the status of learning. This was confirmed via ANOVA which showed that the I_{max} values of replications during the main experiment were not significantly different [$F(4,2254) = 1.5$, $P = 0.2$].

For each stimulus, I_{max} values were determined from the time–intensity profiles of each subject. For normalization, the I_{max} values of all 10 repetitions were divided by the average I_{max} on the 10 repetitions, for each stimulus and subject separately to avoid the intensity effect due to differences of pH. The normalized I_{max} values were then averaged repetition per repetition, for LF and HF subjects separately, collapsing all 11 subjects and 11 stimuli ($n = 121$). For LF and HF subjects, the I_{max} obtained by averaging the I_{max} values of all profiles reached a stable value within four repetitions. The suppression of retronasal olfaction did not modify this I_{max}.

Stimuli

Stimulus materials

Twenty-eight stimuli included a solution of commercial almond (a flavor compound with no taste properties), quinine hydrochloride (qui, 0.4 mM; mol. wt 396.91; Acros Organics, France) and five different acids at various pH levels obtained by dilution (Table 2): acetic acid (ace; mol. wt 60.05; Sigma, France); citric acid (cit; mol. wt 210.1; Sigma, France), DL-lactic acid (lac; mol. wt 90.08; Sigma, France), L-malic acid (mal; mol. wt 134.1; Sigma, France); and HCl (mol. wt 36.46; VWR, France).

Stimuli were all prepared in only one batch of ultraviolet sterilized tap water (Aqua-Stoutz, Actini, France). They

Table 2 Acid stimuli (pH measured after defrosting)

Prepared pH	Acid (p*Ka*)				
	HCl	Acetic acid (4.74)	Citric acid (3.13; 4.76; 6.40)	Lactic acid (3.86)	Malic acid (3.46; 5.10)
1.50	**1.49 ± 0.07; *n* = 10**				
1.70	**1.68 ± 0.11; *n* = 11**				
2.10	**1.98 ± 0.08; *n* = 9**		**2.05 ± 0.10; *n* = 13**	**2.08 ± 0.14; *n* = 11**	**2.14 ± 0.15; *n* = 13**
2.40	**2.35 ± 0.12; *n* = 13**		2.37 ± 0.07; *n* = 8	2.51 ± 0.12; *n* = 10	2.44 ± 0.12; *n* = 8
2.70		2.60 ± 0.17; *n* = 8	2.67 ± 0.09; *n* = 10	2.77 ± 0.15; *n* = 8	2.79 ± 0.13; *n* = 10
3.10		**3.10 ± 0.13; *n* = 13**	**3.01 ± 0.12; *n* = 11**	**3.13 ± 0.13; *n* = 13**	**3.07 ± 0.17; *n* = 11**
3.46		3.40 ± 0.19; *n* = 8	3.46 ± 0.11; *n* = 10	3.52 ± 0.17; *n* = 8	3.72 ± 0.09; *n* = 10
3.86		**3.93 ± 0.12; *n* = 11**	**3.82 ± 0.19; *n* = 11**	**3.93 ± 0.16; *n* = 13**	**3.92 ± 0.13; *n* = 11**

Prepared pH (column 1) and pH measured with an Isfet electrode just prior to the experiment in the defrost vials (mean ± SD; *n*). Bold figures: reduced set for training sessions.

were distributed in 160 ml disposable containers for daily experiments, kept frozen at −22°C and warmed up at room temperature prior to each experimental session. The absence of bacterial development was checked with contact slides (VWR, France) in the remaining solutions of each experimental session.

Solution pHs, obtained by dilution in tap water, were prepared using a glass electrode (#90431; Fisher Bioblock Scientific, France) connected to a WTW pH 330 pH-meter (Fisher Bioblock Scientific, France). Just prior to the experiment, pH was measured again in the defrosted vial with an Isfet electrode (IQ 150, IQ Instruments, USA). This electrode, specific for micro-volumes of solutions, was further used to measure the pH on the surface of the subject's tongue during the experimental runs, further referred to as 'lingual pH'. pH electrodes were calibrated with standard pH 1.68 and 4.01 buffer solutions. The pH of solutions referred to throughout the study is the pH measured in the vials after defrosting (Table 2).

Method of stimulation

The method of stimulation was derived from the technique developed in the laboratory for cerebral imaging of taste in a whole body magnet (Van de Moortele *et al.*, 1997; Cerf, 1998). Two peristaltic pumps (Minipuls 3, Gilson, France) driven by a PC computer (WinTask Version 2.5 software, TaskWare, France) delivered water and stimulus alternately (3.18 ± 0.13 ml/min, $n = 176$). Subjects received water or stimulus via two versilic tubes (1 × 3 mm diameter; VWR, France) joined together in a short versilic tip (5 × 8 mm diameter; VWR, France). Subjects were asked to place their stimulation tip in contact with the upper surface of the tongue as shown in Figure 1 and to keep their mouth closed. The position of the stimulation device remained the same throughout all sessions. A peristaltic (Minipuls 2, Gilson, France) saliva pump (GACD, France) removed the excess of liquid from the mouth to avoid swallowing stimuli and saliva.

Method of excluding retronasal olfaction

An airstream (Rena Air 400 aquarium air pump, Rena, France) was flowed into the subject's nostrils via a plaster molding of the nares. At the beginning of each session, subjects adjusted the airflow rate (~200 l/h) using a dilution of a commercial almond flavor to suppress its olfactory sensation elicited when introduced into the mouth. This solution was originally assessed as strong by every subject, but did not elicit any perception after suppression of retronasal olfaction, showing that it was tasteless and did not stimulate the lingual trigeminal system. Blind controls with this almond solution were conducted at random during all sessions to ensure that each subject correctly suppressed retronasal olfaction. Subjects were allowed to remove their nose from the plaster mold between each experimental run.

Experimental procedure

In-mouth pH measurements

In-mouth pH measurements were obtained from six subjects (three LF and three HF subjects, Table 1). The remaining 16 subjects performed only time–intensity measurements. An Isfet electrode, specific for micro-volumes of solutions, was introduced into the versilic stimulation tube. The pH sensor was placed in contact with the tongue surface (Figure 1) where water and stimuli were delivered to evaluate the actual pH of the solution at the level of the tongue surface when mixed with saliva (time response < 2 s). The evolution of the pH of the tongue surface was recorded throughout the session during stimulation and also during the rinsing period, which ensured that a 'neutral' pH was maintained at the beginning of each sample. Lingual pH recordings were sampled every 2 s.

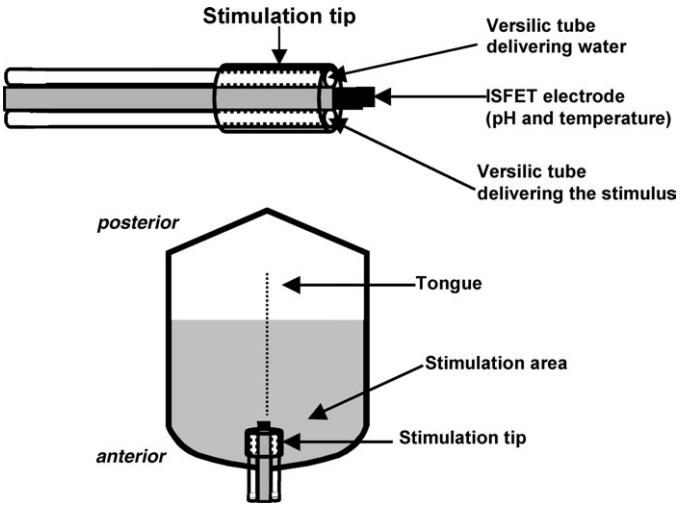

Figure 1 Stimulation device and its localization on the subject's tongue (see text for further details).

Time–intensity measurements

Each subject, in an individual box, produced time–intensity profiles for 18 randomized different solutions during each session. A complete test (72 s) consisted of a 24 s period with tap water (OFF period, reference), a 24 s stimulus period (ON period, stimulus) followed by another 24 s period with tap water (OFF period). This paradigm ensured a steady stimulation for 24 s, which was well adapted to time–intensity evaluation. A rinse period of at least 2 min 25 s was observed between tests, during which subjects had to rinse their mouth with tap water.

Subjects indicated the overall perceived intensity elicited by the stimulus by using the finger-span technique (Lindvall, 1970; Berglund *et al.*, 1978). The perceived intensity was evaluated as the distance between the subject's thumb, which was fixed on the 10 cm linear potentiometer end and his/her forefinger tip attached to the moving cursor. The voltage (maximum: 6 V) was digitalized (DAS-Wizard software, ComputerBoards, Inc., USA) at a frequency of 1 Hz and stored in an Excel spreadsheet for further treatment.

Sessions

Each subject completed at least 16 sessions (including training and main experiment) with two or three sessions per week. The main experiment included 28 stimuli and retronasal olfaction was suppressed. More than one session was necessary for each subject to test all solutions (n = 28), hence five repetitions were obtained in about 10 sessions for each stimulus.

Data analysis

Time–intensity profiles were gathered for 22 subjects, 28 stimuli and at least five repetitions without retronasal olfaction.

Data from the main experiment (time–intensity and lingual pH profiles) were treated for each stimulus separately, subject by subject and on both groups without normalization. Both maximum intensity I_{max} values and minimum lingual pH were collected per subject and further averaged for the two groups.

Results were reported using four criteria: time–intensity profile I_{max}; time to maximum intensity (t_{max}) and rising slopes of time–intensity profiles (calculated between 24 and 28 s), as indicators of the build-up of the perception and of the difficulty of access of stimuli to receptors; and the rinsing slopes of time–intensity profiles (calculated between 54 and 64 s), an indicator of the rinsing capacity of saliva. Time–intensity profiles were in relation to the corresponding pH profiles, and I_{max} values were in relation to the minimum lingual pH.

Data were analyzed with a four-way analysis of variance (ANOVA; SPSS 12.0 software, USA): groups of subjects, acids, pH and replications as fixed factors and I_{max}, t_{max}, rising slopes and rinsing slopes as dependent factors, with a significance level of $P < 0.05$.

Results

Controls

Averaged time–intensity profiles for the taste stimulus quinine used as a non-acid control (pH 7.7) were compared in the LF and HF groups. Although the slope of the profile tended to be steeper for HF subjects, the recorded I_{max} was the same for both groups (Figure 2) showing that both groups used the same intensity scale.

Suppression of retronasal olfaction was verified at each session with the almond odor blind control introduced at random among other stimuli. LF and HF subjects' time–intensity profiles for almond odor, recorded throughout the main experiment (Figure 2), showed that the retronasal olfaction was thoroughly suppressed.

Tongue surface pH evaluation

Individual profiles of lingual pH were averaged for each stimulus and for both groups of three subjects analyzed separately. Minimum lingual pHs of averaged pH profiles for three LF and three HF subjects were determined during the period of stimulation (Table 3, columns 3 and 4). Relative differences (delta %) between lingual pH and stimulus pH for LF and HF groups (Table 3, columns 7–9) indicated that pHs of acid solutions reached a higher value for HF subjects than for LF subjects (averaged HF relative difference: 25.3 ± 8.2%, n = 26, versus LF: 11.3 ± 4.9%, n = 26; averages of columns 7 and 8). The difference of in-mouth pH between LF and HF subjects could reach 1.7 pH unit depending on stimulus (Table 3, columns 3 and 4). A reduced difference between HF and LF subjects lingual pHs was observed for the less acidic solutions, e.g. citric acid, pH 3.8, acetic and lactic acids, pH 3.9.

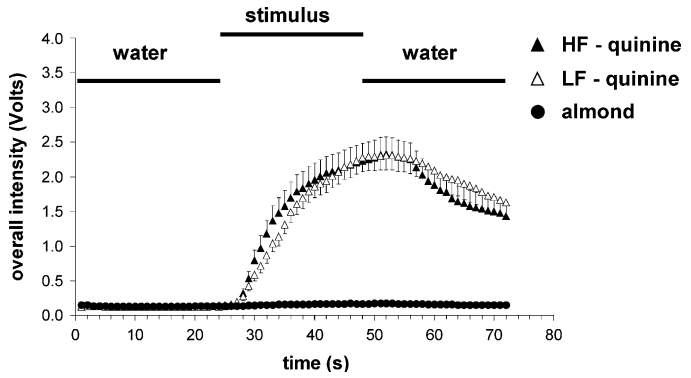

Figure 2 Mean time–intensity profiles of HF and LF subjects for the almond control and quinine–HCl during the main experiment. Profile of the almond solution: n = 401, a little less than 22 subjects × 5 repetitions × 2 (2 is the number of sessions necessary to test all 28 stimuli); profiles of quinine: HF, n = 55, 11 subjects × 5 repetitions; LF, n = 51, a little less than 11 subjects × 5 repetitions. All mean values are given with +1 or −1 SEM. Notice the absence of perception of almond odor, due to the block of retronasal olfaction, and the lack of differences between HF and LF subjects when tested with quinine.

Responses to acids in HF and LF subjects

The effect of pH on the time–intensity response is represented in Figure 3 for malic acid as an example in which lower pH values gave higher ratings of perceived intensity. Time–intensity profiles depended on the acid, the pH and the group of subjects. The corresponding overall I_{max} values for each acid are given in Table 3 (columns 5 and 6).

I_{max} decreased according to pH for both groups of subjects (Figure 4a). Overall perceptions were very low for organic acids at pH 3.5 and above (Figure 4a), so psychophysical responses of the subjects were quantitatively measurable between pH 2.6 and 3.4 for acetic acid, pH 2.1 and 3.1 for lactic acid, pH 2.1 and 3.0 for citric and malic acids, and pH 1.5 and 2.0 for HCl. At equal pH, HCl appeared to be the least efficient acid stimulus and acetic acid the most efficient. At equal concentrations (e.g. 100 mM), the reverse order of efficiency could be established for the organic acids: citric acid > malic acid > lactic acid > acetic acid (Figure 4b).

For each acid (all pHs together), I_{max} values of HF subjects were significantly higher than those of LF subjects [ANOVA: HCl, $F(1,376) = 4.9$, $P = 0.03$; acetic acid, $F(1,350) = 9.0$, $P = 0.003$; citric acid, $F(1,540) = 11.6$, $P = 0.001$; lactic acid, $F(1,541) = 7.8$, $P = 0.006$; malic acid $F(1,529) = 8.0$, $P = 0.005$]. In the case of malic acid (Figure 3), the perceived intensity was higher in HF than in LF subjects for pH 3.0, 2.7 and 2.4.

Maximum intensity as a function of lingual pH

Subjects selected for in-mouth pH measurements (three HF subjects and three LF subjects). In the three LF subjects, pHs of organic acids were increased to pH 2.4 compared with pH 3 and above in the three HF subjects. Delta pH was 0.3 ± 0.1 (n = 26) for the three LF subjects and 0.9 ± 0.3 (n = 26) for the three HF subjects (Table 3, differences between columns 3 and 2, 4 and 2).

Results for malic acid are represented as an example in Figure 5 showing the averaged time–intensity profiles for the three LF and the three HF subjects in relation to their respective averaged lingual pH profiles. Although the averaged lingual pHs of the three HF subjects were higher (less acid) than the averaged lingual pHs of the three LF subjects, time–intensity profiles indicated a higher perceived intensity for the HF subjects than for the LF subjects.

Similarly, for nearly all stimuli the three HF subjects had higher (less acid) lingual pHs than the three LF subjects (Table 3, columns 3, 4 and 9), and conversely, the I_{max} values of the three HF subjects were significantly higher than those of the three LF subjects [for all acids together ANOVA: $F(1,489) = 41.9$; $P < 0.001$].

Eleven HF subjects and eleven LF subjects groups. In the same way, all averaged time–intensity profiles of both the LF and HF groups of subjects for each acid stimulus together with the averaged lingual pH profiles of the three LF and three HF subjects exhibit differences between both groups (Figures 6 and 7). The difference of responses between HF and LF groups was reduced at pHs 3.5–3.8. In the case of citric acid, the difference of lingual pH between HF and LF groups is noticeably lower than others. Table 3 columns 9 and 10 show a variation of the delta % of I_{max} between HF and LF, which is not accounted by the corresponding delta % of lingual pH.

I_{max} values were significantly different for the acids [ANOVA: $F(4,2308) = 176.7$, $P < 0.001$] and for the pHs [ANOVA: $F(7,2308) = 250.2$, $P < 0.001$]. Consequently, the I_{max} elicited by the different acids was significantly different across pHs, as shown by an acid × pH interaction [ANOVA: $F(14,2308) = 4.4$, $P < 0.001$]. I_{max} values were significantly higher for the HF group than for the LF group [ANOVA: $F(1,2308) = 35.3$, $P < 0.001$]. Both groups of subjects rated differently the magnitude of the perceived intensity elicited by the different acids [ANOVA: $F(4,2308) = 2.6$, $P = 0.04$]. Furthermore, the magnitude of the perceived intensity elicited by the different pHs was different across the groups of subjects, as suggested by a significant group × pH interaction [ANOVA: $F(7,2308) = 4.2$, $P < 0.001$].

Time to maximum intensity

Time to maximum intensity (t_{max}, t at stimulus onset = 0) was significantly different between acids [ANOVA: $F(4,2308) = 11.1$, $P < 0.001$] and between pHs [ANOVA: $F(7,2308) = 22.3$, $P < 0.001$]. Consequently, t_{max} elicited by the different acids was significantly different across pHs, as shown by a significant acid × pH interaction [$F(14,2308) = 2.6$, $P < 0.001$].

HF subjects reached their t_{max} (averaged t_{max} = 20.2 s ± 9.4, n = 1258; number of subjects × number of stimuli ×

Table 3 Maximum intensities and lingual pHs in response to various sour stimuli for LF and HF subjects (number of sessions ≈ 36)

Stimulus (pK_a)	Stimulus concentration (M) (1)	Stimulus pH (2)	Minimum lingual pH		I_{max} (V)		Delta % lingual pH versus stimulus pH		(9) Delta % HF lingual pH versus LF lingual pH	(10) delta HF I_{max} versus LF I_{max}
			(3) LF subjects (n = 3)	(4) HF subjects (n = 3)	(5) LF subjects (n =11)	(6) HF subjects (n = 11)	(7) LF subjects	(8) HF subjects		
HCl	0.038	1.5 ± 0.1; n = 10	1.8 ± 0.3; n = 17	2.4 ± 0.5; n = 14	2.1 ± 1.4; n = 49	3.0 ± 1.9; n = 49	17	38	25	30
	0.033	1.7 ± 0.1; n = 11	2.0 ± 0.2; n = 14	2.5 ± 0.7; n = 12	1.8 ± 1.2; n = 47	2.0 ± 1.1; n = 54	15	32	20	10
	0.014	2.0 ± 0.1; n = 9	2.5 ± 0.3; n = 14	3.7 ± 1.1; n = 14	1.3 ± 1.3; n = 48	1.4 ± 1.2; n = 52	20	46	32	7
	0.008	2.4 ± 0.1; n = 13	2.8 ± 0.3; n = 9	4.1 ± 2.0; n = 10	0.9 ± 0.7; n = 52	0.8 ± 0.6; n = 53	14	41	32	−13
Acetic acid (4.74)	0.478	2.6 ± 0.2; n = 8	3.2 ± 0.5; n = 17	3.7 ± 0.4; n = 17	3.5 ± 1.4; n = 41	4.0 ± 1.9; n = 49	19	30	14	13
	0.173	3.1 ± 0.1; n = 13	3.3 ± 0.2; n = 11	3.8 ± 0.8; n = 9	2.0 ± 1.3; n = 54	2.4 ± 1.5; n = 54	6	18	13	17
	0.069	3.4 ± 0.2; n = 8	3.9 ± 0.4; n = 13	4.3 ± 0.4; n = 13	1.6 ± 1.1; n = 37	2.3 ± 1.4; n = 42	13	21	9	30
	0.029	3.9 ± 0.1; n = 11	4.2 ± 0.3; n = 14	4.6 ± 0.7; n = 10	1.1 ± 0.7; n = 50	1.3 ± 0.8; n = 51	7	15	9	15
Citric acid (3.13; 4.76; 6.40)	0.139	2.1 ± 0.1; n = 13	2.4 ± 0.2; n = 19	2.8 ± 0.4; n = 14	3.6 ± 1.6; n = 54	3.8 ± 1.7; n = 53	13	25	14	5
	0.036	2.4 ± 0.1; n = 8	2.7 ± 0.3; n = 8	3.2 ± 0.9; n = 8	2.4 ± 1.3; n = 40	3.2 ± 1.9; n = 49	11	25	16	25
	0.017	2.7 ± 0.1; n = 10	3.0 ± 0.2; n = 13	3.7 ± 0.7; n = 14	1.3 ± 0.6; n = 44	1.9 ± 1.2; n = 47	10	27	17	32
	0.008	3.0 ± 0.1; n = 11	3.4 ± 0.3; n = 10	4.0 ± 0.8; n = 10	1.0 ± 0.9; n = 53	1.1 ± 0.7; n = 55	12	25	15	9
	0.005	3.5 ± 0.1; n = 10	3.9 ± 0.3; n = 13	4.6 ± 0.8; n = 11	0.7 ± 0.6; n = 39	0.9 ± 0.7; n = 42	10	24	15	22
	0.003	3.8 ± 0.2; n = 11	4.2 ± 0.3; n = 10	4.5 ± 0.6; n = 10	0.7 ± 0.8; n = 49	0.7 ± 0.9; n = 55	10	16	7	0
Lactic acid (3.86)	0.431	2.1 ± 0.1; n = 11	2.6 ± 0.5; n = 18	3.1 ± 0.6; n = 18	3.5 ± 1.5; n = 53	3.7 ± 1.7; n = 55	19	32	16	5
	0.093	2.5 ± 0.1; n = 10	2.7 ± 0.3; n = 7	3.1 ± 0.3; n = 6	2.0 ± 0.9; n = 44	2.7 ± 1.8; n = 44	7	19	13	26
	0.039	2.8 ± 0.2; n = 8	3.2 ± 0.3; n = 12	3.8 ± 0.5; n = 14	1.3 ± 0.9; n = 37	2.0 ± 1.4; n = 47	13	26	16	35
	0.023	3.1 ± 0.1; n = 13	3.3 ± 0.3; n = 10	3.9 ± 0.7; n = 10	1.1 ± 0.9; n = 53	0.8 ± 0.5; n = 55	6	21	15	−38
	0.012	3.5 ± 0.2; n = 8	3.9 ± 0.3; n = 13	4.6 ± 0.8; n = 14	0.6 ± 0.6; n = 38	0.5 ± 0.4; n = 46	10	24	15	−20
	0.009	3.9 ± 0.2; n = 13	3.9 ± 0.3; n = 8	4.3 ± 0.6; n = 6	0.6 ± 0.6; n = 54	0.5 ± 0.6; n = 55	0	9	9	−20
Malic acid (3.46; 5.10)	0.240	2.1 ± 0.2; n = 13	2.5 ± 0.2; n = 16	3.0 ± 0.7; n = 17	3.7 ± 1.7; n = 52	4.1 ± 1.6; n = 53	16	30	17	10
	0.049	2.4 ± 0.1; n = 8	2.8 ± 0.4; n = 5	3.4 ± 0.5; n = 8	2.5 ± 1.4; n = 35	3.2 ± 2.0; n = 47	14	29	18	22
	0.022	2.8 ± 0.1; n = 10	3.2 ± 0.2; n = 12	3.8 ± 0.6; n = 11	1.5 ± 0.7; n = 41	1.9 ± 1.0; n = 42	13	26	18	21
	0.012	3.1 ± 0.2; n = 11	3.2 ± 0.3; n = 8	4.0 ± 0.6; n = 13	1.0 ± 0.7; n = 53	1.3 ± 0.7; n = 55	3	21	18	23
	0.005	3.7 ± 0.1; n = 10	4.0 ± 0.3; n = 14	4.6 ± 0.7; n = 12	0.7 ± 0.6; n = 41	0.7 ± 0.5; n = 42	8	20	13	0
	0.004	3.9 ± 0.1; n = 11	4.3 ± 0.4; n = 10	4.8 ± 0.2; n = 7	0.7 ± 0.6; n = 53	0.5 ± 0.5; n = 55	9	19	13	−40
n	26	n_1 = 643	n_2 = 315	n_3 = 302	n_4 = 1211	n_5 = 1301	26	26	26	26

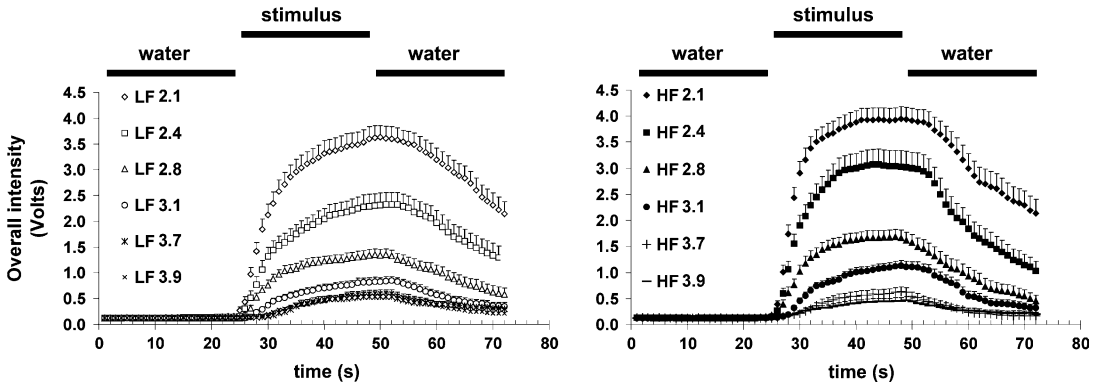

Figure 3 Time–intensity profiles for malic acid at various pHs for LF and HF subjects. Time–intensity profiles (without retronasal olfaction) for each group of subjects (n = 11) were averaged. All mean values are given with +1 SEM. 'LF 2.1': LF subjects, malic acid, pH 2.1; 'HF 2.1': HF subjects, malic acid, pH 2.1

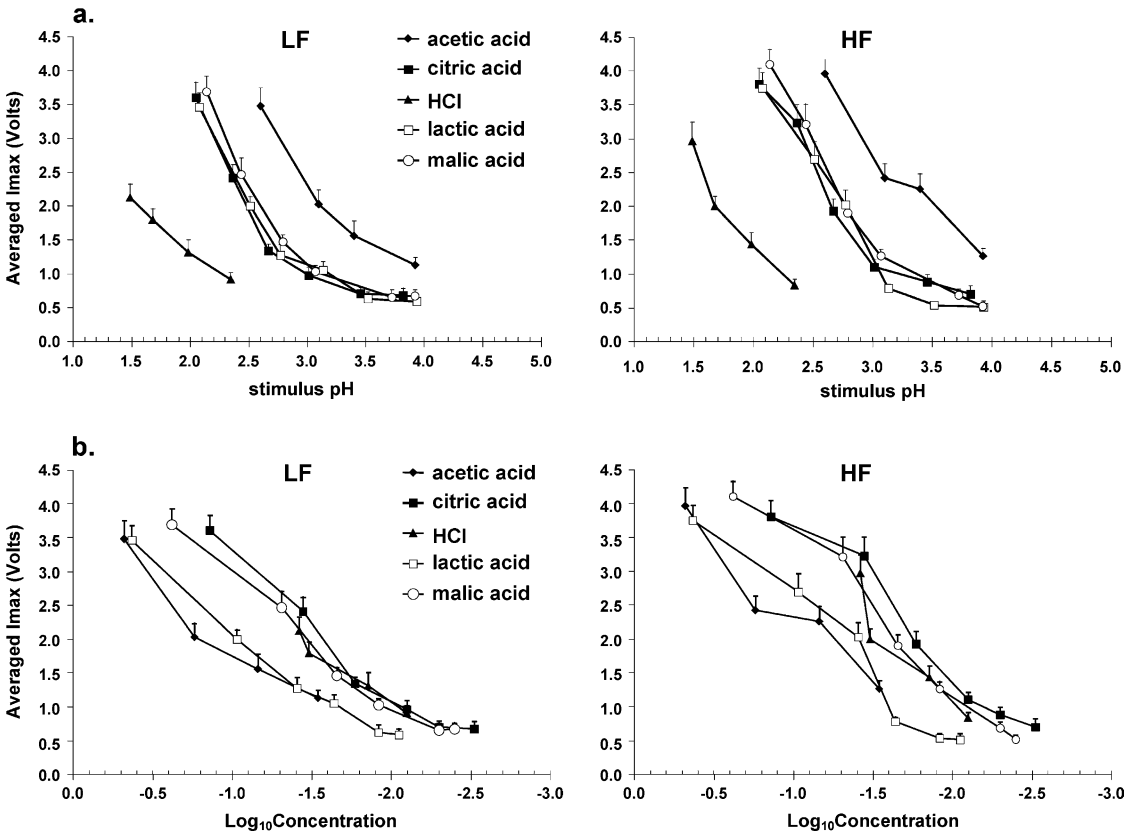

Figure 4 Evolution of averaged I_{max} values of LF and HF subjects related to stimulus pH and Log_{10} concentration. For LF and HF subjects separately, I_{max} values were individually determined and grouped by stimulus (11 subjects × 5 repetitions each). I_{max} and lingual pH are represented with SEM. Averaged I_{max} values for each group of subjects were related to stimulus pH(a) and Log_{10} concentration in molarity(b).

number of repetitions) significantly sooner than LF subjects (averaged t_{max} = 23.4 s ± 9.9, n = 1194; ANOVA: $F(1,2308)$ = 42.5, $P < 0.001$). For acetic acid, pH 2.6, for example, HF subjects reached their maximum intensity 6.3 s sooner than LF subjects (HF mean t_{max} = 18.4 ± 9.6, n = 49; LF mean t_{max} = 24.7 ± 11.6, n = 41; P = 0.006; Student's t-test).

Build up of perception (rising slopes)

Rising slopes of time–intensity profiles indicated that the build up of the perception depended on the LF/HF group of subjects [$F(1,2308) = 26.3$, $P < 0.001$] and varied with acids [ANOVA: $F(4,2308) = 115.3$, $P < 0.001$] and pH [ANOVA: $F(7,2308) = 136.3$, $P < 0.001$]. Significant group × acid

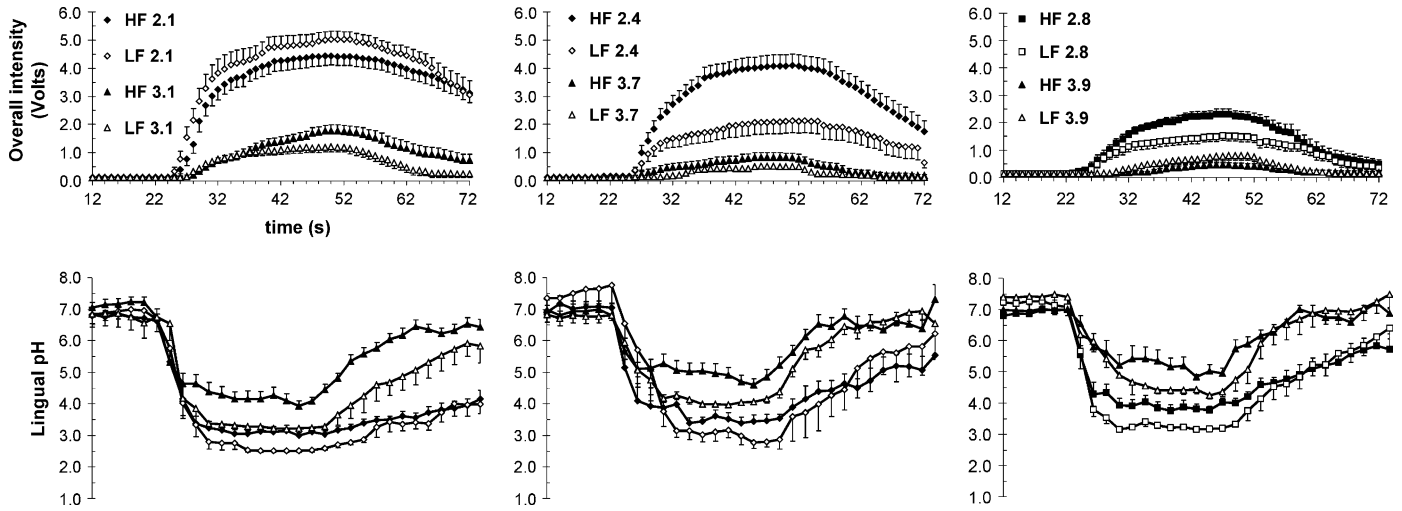

Figure 5 Averaged time–intensity profiles for three LF subjects and three HF subjects for malic acid and their respective averaged lingual pH. Data acquisition: 1 Hz for time–intensity profiles and 0.5 Hz for lingual pH profiles. Filled symbols represent HF subjects; open symbols represent LF subjects. All mean values are given with +1 or −1 SEM. Stimulus onset at 24 s and stimulus rinse at 48 s. 'LF 2.1': LF subjects, malic acid, pH 2.1; 'HF 2.1': HF subjects, malic acid, pH 2.1.

[$F(4,2308) = 5.6$, $P < 0.001$] and group × pH interactions [$F(7,2308) = 4.3$, $P < 0.001$] were found indicating that rising slopes of different acids and pHs were different across groups. Rising slopes of different pHs were different across acids [$F(14,2308) = 10.2$, $P < 0.001$]. A post-hoc LSD test on rising slopes indicated that acids could be separated in three groups: (i) acetic acid; (ii) citric, lactic and malic acids; and (iii) HCl (LSD, $P < 0.05$). HF subject rising slopes were steeper than those of LF subjects: the HF mean of rising slopes fell outside the confidence interval of the LF means.

Decay of perception with rinsing

Rinsing slopes of time–intensity profiles were significantly different between acids [ANOVA: $F(4,2308) = 25.0$, $P < 0.001$] and between pHs [ANOVA: $F(7,2308) = 31.0$, $P < 0.001$]. HF subjects exhibited a better rinse than LF subjects [ANOVA: $F(1,2308) = 37.0$, $P < 0.001$]. HF perception returned to baseline level sooner: mean of HF rinsing slopes fell outside of the confidence interval (95%) of mean of LF subjects.

Rinsing of different acids were different across groups, as shown by a significant group × acid interaction [ANOVA: $F(4,2308) = 2.6$, $P = 0.04$]. Rinsing of various pHs were different across groups, as indicated by a significant group × pH interaction [ANOVA: $F(7,2308) = 5.5$, $P < 0.001$]. No significant interaction was observed between acids and pHs, suggesting that the rinse was equally effective at eliminating the perceived sensation from all acids. Rinsing slopes of time–intensity profiles of nearly all pHs were significantly different (LSD, collapsing stimulus and subjects, $P < 0.05$). However, rinsing slopes of pH 1.7, 2.4, 2.7 and 3.1 were not different, nor were pH 3.5 and 3.9. A better rinse of HF subjects compared with LF subjects was specifically observed for HCl, pH 1.5, acetic acid, pH 2.6, lactic acid, pH 2.1, malic acid, pH 2.1, 2.4 and 3.7 (Figures 3, 6 and 7).

Discussion

Methodological considerations

Peristaltic stimulation, which continuously delivered small quantities to the surface of the tongue, elicited a continuous perception with no adaptation during the 24 s of stimulation. The maximum perceived intensity was lower than with the sip and spit technique, with a smaller stimulated surface of the tongue. Nevertheless, to give an idea of the intensities perceived by the subjects, pH below 2.6 for acetic acid and below 2.1 for citric, lactic and malic acids were unbearable for LF subjects with this technique as well as with the sip and spit. However, the stimulation of the subjects' tongue was controlled more efficiently than in a sip and spit protocol. The great improvement of our method resulted in a relatively constant amplitude for some time, allowing time–intensity measurement during the stimulation. This combination of the two methods, i.e. the continuous delivering of micro-quantities of the stimulus and the finger-span evaluation of perceived intensity over time, produced reliable quantitative measurements in LF and HF subjects as assessed by the quantitative aspect of profiles for the series of pH (Figures 3, 6 and 7).

Effect of saliva

The salivary flow rate was evaluated in this study by collecting the whole saliva, because the proportion and the composition of each saliva constituting whole saliva is known to be modified upon stimulation (Dawes, 1969, 1974;

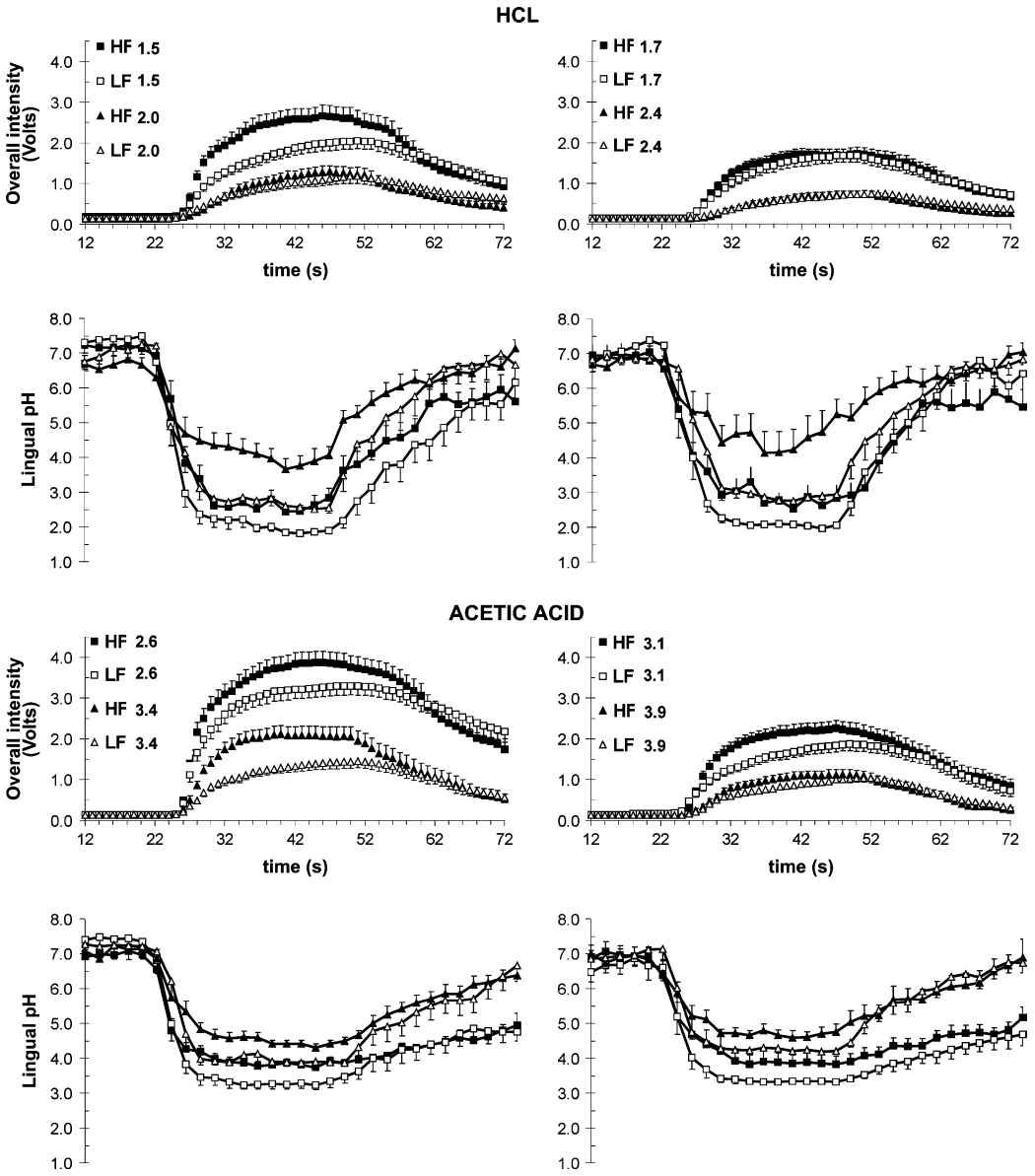

Figure 6 Time–intensity profiles of HF and LF subjects for HCl and acetic acid stimuli associated to the lingual pH variation. Shown are averaged time–intensity profiles for each group of subjects (n = 11) and averaged lingual pH profiles (three LF subjects and three HF subjects). Data acquisition: 1 Hz for time–intensity profiles and 0.5 Hz for lingual pH profiles. Filled symbols represent HF subjects; open symbols represent LF subjects. All mean values are given with +1 or −1 SEM. Stimulus onset at 24 s and stimulus rinse at 48 s. 'LF 1.5': LF subjects, stimulus, pH 1.5; 'HF 1.5': HF subjects, stimulus, pH 1.5.

Benedek-Spät, 1973; Froehlich *et al.*, 1987; Guinard *et al.*, 1998; Dawes and Kubienec, 2004). Large differences in saliva flow rates among subjects were observed for unstimulated state as well as during stimulation. For this study, HF and LF subjects were selected at the extremes of the distribution and constituted two homogeneous groups. Saliva flow rates were similar to those recorded by Christensen (1985) and Christensen *et al.* (1987), which were obtained by chewing an inert gum.

Similarly to Christensen *et al.* (1987), who used HCl at 6 mM, this study did not show any difference of perceived intensity between HF and LF subjects for HCl in the range of low concentrations, but showed a significant difference at pH 1.5 (38 mM). As a whole, lingual pHs of LF subjects during stimulation were lower than those of HF subjects, as already shown by Norris *et al.* (1984) and Christensen *et al.* (1987). Weak acid solutions (at pH 2.1–2.4), for example, induced lingual pHs of 3.0 in HF subjects and 2.4 in LF subjects. This dampening effect could be due to the dilution (Christensen *et al.*, 1987) from a higher volume of saliva (2.2 ± 0.4 vs 0.2 ± 0.2 g/min) or, eventually, a difference in the composition of the saliva. It is known that saliva composition is modified

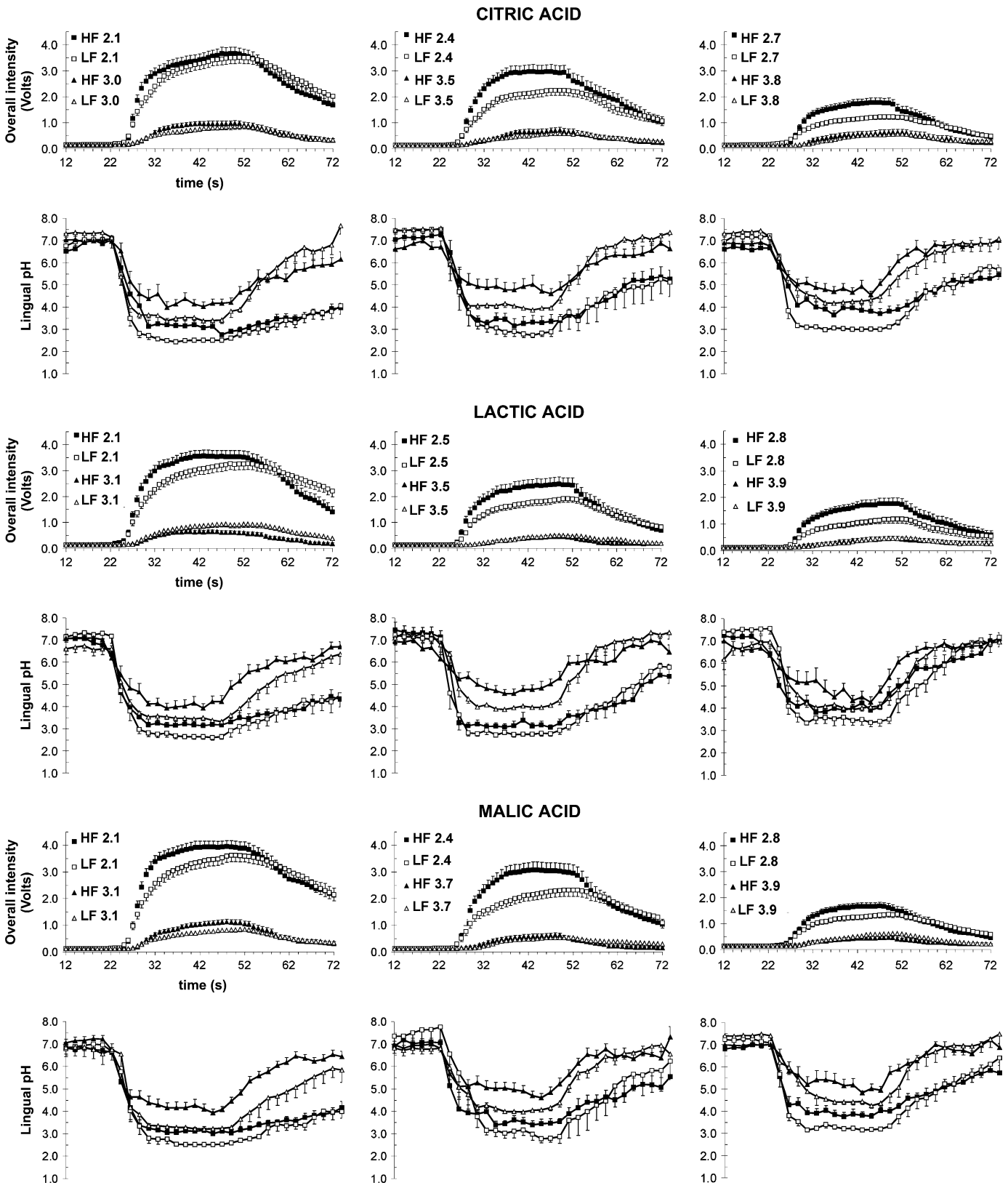

Figure 7 Time–intensity profiles of HF and LF subjects for citric, lactic, and malic acids stimuli associated to the lingual pH variation. Same legends as for Figure 6.

with oral stimulation (Wikner and Soder, 1994). Despite the less acid lingual pH for HF, the time–intensity profiles paradoxically indicated a higher perceived intensity for them, compared with LF subjects, particularly at middle range pHs. Acid stimulation was most efficient in HF than in LF subjects.

A consistent difference in the time course of the developing perceived intensity was also observed between HF and LF subjects, except for the lowest concentrations. After a slower rising phase, LF subjects reached the maximum intensity on average 3 s later than HF subjects for acids as in Bonnans and Noble (1995). For quinine, the perception was also delayed by ~3 s, as in Fischer *et al.* (1994). In addition, for most stimuli and especially for the lowest pH of the most hydrophobic stimuli, the perception lasted longer in LF than in HF subjects who rinsed better and sooner than LF subjects did (Figures 2 and 6). The flow rate of the saliva added some dynamics to the stimulus flow rate. Two mechanisms may be considered: the role of saliva as a vehicle for taste molecules (Matsuo *et al.*, 1997) observed equally for quinine and acids and the ability of the subject's tongue to respond to the stimulus which is different for quinine and acids. During the temporal development of the perception with this system of continuous delivery of small quantities of liquid, it is clear that the saliva flow rate has an effect on the perceived intensity during the rising and rinsing phases for quinine. For acids, the same effect is observed, but the maximum intensity is also higher in HF compared to LF subjects. Hence, for acids, the difference of maximum perceived intensity depends not on the saliva flow rate but on an intrinsic higher sensory response in HF subjects. In conclusion, first, HF subjects exhibit higher responses specifically to acids, and secondly, LF subjects are not able to produce more saliva under acid stimulation compared with basal secretion. The literature clearly shows that the stimulus-induced saliva secretion depends on the trigeminal nerve (Hellekant and Kasahara, 1973; Guinard *et al.*, 1998; Dawes *et al.*, 2000) and the chorda tympani nerve (Hellekant and Kasahara, 1973; Matsuo and Yamamoto, 1989). Hence, a low sensitivity inducing low responses might also fail to produce a high amount of saliva.

Effect of pH

The analysis of time–intensity curves confirms that the perceived intensity of acids decreases according to an increase of the stimulus pH (Harvey, 1920; Norris *et al.*, 1984; Ganzevles and Kroeze, 1987). At equivalent pH, the more hydrophobic acetic acid appeared to be the most efficient acid stimulus and HCl the least efficient (Figure 4a), as previously shown by Beidler (1967). The perceived intensity of citric, lactic, malic acids was less than that of acetic acid, which confirms the studies of Noble *et al.* (1986), CoSeteng *et al.* (1989) and Hartwig and McDaniel (1995). Acetic acid is the only acid giving a response for a stimulus pH of ~4 (lingual pH 4.5 in HF subjects), though at the same pH, the concentration of acetic acid is much greater than the concentration of the three other acids. Hence, the titratable acidity is more representative of the sourness perception than the pH value of the solution.

Effect of concentration

At equivalent total concentrations of acid (equivalent titratable acidity), two groups are clearly defined: citric and malic acids (tri- and dicarboxylic acids), on the one hand, and acetic and lactic acids (monocarboxylic acids) on the other hand. At 100 mM, for example, the order of efficiency of acids was: citric > malic > lactic > acetic acids (Figure 4b). This ranking is correlated with the number of carboxyl groups (from 3 to 1), the lowest pK_a (from 3.13 to 4.74) and the number of carbons (from 6 to 2) in the molecule (Table 4). Hence, the more numerous the carboxyl groups, the lower the pK_a and the higher the carbon number, the more efficient the acid. Citric acid, with potentially 300 mEq of H^+, is more efficient than acetic acid, with potentially 100 mEq of H^+. In this study, solutions were made by dilution with tap water to reach the required pH, so that no other ionic species than the acid molecule and its isolated ions would interfere in the receptor environment. CoSeteng *et al.* (1989), who found a different rank, used a sodium citrate solution (1 M) as a buffer in order to be at equal titratable acidity and pH. It is possible that the ionic environment may have influenced the responsiveness of taste cells.

The role of non-dissociated molecule

At 100 mM, the molecules are not yet dissociated (pH < pK_a−1), which suggests a predominant role of the non-dissociated molecule either as a stimulus entity or as a transporter of protons when diffusing through the tissues as already documented by Ogiso *et al.* (2000) and Lyall *et al.* (2001). ANOVA discriminated the various acids on the basis of time–intensity rising slopes, but no difference in the rinsing slopes between the various acids and pHs was detected by

Table 4 Physicochemical features of different weak acids.

	Acetic acid	Lactic acid	Malic acid	Citric acid
pK_a	4.74	3.86	pK_{a1} = 3.46	pK_{a1} = 3.13
			pK_{a2} = 5.10	pK_{a2} = 4.76
				pK_{a3} = 6.40
Number of carbons	2	3	4	6
Number of carboxyl groups	1	1	2	3
Lipid solubility index ($\log_{10}P$)[a]	−0.17	−0.62	−1.26	−1.72

[a]From Gardner (1980).

the ANOVA, suggesting that only H^+ acts as a stimulus, which is rinsed, and that,neither the molecule nor the anion play any part in the process of rinsing the stimulus off. In these conditions, neither the anion, as already suggested by Ogiso *et al.* (2000), nor the molecule contribute as a stimulus. Hence, the role of the non-dissociated molecule may be understood as a source of H^+ available in the vicinity of the H^+ responsive site.

Effect of hydrophobicity of the molecule

As acids are supposed to stimulate both the gustatory and trigeminal systems at low pH, the order of efficiency of these acids will depend on their relative contributions to both systems. Non-dissociated weak acids with various hydrophobicities may have a different access to taste cells compared with deep trigeminal free nerve endings. At medium range concentration (0.05 M), the tricarboxylic citric acid gives a higher intensity (Figure 4b) than the monocarboxylic acetic acid in HF and in LF subjects (r = citric acid intensity/acetic acid intensity = 1.9 in HF and LF subjects). At higher concentrations (0.13 M), the ratio of relative intensities is more in favor of acetic acid compared with citric acid in HF (r = 1.6) than in LF subjects (r = 1.9). The same is observed for other combinations of hydrophilic/hydrophobic acids (citric/lactic: 1.3 vs 1.5; malic/acetic: 1.6 vs 1.7; malic/lactic: 1.3 vs 1.4). The results of the present study show a relatively more efficient stimulatory property of hydrophobic molecules in HF compared with LF subjects and suggest a more important contribution of trigeminal sensitivity, at high concentrations, in HF than in LF subjects. Gardner (1980) suggested that neutral molecules could penetrate lingual epithelium according to their lipid solubility and acidify taste receptor cells internal pH. But non-dissociated acid molecules could also reach and stimulate the trigeminal free nerve endings, which are located deep in the lingual epithelium (Figure 8). The difference in sensitivity between HF and LF subjects might be due to a higher permeability of epithelial tissue to hydrophobic molecules. This is in accordance with Nasrawi and Pangborn (1990), who observed that capsaicin, a trigeminal stimulus, increased salivary flow in HF subjects but not in LF subjects. Electrophysiological recordings in animals will help discriminating between the stimulus properties that contribute to a more efficient stimulation on the taste cells or on trigeminal free nerve endings. The psychophysical evaluation of acid taste must be understood as a global result of stimulating both sensory systems.

To summarize, it is clear that the saliva of HF subjects can modify the pH of an acid solution more efficiently than the saliva of LF subjects does, thanks either to a dilution effect or to a difference in buffering capacity of HF and LF saliva. However, HF subjects present a steeper slope of rising perception and a quicker rinsing phase, indicating more efficient ON and OFF effects. Furthermore, the HF subjects may present a greater sensitivity to acids compared with LF subjects, and the acid solution HCl, pH 2.5, is unable to stimulate saliva production in LF subjects, unlike in HF subjects. The non-dissociated acid molecule may enter the epithelium and liberate protons in the vicinity of both receptor cell and trigeminal receptors. The relative contribution of both sensory systems may be different in HF and LF subjects. Electrophysiological recordings in animals, together with a psychophysical quality description of the perception in humans, are in progress to address this question.

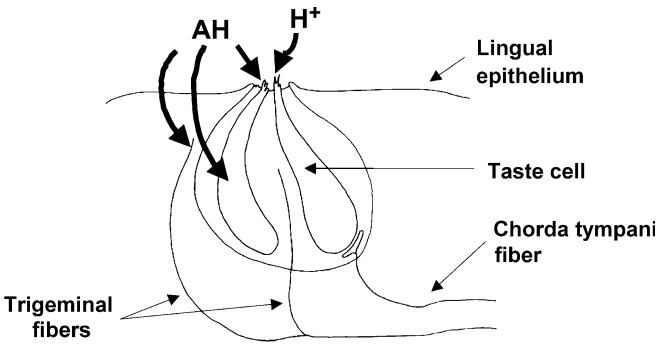

Figure 8 Different pathways for acid stimulation. In addition to H^+ stimulation on the apical membrane, the non-dissociated molecule uses the transcellular pathway towards basolateral membrane of taste cells and trigeminal free nerve endings. Figure redrawn from Yamasaki *et al.* (1984).

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