The Japanese Journal of Physiology
19, pp. 670-681, 1969

EFFECTS OF pH ON TASTE RESPONSES IN THE
CHORDA TYMPANI NERVE OF RATS

Hisashi OGAWA

Department of Physiology, Kumamoto University Medical School, Kumamoto, Japan

Effects of pH on neural taste responses in insect chemoreceptors have been studied electrophysiologically by several investigators. EVANS and MELLON8), and GILLARY9) failed to find a pKa value of Na receptors of the blowfly, because they found that the magnitude of response to NaC1 was abruptly diminished when pH of the taste solution was lowered below ca. 3.0. MORITA12) studied pH dependency of sucrose receptors of the fleshfly, and reported that the magnitude of response to sucrose was reduced linearly with a decrease in pH of sucrose solution below 3.0 and that in this reaction pKm (Km Michaelis constant) depended on pH in alkaline solution in a complicated manner.
BEIDLER2) investigated effects of pH change on responses to NaCl in the chorda tympani nerve of rats: He found that the magnitude of response to 0.5 M NaCl was independent of pH in the range between 3.0 and 11.0. The present author, however, showed in the preceding paper13) that the magnitude of response to 0.1 M NaCl decreased when pH of test solutions was lowered below 4.0, and that the magnitude of response to 0.5 M sucrose was also decreased slightly by lowering pH of the test solution below 4.5, but that responses to these two solution were independent of pH over the range from 4.0 to 7.0 and from 4.5 to 7.0, respectively.
In the present experiment effects of pH on taste responses to NaC1, KC1, sucrose and quinine in the chorda tympani nerve of rats have been investigated more in detail.

MATERIALS AND METHODS

Female albino rats of Sprague-Dawley stock, weighing 180-200 g, were used in the
experiments. The experimental procedures were essentially the same as those used in the previous paperlu.
As test solutions, NaCl, KC1, sucrose and quinine were used. pH in test solutions
was changed between 3.0 and 11.0 by adding HC1 or NaOH. However, pH in quinine

Received for publication December 20, 1968
小川 尚

670

pH EFFECT ON TASTE RESPONSES 671

solution was not possible to elevate above 6.2, because quinine precipitates at pH
higher than 6.2. Test solutions were used at the optimum temperature where respon.-
ses in the chorda tympani nerve of the rat to these solutions show a greatest mag-
nitude: KCI, sucrose and quinine solutions were applied to the tongue at 30•Ž,
whereas NaCl solutions were used at 30•Ž and 10•Ž because the magnitude of response
to 0.1-1.0 M NaCl reaches a maximum at 30•Ž and that for NaCl solution below 0.1 M
increases with decreasing temperature, reaching a saturated value at 10•Ž15). For the
experiments at 10•Ž the preadaptation method was employed in order to avoid thermal
responses in the chorda tympani nerve.
Magnitudes of all responses were measured at 10 sec after stimulation. Km or
Michaelis constant in the reaction between taste stimuli and receptors was determined
from Beidler's taste equation2) where the constant K corresponds to the reciprocal of
Km.

Dixon's method for obtaining pK value in enzyme reactionG) was applied to taste
reactions in order to obtain pK value of the components in taste reactions such as
taste substances, taste receptors and their complexes.

RESULTS

Since it has scarcely been investigated how pH affects responses to vari-

ous solutions, the following experiment was carried out as a preliminary study, using 0.1 M NaCl, 0.1 M KCl, 0.5 M sucrose and 0.02 M quinine as test solu-tions and changing pH of these solutions between 3.0 and 11.0 by adding HC1 or NaOH. However, the pH of quinine solution was limited to the range from 3.0 to 6.2. Results of the experiments are presented in FIG. 1, where the magnitude of response to each solution, expressed relative to the control response magnitude at pH 7.0 or in the case of quinine at 6.2, is plotted against pH. Open circles in this figure represent magnitudes of responses to test solutions, whereas solid circles those of responses when the magnitude of response to the buffer solution was subtracted from that for the test solu-tion. The magnitude of response to 0.1 M NaCl was constant at the pH range from 4.0 to 11.0, but it was depressed gradually by lowering pH of test solu-tions below 4.0 (FIG. 1 A). However, the magnitude of response to 0.1 M KC1 was almost independent of pH in the range from 3.0 to 11.0 (FIG. 1 B). Changes in pH did not affect the magnitude of response to 0.5 M sucrose in the pH range from 4.5 to 11.0, but the response magnitude was reduced slightly

below pH 4.5 (FIG. 1 C), while the magnitude of response to 0.02 M quinine was almost linearly increased with a decrease in pH from 6.2 to 3.0 (FIG. 1 D). It is considered that HCl or NaOH does not affect properties of NaC1

,

KC1 and sucrose, but that they may possibly change properties of quinine since quinine acts as a base in an acid solution. As the mechanism, by which acids, especially organic acids, produce taste responses, are still unknown11, magnitudes of responses to buffer solutions were not subtracted from those of responses to quinine, whereas the former was subtracted from magnitudes of responses to NaCl, KCI and sucrose in the following analyses

.

672 H. OGAWA

In order to see whether pH affects taste receptors or taste stimuli, ef-
fects of pH on neural responses to taste solutions of varying concentrations
were investigated. pH was changed only in the acidic side where magnitudes
of responses to almost all the above mentioned stimuli were affected except for that of response to 0.1 M KCl.

A

B

C

D

FIG. 1. Relationship between the response magnitude and pH. Open circles re-present magnitudes of responses to test solutions and solid circles the response mag-
nitudes when those for buffer solutions were subtracted from those for test solutions.
Each point represents an average of several experiments. A: 0.1 M NaCl, B: 0.1 M
KCl, C: 0.5 M sucrose and D: 0.02 M quinine.

Effects of pH on the concentration-response relationship for NaCl. Inte-
grated responses to NaCl solutions of varying concentrations at 30•Ž were
recorded, when pH in test solutions was changed. The magnitudes of res-
ponses were plotted in FIG. 2, A and B, against NaCl concentration (A) and
pH of test solutions (B), respectively. Magnitudes of responses to 0.03 M and
0.1 M NaCl were reduced linearly when pH of test solutions was lowered
from 4.0 to 3.0, whereas they hardly changed in the pH range from 7.0 to
4.0. The response to 0.01 M NaCl was slightly reduced in magnitude by
decrease in pH f rom 7.0 to 4.0 and 3.7. However, it slightly increased with
further decrease in pH from 3.7 to 3.3 and 3.0. The magnitudes of responses
to 0.3 M and 1 M NaCl were almost independent of pH in the range from 3.0
to 7.0, but they were slightly increased with a decrease of pH and again de-
creased suddenly at pH 3.0. In one case out of six experiments carried out,

pH EFFECT ON TASTE RESPONSES 673

responses to NaCl solutions of varying concentrations were found to be almost
independent of pH.

A B

FIG. 2. Relationship between the response magnitude for NaCl at 30•Ž and con-
centration of NaCl (A) or pH (B). Each point represents an average of five
experiments.

Magnitudes of responses to NaCl solutions of varying concentrations at
10•Ž were plotted in FIG. 3 against NaCl concentration (A) and pH (B). The
slope, at which the magnitude of responses to NaCl increased with a logari-
thmic increase in NaCl concentration, is smaller at 10•Ž than at 30•Ž, and
the threshold of NaCl response is also lower at 10•Ž than at 30•Ž. These
are consistent with YAMASHITA and SATO'S observationsi15). The magnitude
of responses to 0.01 M, 0.03 M and 0.1 M NaCl at 10•Ž was decreased linearly
with a decrease of pH in test solutions from 4.0 to 3.0, but it was independent
of pH in the range from 4.0 to 7.0. The magnitude of response to 0.003 M
NaCl at 10•Ž was changed with pH in a similar way as that of response to
0.01 M NaCl at 30•Ž, but it was decreased slightly by lowering pH from 4.0
to 3.3. However, the magnitude of 0.3 M NaCl was almost independent of pH.
Depressive effects of pH on the magnitude of response to NaCl were more
intense in the degree and broader in the concentration range at 10•Ž than
at 30•Ž (compare FIG. 2 with FIG. 3).

674 H. OGAWA

A B

FIG. 3. Relationship between the response magnitude for NaCl at 10•Ž and con-centration of NaCl (A) or pH (B). Each point represents an average of four experiments.

Effects of pH on the concentration-response relationship for KCl. Though
the magnitude of response to 0.1 M KCl was almost independent of pH in the range from 3.0 to 11.0 (FIG. 1 B), effects of pH on responses to KCl were studied using the same concentration and the same pH of KCl solutions as those of NaCl solutions in order to campare pH dependency of responses to KCl with that of NaCl responses. The results of the experiments were pre-sented in FIG. 4. Magnitudes of responses to 0.03 M and 0.1 M KCl were constant regardless of pH change and that for 0.01 M KCl was almost inde-pendent of pH except at pH 3.0 where the magnitude was slightly increased, whereas magnitudes of responses to 0.3 M and 1 M KCl were greatly increased by lowering pH below 4.0. This was in marked contrast to the pH depen-dency of NaCl responses (FIG. 2 and 3). The temporal pattern of responses to 0.3 M and 1 M KCl was altered little by pH change, but the concentration-response curve was changed by pH. In the pH range from 7.0 to 4.0 the response magnitude for KCl was linearly increased from 0.01 M to 0.3 M and was abruptly increased at 1 M with characteristic pattern as already pointed out by YAMASHITA and SATO15). However, at the pH range lower than 4.0, the magnitude of response to KCl was almost linearly increased from 0.01 M to 1.0 M.

Effects of pH on the concentration-response relationship for sucrose. A few experiment was conducted to see effects of pH change on the magnitude

pH EFFECT ON TASTE RESPONSES 675

A B

FIG. 4. Relationship between the response magnitude for KCl and concentration
of KCl (A) or pH (B). Each point represents an average of five experiments.

of responses to 0.1 M, 0.2 M, 0.5 M and 1 M sucrose. pH was changed from 7.0 to 3.3. The results of the experiments were presented in FIG. 5. Magni-tudes of responses to 0.1 M and 0.2M sucrose were slightly reduced in the pH range lower than 4.5, whereas that of responses to 1 M sucrose was decreased abruptly at pH 3.3.

Effects of pH on the concentration-response relationship for quinine. Several experiments were conducted in order to see whether or not lowering of pH of test solutions from 6.2 to 3.0 increases the magnitude of responses to qui-nine of varying concentrations. Results of the experiments were presented in FIG. 6. Magnitudes of responses to 0.002 M-0.02 M quinine were increased monotonously as pH in test solutions was lowered from 6.2 to 3.0. This is quite similar to the response to KCl which was also increased by a decrease in pH. However, the magnitude of response to quinine was increased in a broader range of concentration and of pH than that of response to KCl, the

676 H. OGAWA

A B

FIG. 5. Relationship between the response magnitude for sucrose and concentra-tion of sucrose (A) or pH (B). Each point represents an average of three experiments.

increase of which was seen only at concentrations higher than 0.1 M and in
the pH range from 4.0 to 3.0.

Relation between pH and Km in taste reactions. Km values of taste reac-
tions between the four chemical substances and receptors were calculated
from Beidler's taste equation as a reciprocal of the constant K. Since Bei-
dle's equation cannot be applied to these reactions at full range of the
concentration, results obtained at the following concentrations were used for
calculation: 0.1-1.0M NaCl (at 30•Ž), 0.01-0.3M NaCl (at 10•Ž), 0.03-0.3M KCl,
0.1-1.0 M sucrose and 0.001-0.02 M quinine. The relationship between pKm
(-log10Km) and pH of taste solution is shown in FIG. 7.
pKm of the reaction between NaCl and receptors was constant between
pH 4.0 and pH 7.0, but it was decreased monotonously with a decrease in pH
below 4.0, both at 30•Ž and 10•Ž (FIG. 7 A). However, pKm of the reaction
between KCl and receptors was slightly decreased below pH 4.0 (FIG. 7 B).
pKm of sucrose responses was almost constant at the pH range from 4.0 to
7.0, but it was slightly decreased between 4,0 and 3.3 (FIG. 7 C). In the case

pH EFFECT ON TASTE RESPONSES 677

A B

FIG. 6. Relationship between the response magnitude for quinine and concentra-
tion of quinine (A) or pH (B). Each point represents an average of five experiments.

A

B

C

D

FIG. 7. Effects of pH on Km in various kinds of taste reactions. A: NaCl, B: KCl, C: sucrose and D: quinine.

of quinine, pKm was increased as pH was decreased below ca. 4.0 (FIG. 7 D). According to Dixon's theory that pKa of the components in enzyme

678 H. OGAWA

reactions can be given as the pH value where pKm changes by 0.3 units,6)
pKa (Ka: the ionization constant of the acid) of the components in the reac-
tion between NaCl or quinine and gustatory receptors was obtained from the
graph of pKm versus pH. In FIG. 7, A and D, two straight lines, one with
zero unit slope and another with one unit slope (dashed lines) were drawn to
intersect at the pH where pKm was changed by 0.3 units. In the case of
NaCl, the graph of Km concaves downwards, whereas it shows an upward
concavity in the case of quinine. Thus, the former curve is considered to
be produced by pKa of Na receptors because NaCl is fully ionized at any pH,
and the latter curve is attributable to the ionizing group of the quinine re-
ceptorquinine complex. The pKa of Na receptors was found to be 2.9 at
30•Ž, and 3.2 at 10•Ž, while the pKa of the quinine receptor-quinine complex
is 2.8. Effects of pH on Rm (maximum response magnitude) in Beidler's taste
equation was also investigated, because Rm is considered to be proportional
to Vm (maximum velocity of the reaction), the decrease of which gives infor-
mation for a pKa value of the receptor-chemical complex. However, log10Rm
or log10Vm was found to be almost constant in all the reactions mentioned
above in spite of change in pH.
pKm of the reaction between KCl and receptors was reduced at low pH,
though the magnitude of response to KCl was hardly decreased but it was
increased at 0.3M and 1.0M KCl at low pH. Therefore, the decrease in the
pKm value should be attributed to the error which may be brought about by
the application of Beidler's taste equation to the complicated receptor system
for KCl.

DISCUSSION

It has commonly been considered that there is only one kind of receptor
for NaCl in the area of the tongue of rats innervated by the chorda tympani
nerve, mainly because Beidler's taste equation is applicable to the concentra-
tion-response magnitude relationship for NaCl at room temperature3). Howe-
ver, it is well known that its application is limited at the range of the
concentration more than 0.1M3). Present experiments showed that in most
cases responses to NaCl are approximately independent of pH in the range
from 4.0 to 7.0, but they are depressed at low pH. It is possible that H ions
compete with Na ions in occupying the receptor for the latter: in other
words, an increase of H+ in taste solutions (a decrease of pH) may cause
the parallel shift of the log concentration-response curve toward the right
side. The curves in FIGS. 2A and 3A show the shift in the middle portion
to the right side but the lower and upper portions of the curve do not show
any shift. According to the above hypothesis, response magnitudes for 0.01
M NaCl (at 30•Ž) and 0.003 M NaCl (at 10•Ž) were expected to be reduced

pH EFFECT ON TASTE RESPONSES 679

nearly to zero at pH 3.0, but in actuality they were slightly increased. Such a discrepancy may be explained by assuming two kinds of Na receptors; one is dependent on pH, while another independent of pH in the range from 3.0 to 7.0. It is also assumed that the pH-independent receptor has lower threshold than the pH-dependent receptor and that in the latter receptor system the log concentration-response curve may show parallel shift toward the right side with lowering of pH of test solutions below 4.0, while the former receptor system may be left unaffected. Consequently the response to NaCl solution of a low concentration, which can be attributed to that of the pH-independent receptor, may not be influenced by lowering of pH even at 3.0. Quite recently S. YAMASHITA and T. KIYOHARA (personal communi-cation) have found two kinds of chorda tympani nerve fibers of rats differing in pH dependency of the response to 0.1M NaCl from each other: one was independent of pH while in another fiber the response magnitude was depres-sed sharply by lowering of pH of test solutions below 4.0. Their experiments are consistent with the present experiments.
Present experiments show that responses to 0.3M and 1M KCl were increased at low pH, whereas those to KCl lower than 0.3M were independent of pH. This fact suggests that there are two kinds of K receptors: one is not sensitive to pH change but another is so sensitive to pH change that the affinity for K is increased by lowering of pH of test solutions below 4.0. The first receptor type may produce the ordinary concentration-response magnitude relationship at concentrations lower than 0.3M. Beidler3) pointed out the ex-istence of two kinds of K receptors: one is the receptor with the higher affinity for K (K=15) and the smaller maximum response magnitude, and another is the receptor with lower affinity for K (K=0.3) and the larger maximum response (where K is the constant in Beidler's taste equation). Present study also suggests that there are two kinds of K receptors.
It has been shown that cations determine intensity of salt to stimulate
salt receptors and that the order of affinity of univalent cations for salt receptors is Li>Na>K>Rb>Cs3). Based on this fact as well as the indepen-dency of pH of the response to 0.5M NaCl, Beidler2) has supposed that the active chemical group of salt receptors might be the phosphate and sulfate radicals of such natural polyelectrolytes as nucleic acids and certain poly-saccharides. However, results of the present experiments show that pKa in one kind of Na receptor is around 3.0, although in another kind of receptor the response magnitude is independent of pH in the range from 3.0 to 7.0 and the affinity of salt receptor for K is not decreased even at pH 3.0. These facts suggest that besides phosphoryl or sulfonyl group, the receptors have the active chemical group with higher pKa value than that of the phosphoryl or sulfonyl group such as the carboxyl group in the protein be-cause pKa of the carboxyl group of protein is between 3.0 and 5.07) and the

680 H. OGAWA

order of its selectivity for univalent alkali cations runs Li>Na>K4).
pKm of the reaction between sucrose and its receptor was slightly de-creased at pH 3.3 and 3.7. However, it is not possible to determine whether or not the decrease of pKm is attributable to the ionization group of this
receptor. Recently Dastoli and Price5) have succeeded in extracting a sweet-
sensitive protein from the epithelium of bovine tongue. They investigated
effects of pH on activity of the protein to bind with sweet substances and showed that its affinity for 0.1M fructose was decreased progressively as pH
of test solutions was lowered below 5.5, although it was independent of pH
in the range 5.5-10.0. However, it cannot be determined from the present
study whether the slight decrease in pKm below pH 4.0 corresponds with the depression of affinity of their protein for fructose.
Quinine contains two atoms of nitrogen, one belonging to the quinoline nucleus, the other to the part of the molecule commonly referred to as the

"second half" or the quinuclidine nucleus. Both atoms of nitrogen are able
to dissociate into ions. The quinuclidine nucleus of this alkaloid is, however,
more basic than the quinoline nucleus: pKa of the former is 9.7 and that
of the latter 5.0710). In the neutral solution the nitrogen atom of the quinu-clidine nucleus ionizes fully, and that of another part ionizes progressively as pH of quinine solution is lowered. Facilitation of quinine response would
have been due to the ionization of the nitrogen atom of quinoline nucleus.
However, the relationship of pKm versus pH in FIG. 7D suggests that its
concavity is attributed to the ionizing group of quinine receptor-quinine
complex, but not to that of quinine itself, although log10Rm or log10 Vm was
independent of pH.
Present study showed that the magnitudes of responses to KCl and qui-
nine were increased as pH of test solutions was lowered. Since single fiber
analysis of chorda tympani nerve responses has revealed that responses to KCl and quinine are highly and positively correlated with that to Hal14), the
facilitation may be ascribed to the synergism between KCl or quinine and
HCl through the interdependent receptor system1).

SUMMARY

1. Effects of pH on chorda tympani nerve responses to NaCl, KCl, sucrose
and quinine of varying concentration and of the optimum temperature were
investigated in rats.
2. Magnitudes of responses to NaCl in concentrations of 0.1M or lower were
decreased linearly with lowering of pH of test solutions below 4.0, whereas
those for 0.3M and 1M NaCl were independent of pH at 30•Ž. Depression
of responses to NaCl by low pH were greater at 10•Ž than at 30•Ž. Magni-
tudes of responses to 0.01M, 0.3M and 1M KCl were increased as pH of test

pH EFFECT ON TASTE RESPONSES 681

solutions was decreased below 4.0, but those of responses to 0.03M and 0.1M were independent of pH. pH dependency of the response to KCl was in marked contrast with that of the response to NaCl.
3. Magnitudes of responses to sucrose of varying concentrations were almost independent of pH, but slight decrease in the response magnitude was seen below pH 4.0.
4. Magnitudes of responses to quinine of varying concentrations were mono-tonously increased as pH of test solutions was decreased from 4.5 to 3.0.
5. Km or Michaelis constant of taste reaction was obtained from Beidler's

taste equation and pH dependency of Km was examined. Thus, depression of responses to NaCl was attributed to the ionization of the receptor for NaCl, whereas facilitation of the responses to quinine was ascribed to that of the quinine receptor-quinine complex.

This work was carried out under the supervision of Professor M. SATO. The author expresses his sincere gratitude for his helpful advice and discussion in carry-ing out the experiment and in preparing the manuscript. The author's grateful thanks are also due to Dr. H. MORITA, Department of Biology, Faculty of Science, Kyushu University, for his valuable criticism in preparing the manuscript.

REFERENCES

1) ARIENS, E. J.: Molecular Pharmachology, 1, Academic Press: New York (1964)

.

2) BEIDLER, L.M.: J. gen. Physiol., 38, 133-139, (1954).
3) BEIDLER, L.M.: Progr. Biophys. biophys. Chem., 12, 107-151, (1962).
4) BREGMAN, J.L.: Ann. N.Y. Acad. Sci., 57, 125-143, (1953).
5) DASTOLI, F.R. AND PRICE, S.: Science, 154, 905-907, (1966).
6) DIXON, M.: Biochem., 55, 161-170, (1953).
7) DIXON, M. AND WEBB, E.C.: Enzymes, Longmans, Green and Co., London (1958).
8) EVANS, D.R. AND MELLON, D. JR.: J. gen. Physiol., 45, 651-661, (1962),
9) GILLARY, H.L.: J. gen. Physiol., 50, 337-350, (1966).
10) The Merck Index of Chemicals and Drugs, 2nd Ed., Merck & Co., Inc.: Rahway (1960).
11) MONCRIEFF, R.W.: The Chemical Senses, 2nd Ed., Leonard Hill Limited: London (1951).
12) MORITA, H.: In Kankaku-Joho 2, ed, by M. Kuwabara, Kyoritsu-Shuppan: Tokyo (1967) (in Japanese).
13) OGAWA, H.: Jap. J. Physiol., 19, (1969) in press.
14) OGAWA, H. SATO, M. AND YAMASHITA, S.: J. Physiol., 199, 223-240, (1968).

15) YAMASHITA, S. AND SATO, M.: J. cell. comp. Physiol., 66, 1-18, (1963).