Possible Involvement of Undissociated Acid Molecules in the Acid Response of the Chorda Tympani Nerve of the Rat

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Ogiso, Kazuma, Yasutake Shimizu, Ken Watanabe, and Keichi Tonosaki. Possible involvement of undissociated acid molecules in the acid response of the chorda tympani nerve of the rat. J. Neurophysiol. 83: 2776–2779, 2000. To test whether undissociated acid is capable of exciting the chorda tympani nerves in rats, we have used buffered acid solutions as taste stimuli. These solutions were prepared by adding alkali to weak acids, such as acetic acid, so that the proportion of undissociated and dissociated acids was varied whereas keeping the total acid concentration constant. When acetic acid solutions, adjusted to wide ranges of pH by NaOH, were applied to the tongue, the response magnitude of the chorda tympani nerves was not varied systematically with pH changes. However, if the sodium effect was eliminated by amiloride or replacement of cation by potassium or Tris(C(CH$_2$OH)$_3$ (Tris-base), the chorda tympani response was reduced systematically as pH increased. Similar results were obtained with citric acid and ascorbic acid. This pH-dependent change in taste nerve response to acid cannot be solely attributed to the proton gradient because the response magnitude induced by hydrogen itself, which was estimated from responses to strong acids, was much smaller than that by equi-pH acetic acid (~85%). Thus we cannot explain the pH-dependent responses of the chorda tympani nerves to weak acids unless effects of undissociated acid molecules are postulated. It is therefore concluded that undissociated acids in weak acid solutions can be a stimulant to taste receptor cells.

INTRODUCTION

It is generally accepted that the perception of chemical stimuli as sour by taste receptor cells depends on the concentration of protons in the acid solution (Lindemann 1996; Settle et al. 1986; Stewart et al. 1997; Tonosaki and Funakoshi 1984). Despite the wide simplicity of the acid stimuli, there is a wide variety of mechanisms by which acids can cause the depolarization of the taste cells. Mechanistic proposals for acid transduction include blockage of apical potassium channels by proton in *Necturus* (Kinnaman and Roper 1988; Kinnamon et al. 1988), gating of calcium channels by protons in the frog (Miyamoto et al. 1988), depolarizing acid currents through apical amiloride-sensitive sodium channels in the hamster (Gilbertson et al. 1992), and depolarizing chloride efflux through basolateral NPPB-sensitive chloride channels in the mouse (Miyamoto et al. 1998). More recently it has been suggested that a proton-gated amiloride-sensitive cation channel, isolated from rat circumvallate papilla, may act as a sour-taste receptor (Ugawa et al. 1998).

All these mechanisms for acid-sensing focus on free protons in the acid solution as essential stimulatory element. However, the acid sourness is not necessarily related to proton concentration. For instance, HCl is perceived as less sour than acetic acid when they were applied to tongue at equal proton concentrations (pH) (Ganzevles and Kroeze 1987). Furthermore, it has been demonstrated that rat chorda tympani nerve responses to various acids are not a simple function of the pH of the stimulatory acid solution (Beidler 1967, 1971). Although chorda tympani nerve responsiveness cannot be attributed as equivalent to sourness intensity in rats, these results do illustrate that free hydrogen is not the exclusive taste stimulant in acid solutions. In fact, Ganzevles and Kroeze (1987) have suggested in human psychophysical study that undissociated acid may participate in some process of acid sensing.

In this study we have used buffered acid solutions to test whether undissociated acid is capable of exciting the chorda tympani nerve. Buffered acid solutions were prepared by adding NaOH, KOH, or Tris[hydroxymethyl]aminomethane; NH$_2$C(CH$_2$OH)$_3$ (Tris-base) to weak acids including acetic acid, citric acid, and ascorbic acid in a manner that kept the concentrations (pH) (Ganzevles and Kroeze 1987). Furthermore, it has been demonstrated that rat chorda tympani nerve responses to various acids are not a simple function of the pH of the stimulatory acid solution (Beidler 1967, 1971). Although chorda tympani nerve responsiveness cannot be attributed as equivalent to sourness intensity in rats, these results do illustrate that free hydrogen is not the exclusive taste stimulant in acid solutions. In fact, Ganzevles and Kroeze (1987) have suggested in human psychophysical study that undissociated acid may participate in some process of acid sensing.

METHODS

Subjects

Male Wistar rats weighing 250–300 g were housed in plastic cages at 22 ± 1°C with a 12:12 h light:dark cycle (light on 0700–1900 h). They were given free access to laboratory chow (LABO MR Stock, Nihon-Nosan, Yokohama, Japan) and water.

Neural recording procedure

Chorda tympani nerve responses were recorded as previously described (Shimizu and Tonosaki 1999). Briefly, each rat was deeply anesthetized by an intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight) and the trachea was cannulated. The chorda tympani nerve was exposed through a mandibular approach and placed on a pair of silver wire electrodes. The electrical activity of the whole chorda tympani nerve was fed to an AC amplifier and displayed on an oscilloscope screen. Neural responses resulting from chemical stimulation of the tongue were integrated (time constant: 1.0 s) and recorded on a chart recorder. The tongue was gently extended with a hook and test solutions were applied for 20 s at a constant rate of 0.5 ml/s by a gravity flow system. Bottles containing distilled water and taste solutions were first incubated in the water bath at 30°C and then moved to the gravity flow system immediately before each application. When the epithelial sodium transport blocker amiloride was
used, it was applied 20 s before every taste stimulation. The solvent for taste solutions and the rinse were mixed with 500 μM amiloride.

The steady-state phase of the integrated chorda tympani neural response, which was measured at 10 s after the onset of stimuli, was normalized to the response to 0.1 M NH₄Cl, which was taken as unity (1.0). The stability of the nerve responses was monitored by periodic application of 0.1 M NH₄Cl.

Solutions

Acids, including acetic acid, citric acid, and ascorbic acid, were prepared to 0.2 M solution by diluting in distilled water before pH adjustment. They were titrated to the desired pH with NaOH, KOH, or Tris-base. After pH adjustment, final concentration of acids were adjusted to be 0.1 M by adding distilled water. Changes in pH during the dilution for final concentration adjustment were negligible. For the pH adjustment of strong acids such as HCl, H₂SO₄, and HNO₃, the concentrated acid solution was diluted by water without using alkali. Molar concentration of HCl, H₂SO₄, and HNO₃ at pH 3.0 was 1.04, 0.65, and 1.03 mM, respectively. All of the test solutions were made fresh each week and refrigerated when not in use. Amiloride solution (500 μM) was prepared immediately before use.

Statistical analysis

All values are means ± SE. Statistical significance was examined by two-way analysis of variance (ANOVA), with post hoc testing by means of Duncan’s multiple range test. Comparisons between groups were made by Student’s t test.

RESULTS

Representative recordings of the integrated chorda tympani neural response to 0.1 M acetic acid solutions adjusted to wide ranges of pH using NaOH and mean responses, normalized to 0.1 M NH₄Cl, are shown in Fig. 1. Response magnitudes varied as U-shaped function with a minimum at pH 4.0. The complexity of pH-response relationship may be a result of the ionic composition of the test solutions because the pH gradient and sodium gradient inversely coexisted. In attempt to negate the influences of the sodium gradient, solutions were applied in the presence of 500 μM amiloride. With amiloride, the nerve responses to acetic acid solutions varied as a decreasing monotonic function of increasing pH (Fig. 1). Even when the contaminating cation was replaced from sodium to either potassium or Tris-base, the same pH-response rela-
tion was accurately reproduced (Fig. 2). After elimination of the sodium effect by either replacement by potassium or Tris-base or by amiloride application, acetic acid solutions at pH 7.0 elicited no tonic responses (Figs. 1 and 2).

We estimated the response magnitude of the chorda tympani nerves that might be solely induced by hydronium ion by testing strong acids. HCl at pH 3.0 induced much smaller (~85% less) responses compared with equi-pH acetic acid (Fig. 3). Similar results were obtained when H2SO4 or HNO3 were applied at pH 3.0 (Fig. 3). An equivalent response to acetic acid required almost a 10-fold higher concentration of HCl (data not shown).

The chorda tympani nerve response to citric acid or ascorbic acid varying in pH from 3.0 to 7.0 at a constant molar concentration (0.1 M) were also recorded (Fig. 4). In this experiment, acid solutions were titrated by Tris-base. As pH increased, response magnitudes to these acid solutions were reduced constantly. The slope of the pH-response relationship on these acids was similar to that in Fig. 2.

### Discussion

It has been suggested that the undissociated form of weak acids may be involved in acid detection in human psychophysical studies (Ganzevles and Kroeze 1987; Gardner 1980). In support of this prediction, we present here neurophysiological results in rats demonstrating that undissociated acid molecules can stimulate the chorda tympani nerves. Although it is unclear how undissociated acids induce taste nerve responses at present, our results should help toward a comprehensive understanding of the mechanism for acid sensing.

The most obvious common factor of gustatory stimuli that are perceived as sour is free proton (Lindemann 1996; Stewart et al. 1997). However, factors other than the concentration of the free protons may be important in the gustatory sensing of acid. For instance, acetic acid induces greater chorda tympani nerve responses than does HCl at an equal free proton concentration (Beidler 1967, 1971). One of the candidates for the difference between acetic acid and HCl would be the species of anion in these acid solutions. Acetic acid adjusted to pH 7.0 by NaOH enhanced the nerve responses markedly, but the solution produced only a negligible response when the sodium effect was eliminated by amiloride or replacement by potassium or Tris-base (Figs. 1 and 2). Considering that most of the acetic acid molecules dissociate at pH 7.0, these results indicate that the anion (i.e., CH3COO−) would not be a major factor, at least up to 0.1 M. In contrast, Cl− has stimulatory effect by itself in the chorda tympani nerve (Formaker and Hill 1988). Taken together, the greater taste nerve response to acetic acid than to HCl cannot be attributed to the difference in anions.

The pKa value for acetic acid is 4.74. Thus the undissociated and dissociated molecules equally exist when pH of the solution is 4.74 and the undissociated form increases exponentially as pH decreases whereas it decreases as pH increases. In this study, buffered solutions varying in proportion of undissociated and dissociated acids at a constant molar concentration were used as taste stimuli to elucidate the possible stimulatory effects of the undissociated form of acetic acid. One of the problems with this approach is the addition of the cation that is inevitably mixed from an alkaline solution during pH adjustment. If the added cation is capable of stimulating the chorda tympani nerve, it is difficult to distinguish which stimulatory factor is affecting neural activity. In fact, pH dependency of the nerve responses was complicated as a result of the presence of sodium added from NaOH during pH adjustment (Fig. 1) because sodium is a strong taste stimulus in rats (Formaker and Hill 1988; Lindemann 1996; Stewart et al. 1997). We removed the effect of sodium by amiloride. The efficiency of amiloride treatment is evidenced by the fact that acetic acid solution adjusted to pH 7.0 produced little tonic response when it was applied with amiloride. Similarly, acetic acids titrated with KOH or Tris-base at pH 7.0 produced almost no neural response, showing that the processes for removal of cation effects was efficacious at least in the present condition. It is therefore considered that pH-dependent responses seen after removal of cation effects are related to proton concentration and/or undissociated acid, both of which increase as pH falls.

We cannot differentiate between the effects of proton and undissociated acid because these vary simultaneously depending on the amount of alkaline solution added. To estimate the effect of undissociated acid, response magnitude to the proton itself was obtained by using strong acids such as HCl, H2SO4, and HNO3. All these strong acids induced the neural responses but the response magnitudes were much less than those induced by equi-pH acetic acids. For example, HCl-induced nerve response was only 15% of acetic acid-induced one at pH 3.0 (Fig. 3). It should be noticed that Cl− has its own effect for stimulating the nerves whereas CH3COO− does not (Formaker and Hill 1988; Shimizu and Tonosaki 1999). Furthermore, SO42− and NO3− also possess stimulatory effects similar to Cl− (unpublished observation). These results indicate that the response magnitude caused by the proton would be overestimated if responses induced by the strong acids were regarded as a pure proton response. Accordingly, the proton gradient cannot solely explain the pH-dependent changes in taste nerve responses to acetic acids. In agreement with this, acetic acid at pH 4.5 substantially enhanced the nerve activity whereas HCl had no effect at this pH (Fig. 3). Alternatively, it would be reasonable to assume that the undissociated form of acetic acid acts as a suitable stimulus for the chorda tympani nerves. Our results may provide experimental evidence for the involvement of undissociated acids in sourness perception which has been previously suggested from human psychophysical studies (Ganzevles and Kroeze 1987; Gardner 1980).

### Figure 4

**pH-response relationships for ascorbic and citric acid solutions adjusted to wide ranges of pH with Tris-base.** Ascorbic and citric acid solutions adjusted to wide ranges of pH using Tris-base were applied and the chorda tympani nerve responses were recorded. Final molar concentration of these acids was 0.1 M. Steady-state responses were normalized to response to 0.1 M NH4Cl, which was taken as unity (1.0), and values presented as means ± SE (n = 5).
The mechanism by which the undissociated acetic acid enhances the chorda tympani nerve activity is not clear at present. This mechanism does not necessarily need to be specific for acetic acid but may be common for all weak acids because analogous effects were observed in the case of citric acid and ascorbic acid (Fig. 4). In our preliminary experiments, the nerve responses to acids were not influenced by pretreatment of the tongue surface with protease whereas that to sucrose were reduced effectively (Hiji 1975). This indicates that, unlike sucrose sensing, membrane receptors are not involved in undissociated acid perception. Alternatively, it has been suggested that cytoplasmic acidification of the taste cells can be related to acid detection (Lyall et al. 1997). In accordance with this, Bigiani and Roper (1994) observed that electrical coupling between taste receptor cells in Necturus were reduced in response to cytoplasmic acidification. This observation is supported by the evidence that a decrease in cytoplasmic pH impairs electrical communication via gap junctions (Spray and Bennett 1985; Spray et al. 1981). Weak acids can penetrate cell membranes in the undissociated form and are expected to dissociate intracellularly leading to cytoplasmic acidification (Gardner 1980; Spray and Bennett 1985). It is therefore most probable that undissociated acid perception is mediated by its penetration into taste cells. This assumption is quite consistent with the report by Gardner who pointed out the correlation between lipid solubility (i.e., membrane permeability) and sourness of the weak acids (Gardner 1980).

In summary, we have shown neurophysiological evidence that undissociated acid molecules may stimulate the chorda tympani nerve responses in rats. As mentioned above, penetration and subsequent acidification of taste cells possibly contributes as a perceptual mechanism of undissociated acids at least in part. However, this does not necessarily rule out other mechanisms for detection of undissociated acids. Further studies are now in progress to clarify the critical mechanism for acid sensing.

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