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TASTELESSNESS: A POORLY DEFINED CONCEPT—
THE EXAMPLE OF QUININE ETHYLCARBONATE¹

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TASTELESSNESS: A POORLY DEFINED CONCEPT— THE EXAMPLE OF QUININE ETHYL CARBONATE¹

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Tastelessness has become an important concept for theories which attempt to relate molecular structure and gustatory properties of compounds, but it has not been operationally defined. 3 experiments compared the gustatory properties of allegedly tasteless quinine ethylcarbonate (QEC) and a sapid analogue, quinine monohydrochloride (QHCl); 2 bottle preference tests with rats showed equivalent rejection of both compounds. Electrophysiological records from rats showed that responses to low concentrations of QEC and QHCl could be obtained. Psychophysical scaling and cross-adaptation in humans showed similar gustatory properties for both compounds. QEC is not tasteless but merely less soluble than QHCl. A classification of compounds as insoluble, tasteless, or sapid is proposed in order to make tastelessness a useful concept in theoretical arguments.

The concept of tastelessness has become increasingly important as attempts are made to build theories which relate the molecular structure of compounds to their gustatory properties (e.g., Dzendolet, 1968; Kubota & Kubo, 1969; Moncrieff, 1967). The word "tasteless," of course, means having no taste but may properly be used for several different reasons. Adaptation to a sapid solution makes solutions of the adapting compound, in a range of concentrations around the concentration of the adapting solution, tasteless (Bartoshuk, McBurney, & Pfaffmann, 1964). Certain agents such as gymnemic acid have the effect of making normally sapid solutions tasteless (e.g., Warren & Pfaffmann, 1959). Substances such as glass, which are totally insoluble in physiological media, are also tasteless. Subthreshold concentrations of sapid compounds may be considered tasteless (Koh & Teitelbaum, 1961). The cases of importance to theories of the molecular bases for gustatory properties are those in

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which compounds dissolved in physiological media are tasteless to a normal individual. The argument presented by such theories is that all compounds which have some particular physical-chemical characteristics will have some particular gustatory property, and other compounds will not. Presumably, then, sapid and tasteless compounds interact differently with taste receptors, and this is the basis for their different gustatory properties.

Unfortunately, the statement that a compound is sapid or tasteless is often based on the judgment of the theorists, and no indication of the procedures used in such a determination is given (Kubota & Kubo, 1969; Moncrieff, 1967). This omission seems especially hazardous since individual differences in taste sensitivity are well known (Fischer & Griffin, 1964; Kare & Ficken, 1963; Meiselman & Dzendolet, 1967). Furthermore, compounds with different physical-chemical characteristics may differ greatly in solubility, but this factor has received little consideration in the theoretical reports.

The initial object of the present study was to confirm the tastelessness of some compound for use in further studies, as suggested by Hagstrom (1957). The compound chosen was quinine ethylcarbonate (QEC), which has been called "euquinine" or "tasteless quinine" in the chemical literature (Lucchini, 1913; Biginelli, 1914; Stecher, 1968). Three experiments were designed to compare the gustatory properties of QEC and a sapid analogue, quinine monohydrochloride (QHCl): (a) a two-bottle preference test using rats, (b) an electrophysiological study on rats, and (c) a psychophysical scaling procedure and a cross-adaptation procedure using humans.

General Methods

The QEC was obtained from Pfaltz and Bauer. Its melting point was determined to be 91.5 — 92.5° C, in agreement with reported values (Page, 1934). Also, its ultraviolet absorption spectrum was very similar to that of an equimolar solution of QHCl, in agreement with reported observations (Hicks, 1930). QHCl was N.F. grade. All solutions were made up in glass distilled water (refractive index = 1.3330, conductivity < 6×10^{-5} mhos); all distilled water used was of this quality. QEC and QHCl solutions were stored in airtight, light-protected containers, and refractive index, conductivity, pH, and fluorescence were checked regularly for stability.

EXPERIMENT 1: RAT PREFERENCES

Methods

The Ss were six male Wistar (Camm Research) rats weighing approximately 450 g. each. They were individually housed in 9½" x 7" x 7" metal cages with open mesh fronts and bottoms and solid sides and backs. The cage fronts were fitted with two metal holders for 100 ml. calibrated glass drinking tubes (Wahmann Mfg. Co., #LC-274) so that

the spouts, which projected inside the cage, were about 1" from the sides, front, and bottom of the cage. The holders were mounted as an angle of about 15° with respect to the plane of the cage front to facilitate drinking. Food (Agway Rat and Mouse Diet) was available ad lib. Either QEC or QHC1 and distilled water were placed on the cage following restricted random sequences so that a compound was never placed on the same side more than twice in a row, the same compound was never used more than twice in a row, neither compound appeared on only one side in a block of trials, and each compound appeared the same number of times in a block of trials.

The concentrations used were, in order: 0.05, 0.005, and 0.01 mM. One concentration was used in a 10-day block of trials in which the QEC and QHC1 concentrations were the same. Daily trials were 23.5 hrs. long. Intake volumes were recorded to the nearest ml. QEC and QHC1 intakes and the water intakes on the QEC and QHC1 trials were summed to give total intake volumes. Percentage preference was calculated for each trial as quinine intake divided by total intake, and a median percentage preference was determined for each animal for each solution.

Results and Discussion

Rejection was defined as significantly less quinine intake than water intake. The Ss rejected 0.01 and 0.05 mM QEC and QHC1 (Sign test, one-tailed, $p = 0.016$) but did not reject the 0.005 mM solutions. QEC

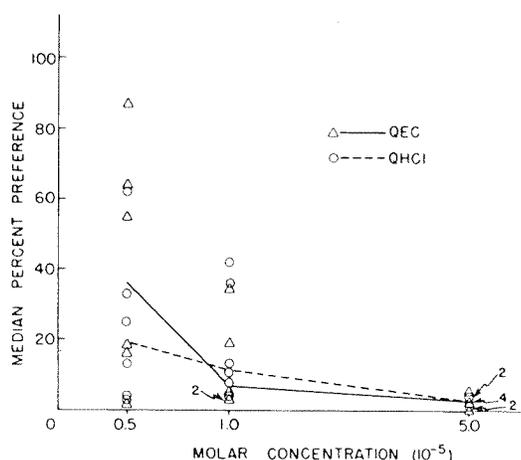


Fig. 1. Rat preferences for QEC and QHC1 as a function of solution concentration. The lines are drawn through the medians of the distributions. The abscissa is a logarithmic scale.

and QHC1 were compared directly with the median percentage preferences, but no difference between the two was observed at any concentration. The preference functions for QEC and QHC1 are shown in Fig. 1.

TABLE 1
Two-Bottle Preference Threshold for Normal Rats

| Experimenter | Compound | Water Procedure | Length of Test | Subjects | Rejection Threshold | Threshold $\times 10^{-5M}$ |
|----------------------------------|------------------|-----------------|----------------|---|---|--|
| Wedell, 1936 | QSO ₄ | D Tap | 15-20 hours | 3m, 3f, albino, 3 months 9 albino | w/q greater than 1.9 | 0.96 |
| Patton & Ruch, 1944 | QHC1 | D Tap | --- | 9 albino | q/q+w less than 0.1 | 0.75 |
| Pfaffmann, 1952 | QSO ₄ | D Tap | 48 hours | 5m albino, Brown colony 4-6 months | statistically significant difference between w & q intake | 1.34 |
| Benjamin & Pfaffmann, 1955 | QHC1 | D --- | 48 hours | 5m, albino, 3-4 months | q/q+w = 0.25 | 1.65 |
| Benjamin, 1955a | QHC1 | D --- | 48 hours | 16m, albino, 3-4 months | q/q+w less than 0.25 | 2.14 |
| Benjamin, 1955b | QHC1 | D --- | 48 hours | 18m, albino, 3-4 months | q/q+w less than 0.25 | 3.5 |
| Benjamin, 1959 | QHC1 | D Tap | 48 hours | 6m, albino, 110-148 days | q/q+w less than 0.25 | Test 1 = 3.0 |
| Benjamin & Akert, 1959 | QHC1 | D Tap | 48 hours | 18 | q/q+w = 0.25 | Test 2-4 = 1.0 |
| Ables & Benjamin, 1960 | QHC1 | D Tap | 48 hours | 12 albino | q/q+w = 0.25 | 1.85 |
| Oakley & Pfaffmann, 1962 | QHC1 | D Dist. | 24 hours | 8f, Sprague-Dawley | q/q+w = 0.25 | 0.51 |
| Young, Burright & Tromater, 1963 | QHC1 | A Dist. | 15 minutes | 10f, Holtzman, 2-12 months | q/q+w less than 0.25 | 217.0 |
| Cicala & McMichael, 1964 | QHC1 | D,R Dist. | 23 hours | 24m, Wistar, 22 days 95-105 days, 300 days | w-q/w+q = 0.5 | 22 days = 5.0 95 days = 1.0 300 days = 0.8 |
| Blomquist & Antem, 1967 | QHC1 | A,D Dist. | 48 hours | 9m, Sprague-Dawley | q/q+w = 0.25 | 3.2 |
| Mook & Blass, 1968 | QHC1 | D Dist. | 48 hours | 2m, Sherman, 90 days | q/q+w less than 0.25 | 1.0 |
| Goodrick, 1969 | QSO ₄ | D Tap | 48 hours | 80m, Wistar, 1,5,15,25 months | undefined, but using q/q+m = 0.25 | 1.5,15 months = 0.34 |
| The present study | QHC1 QEC | R Dist. | 23.5 hours | 6m, Wistar | statistically significant difference between w & q intake | 25 months = 1.34 1.0 |

Abbreviations: A = ascending series presentation; D = descending series presentation; F = Random presentation; f = female; m = male; q = quinine intake; w = water intake.

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The results indicate that the rat rejects QEC and QHCl to the same degree. The lowest concentration of QEC and QHCl significantly rejected is very close to that reported by others as shown in Table 1. Only one study, using a relatively short test length, obtained a value very different from 0.01 mM.

At least for the rat, QEC appears not to be "tasteless quinine." It is possible, though unlikely, that QEC is detected by internal chemoreceptors rather than by taste receptors since a long-term preference test was used. An electrophysiological study was undertaken to determine whether or not QEC is a gustatory stimulus.

EXPERIMENT 2: RAT ELECTROPHYSIOLOGY

Methods

The Ss were three male Wistar (Camm Research) rats which were housed similarly to those in Experiment 1 except that cages were not fitted with the calibrated drinking tubes, and tap water was available ad lib. The animals were anesthetized with sodium penobarbital, and previously described surgical procedures were used (Halpern, Bernard, & Kare, 1962). Multiunit electrophysiological responses were led off the chorda tympani nerve with silver-silver chloride electrodes, passed through a differential a.c. preamplifier, and processed by a digitally controlled electronic summator (Brush & Halpern, 1970). Arrival of solutions (10 ml, $24 \pm 1^\circ\text{C}$) at the tongue was detected by a reflection phototransistor. The stimulus solutions were 0.05 mM QEC, 5.0 mM and 0.5 mM QHCl, 10 mM A. R. grade NaCl, and distilled water. Median response magnitudes were determined for each stimulus.

Results and Discussion

In two Ss the responses to QEC and to 0.5 mM QHCl were at the recording noise level as represented by the responses to distilled water. In the third S, small but consistent responses to QEC could be recorded above noise level as shown in Table 2.

TABLE 2

Gustatory Response Magnitudes Recorded from a Rat Chorda Tympani

| Chemical | H ₂ O | QEC | QHCl | | NaCl |
|--------------------|------------------|------|------|---------|---------|
| Concentration (mM) | | 0.05 | 0.5 | 5 | 10 |
| Median Magnitude | 1.5 | 2.5 | 3.5 | 18 | 31 |
| Range | 0-5 | 0-5 | 0-6 | 13-24.5 | 20-33.5 |
| N | 19 | 7 | 9 | 26 | 4 |

Note. — Table shows maximum magnitudes using 1 sec. integrating times. A response magnitude of 10.5 is produced by 350 millivolt, 0.5 msec. duration rectangular calibration pulses, occurring 20 times per sec. at the digitally controlled summator input, and by 20 microvolt square wave calibration pulses at 50 Hz, delivered to the neural response amplifier (gain = 100,000).

While larger neural responses might have been recorded from the glossopharyngeal nerve (Frank & Pfaffmann, 1969; Halpern & Nelson, 1965; Oakley, 1967), even small responses were sufficient to suggest that QEC is a gustatory stimulus. The concentration used was shown

to be rejected in Experiment 1, and this experiment (2) demonstrated that neural responses could be obtained under favorable recording conditions. Although these results do not negate the possibility that QEC is detected by internal chemoreceptors, they do suggest that QEC is a gustatory stimulus and cannot be considered tasteless to the rat.

EXPERIMENT 3: HUMAN SCALING AND CROSS ADAPTATION

Methods

The Ss were six non-smoking male summer students at Cornell University chosen from a larger group on the basis of their responses to a gustatory screening task (Meiselman & Dzendolet, 1967). All six Ss served to establish the psychophysical functions, and four of these served in the cross-adaptation experiment.

All solutions were maintained in a water bath at 32°C for presentation to Ss. Psychophysical functions were obtained using 0.008, 0.018, 0.040, 0.0875, and 0.200 mM QEC and QHCl. Stimulus presentation consisted of the S's pouring into his mouth 10 ml. of the stimulus from a small disposable plastic cup. First, 0.040 mM QHCl was presented twice separated by 2 min. The S was instructed to treat the intensity of this stimulus as a standard and assign it a value of 10. The strength of all later stimuli were judged in proportion to the standard in the manner of magnitude estimation (Stevens, 1957). One test compound, QHCl or QEC, was presented per session, and each of the five concentrations was presented four times. The order of the 20 stimuli was randomized. There was a 2-min. rest interval between successive stimuli. No rinses were used.

In the cross-adaptation experiment, 0.040 mM was used as the adapting concentration for both QEC and QHCl, and the test stimuli were the same concentrations of QHCl as were used in the psychophysical scaling. The adapting solutions were presented through a whole mouth flow system (Abrahams, Krakauer, & Dallenbach, 1937; Meiselman, in press) consisting of inflow and outflow tubes embedded in dental impression compound. At the beginning of each session, the standard of 0.040 mM QHCl was presented and assigned a value of 10. Only one of the two adapting solutions was tested in any one session. Three min. after the standard, the adapting solution was flowed through the mouth continuously for 2 min., and then the flow was switched to one of the five test stimuli for 3 sec. It was the later, short-duration test stimulus which was judged. The Ss were asked to categorize the taste as sour, salty, bitter, or sweet and to estimate the magnitude as described above. Each test stimulus was tested twice during a session, and the 10 test stimuli were presented in random order. Rest periods of 2 min. were interposed between test stimuli, and no rinses were used. After this cross-adaptation experiment was completed, the same procedure was used to test three lower concentrations of QHCl to extend the functions. Three replications for each of these lower concentrations were obtained.

Results and Discussion

The psychophysical functions for QEC and QHCl are approxi-

mately linear on log-log coordinates indicating power functions as shown in Fig. 2. Least-squares determinations of the slopes provide ex-

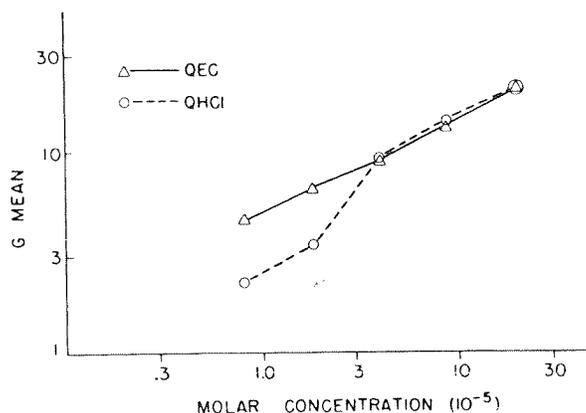


Fig. 2. Psychophysical functions for QEC and QHCl. Each point is the geometric mean magnitude estimate (G MEAN) of 24 measurements, four presentations to each of six Ss. Both axes have logarithmic scales.

ponents for the power functions: 0.47 for QEC and 0.73 for QHCl. These values are close to recent values reported for stimulation of the entire mouth with QHCl or quinine sulfate (QSO_4) (Moskowitz, 1968; Meiselman, in press). Clearly, QEC is not tasteless to humans, and its intensity increases with concentration in a way similar to QHCl and QSO_4 .

The cross-adaptation procedure showed no systematic differences between the taste qualities or magnitudes of QHCl solutions preceded by prolonged exposure to QHCl or QEC as shown in Fig. 3. This was the expected result, since many bitter substances, with the exception of urea but including QHCl and QSO_4 , have been shown to cross-adapt (McBurney, 1969). It is generally assumed that cross-adaptation indicates that the adapting and test stimuli share common receptor mechanisms (McBurney, 1969; Meiselman, 1968; Smith & McBurney, 1969). These procedures have demonstrated that QEC not only tastes like QHCl but may share, at least in part, the same receptor mechanisms.

GENERAL DISCUSSION

These experiments have demonstrated that QEC is tasteless neither to humans nor rats in spite of the label "tasteless quinine." Because of its limited solubility in water, there is an upper limit on the intensity of its taste in water. This is true for all compounds, but in the case of QEC the upper limit is low compared to QHCl. Marfori has suggested that the absence of bitter taste of QEC is due to its insolubility (Lucchini, 1913), but the present experiments indicate that to the extent of its solubility, QEC is as sapid as QHCl.

As indicated in the introduction, a variety of circumstances, such as the limited solubility of QEC, lead to use of the word tasteless. It appears, therefore, that a classification system is required to separate the trivial from the interesting cases. One useful system would separate all compounds into one of three categories depending on solubility and

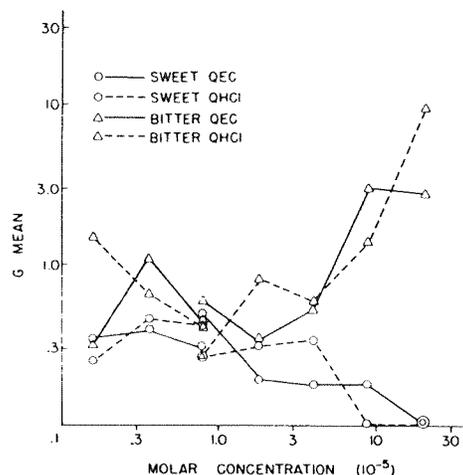


Fig. 3. Geometric mean magnitude estimate (G MEAN) of sweet and bitter responses to QHCl stimulation after adaptation to QEC and QHCl. Both axes have logarithmic scales.

gustatory properties: (a) insoluble and therefore tasteless, (b) soluble, tasteless at all concentrations, and (c) soluble, sapid at some concentrations. The solubility criterion refers to solubility in the physiological media, such as saliva, of the gustatory receptors. Insoluble compounds such as glass are of little importance to the molecular theories of gustatory properties and should be called insoluble rather than tasteless.

Soluble compounds may be tasteless or sapid. Soluble compounds which are tasteless may not have access to receptors or may not interact in a functional way with receptors. Soluble compounds which are sapid have access to receptors and interact with them in a functional manner. A large number of factors determine whether or not a compound is actually tasted, however. The concentration of the solute, the solvent, and the characteristics of the organism such as species, age, sex, hereditary factors, etc., are involved in the production of a taste. Solubility and related factors have only rarely been considered systematically in their relation to gustatory properties (Marstrom, 1967). It should be noted that the effects of drugs and adaptation which render solutions tasteless are affecting the particular *S* involved rather than the solute. Since these effects do not change the physical-chemical characteristics of the compound, this form of tastelessness should be grouped with the other organismic variables that affect sapid compounds.

Finally, it should be recognized that it is much easier to demonstrate that a compound is sapid than to demonstrate that it is tasteless. A solution of a tasteless compound should taste the same as the solvent alone over a wide range of concentrations. Any of several psychophysical and animal behavioral procedures should be useful in determining whether or not a compound has a taste. Unequivocal identification of soluble but tasteless compounds will improve our understanding of the relationship between molecular structure and the gustatory properties of taste stimuli.

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