ANALYSIS OF THE SINGLE UNIT ACTIVITY OF GUSTATORY RECEPTORS IN THE FROG TONGUE

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Specific receptors for acid and salty taste were first observed in the tongue of the frog by Pumphrey (1935). Zotterman (1949), Anderson, Landgren, Olson and Zotterman (1950) and Anderson and Zotterman (1950) have found that there are three types of taste fibres or gustatory receptors; the first responds specifically to water and to dilute NaCl; second to acid; and the third to NaCl and CaCl₂. Koketsu (1951) has shown that frog tongue responds to quinine; and Kusano and Sato (1957) have pointed out that it also responds to sucrose.

Recently, however, analyses of gustatory receptor activity have been made by recording impulses in single gustatory nerve fibres of mammals (Cohen, Hagiwara and Zotterman, 1955; Fishman, 1957; Pfaffmann, 1955). The results obtained from cats, rats and rabbits indicate that most of the single gustatory receptor units respond to more than two kinds of taste stimuli.

On the other hand, Kusano and Sato (1958) and Kusano (1958a, b; 1959a) have suggested that there are two distinctly different receptor mechanisms for initiating gustatory impulses: one is the response to monovalent salts which may be produced by movements of ions across the membrane of gustatory receptors and the other is the response to divalent salts, sucrose, quinine and acetic acid which may be produced by some chemical reactions on the receptor surface.

The present experiments were therefore undertaken to examine whether single unit of gustatory receptors in the frog's tongue responds to various kinds of taste stimuli; and to investigate whether or not two distinct units corresponding to the two mechanisms mentioned above can be obtained.

METHOD

The tongue with the glossopharyngeal nerve was isolated from a Japanese common frog (Rana nigromaculata), and a few functional gustatory nerve fibres were dissected out over 1.0-2.0 mm length at the point of 5-10 mm from the tongue, using sharpened needles and a binocular dissecting microscope. The tongue was

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placed in one of three Ringer pools, which were separated by two air gaps, and a dissected portion of the nerve was mounted on one of the air gaps, as shown in Fig. 1. On another gap, the width of which was about 2 mm, was mounted an intact part of the glossopharyngeal nerve. Afferent impulses in the glossopharyngeal nerve were recorded by a cathode-ray oscilloscope through a c.r.-amplifier. A loud speaker was used to detect the nerve activity.

Using distilled water as solvent a series of solutions of different concentrations were prepared and about 0.5 ml of each was applied to the tongue with a small pipette; this served to stimulate a region of the tongue of 3-4 mm² innervated by a particular nerve fibre under observation. The chemicals used for stimulation were: NaCl, NaBr, NaI, NaNO₃, saccharin sodium (C₇H₄O₃NSNa), KCl, KBr, KI, LiCl, CsCl, NH₄Cl, MgCl₂, CaCl₂, SrCl₂, BaCl₂, 1/4 M sucrose in distilled water (M/4 Suc-w), 1/4 M sucrose in Ringer (M/4 Suc-R), 1/256 M quinine hydrochloride (M/256 Q-HCl), and 1/64 N acetic acid (N/64 CH₃COOH) and distilled water (water). But it was very difficult to use varying concentrations of all these chemical solutions on each preparation. Therefore, for analysis of the activity of single units taste solutions of the following concentrations were usually employed: distilled water, 1/4 M NaCl, 1/8 M KCl, 1/8 M MgCl₂, 1/128 M CaCl₂, 1/4 M sucrose in distilled water, 1/4 M sucrose in Ringer, 1/256 M quinine hydrochloride in distilled water, 1/64 N acetic acid in distilled water. But it is very dangerous to use only taste solutions of such a restricted range of concentration as stimuli. Therefore such stimuli as described above were used for rough analysis of activity of the single unit and chemical stimuli of wider range of intensity were employed for further analysis of activity of each single unit.

The tip of the pipette was usually held at a distance about 5-10 mm over the surface of the tongue. After application of each taste solution, the tongue was washed sufficiently with normal Ringer.

Some of these experiments were performed during the winter of 1956 when frogs hibernated, but nearly all the experiments were made from March to October of 1958 under room temperature (18°-27° C) when frogs were active.

FIG. 1. Schematic representation of the experimental set-up. The width of the middle pool is about 3 mm, while the air gaps are 0.5 mm (left) and 2 mm (right) in width. Each pool is filled with Ringer solution.
RESULTS

As previously reported (Kusano, 1958a), certain difference was found in the response patterns of the frog's tongue to the various taste stimuli according to the season. Activity of the whole nerve to the taste stimuli is shown in Fig. 2. Responses of the summer frog to distilled water and divalent salts are more marked than those of the winter frog, but responses to potassium salts and acetic acid are less in the former than those in the latter.

![Gustatory impulses of the whole glossopharyngeal nerve of the summer frog evoked by various taste stimuli.](image)

Fig. 2. Gustatory impulses of the whole glossopharyngeal nerve of the summer frog evoked by various taste stimuli. The taste solutions applied to the tongue in each record are: A: water, B: 1/4 M NaCl, C: 1/4 M KCl, D: 1/8 M NH₄Cl, E: 1/8 M MgCl₂, F: 1/8 M SrCl₂, G: 1/128 M CaCl₂, H: 1/4 M sucrose in distilled water, I: 1/4 M sucrose in Ringer, J: 1/256 M quinine hydrochloride, K: 1/64 N acetic acid. Two successive traces in each frame of this and subsequent figures show photographs taken at the beginning of the discharge and at approximately 5 sec after, indicating the rate of its adaptation. Time signal: 10 msec.

Activities of 105 units responding to the taste stimuli were recorded in this experiment. From the pattern of responses obtained, the gustatory receptor units in the frog tongue were classified in the following way.

1. Units responding to all taste stimuli employed; Only 3 units responded to all taste stimuli employed, but none of these showed the same degree of sensitivity to each taste solution, the impulse number ranging from about 70 to only 1 during initial 2 sec after application of taste solutions. Usually, in this experiment, almost all units did not show any response to application of Ringer solution except a few units, which produced a few impulses during initial 2 sec after application of
Ringer solution; and each unit had different sensitivity to various kinds of taste stimuli.

2. Units responding to only one kind of taste stimuli; Units which responded specifically to a particular substance were observed in 11 examples. Among them 5 units responded to 1/256 M quinine hydrochloride alone, 2 units responded to 1/64 N acetic acid, 3 units responded to water and 1 unit responded to 1/4 M saccharin. But it can not be concluded that they have specific characters responding to a particular substance only, because each specific unit was very few in number in the present experiments. In other words, the specific units observed in cats (COHEN, HAGIWARA and ZOTTERMAN, 1955) were scarcely found in the frog.

3. Units responding to divalent salts, sucrose and water; Sixty seven units responded well to divalent salts, sucrose and water; some of them were also sensitive to monovalent salts, quinine or acetic acid, but some were not.

   a. Twenty seven units among 67 responded massively to divalent salts, sucrose and water, but hardly to NaCl, KCl, quinine and acetic acid (Figs. 3 and 4 A).

   b. Twenty four units among 67 responded to some monovalent salts to some extent, but hardly to quinine and acetic acid (Fig. 4 C).

   c. Sixteen units among 67 responded well to either 1/256 M quinine hydrochloride or 1/64 N acetic acid and some of them also responded to some monovalent salts (Fig. 4 B and E).

   Generally speaking, these 67 units may be called D-units (SATO and KUSANO, 1960) and one may call 3(a) genuine D-units, 3(b) D+M-units, and 3(c) D+Q-units or D+A-units. Units of the types described here were most commonly observed in the frog tongue. Although the author could not measure the diameter of each glossopharyngeal nerve fibre under the microscope, the fibre of D-units seems to have a thick diameter, because the spike height of the units belonging to 3(a) group is generally larger than that of other units, which will be described afterwards.

   Some of these units responded intensely to distilled water, but some did not (compare Fig. 3 with Fig. 4 A). As reported previously (KUSANO, 1958a), the impulse discharge of almost all units of 3(a) showed a tendency to increase in frequency following successive application of water, but other units as illustrated in Figs. 4 C, D, E, F, G and Fig. 6 did not show such properties. Since most of the units responding to water have a large spike and other units rarely respond to water, the so-called “water-fibre” (ZOTTERMAN, 1950) should be included in these units. On the other hand, it should be noted that the units which belong to 3(a) respond well to neither acid nor quinine as compared with the water-fibre observed on cats tongue by COHEN et al. (1955), but the latter has properties which are more similar to the units 3(c) than to the units 3(a).

   In this experiment, the order of stimulating effectiveness of various salts could
not be determined (KUSANO and SATO, 1957; KUSANO, 1958a), because, although almost all the units in 3(a) showed massive discharges to various divalent salts, they did not show responses to monovalent salts; and each unit had a different sensitivity to each of these types of salts. Therefore, when the order of stimulating effectiveness of divalent salts and monovalent salts on these units of 3(a) type was measured, the order of effectiveness of divalent salts obtained was Ca++ > Sr++ > Mg++, but that for monovalent salts could not be measured. However, these units responded to dilute monovalent salt solutions of less than 1/256 M. This fact indicates that in these units the water response is inhibited by monovalent salts when their concentration is higher than a certain value (Fig. 5 C and D).

The units of 3(b) type always produced impulses in response to both concentrated and diluted solutions of divalent salts and monovalent salts (Fig. 5 B). The discharge by solutions of a high concentration is the response to salt and that by diluted solution is the response to water. Therefore, in these units all salts except CaCl₂ showed depressing action on water discharge.
FIG. 4. Figures showing the sensitivity of 7 gustatory receptor-units of frog to various taste stimuli.
Fig. 5. Concentration effect of various salts on 4 different units which respond to salts. Figure A is obtained from M-unit and B, C, D are obtained from D-units. In each figure number of impulses in 2 sec after application of the taste solutions is plotted as an ordinate against the molar concentration of salts as an abscissa.
On a few units responding to various salts, the stimulating effectiveness of various cations and anions was compared. Results are summarized in Table 1. Though there is a scatter in the impulse number of an individual unit in 2 sec after application of various salts solutions, the sum of 4 units presented in Table 1 clearly indicates that the number of impulses per 2 sec is influenced very much by cations, the order being \( \text{NH}_4^+ > K^+ > \text{Cs}^+ > \text{Na}^+ > \text{Li}^+ \) for monovalent cations and \( \text{Ca}^{++} > \text{Sr}^{++} > \text{Mg}^{++} \) for divalent cations, while anions do not influence the impulse number much. The order of the effectiveness of cations described here is in good agreement with the results obtained by determining the threshold concentrations (KUSANO, 1958a), but that of anions do not agree with the result obtained previously (KUSANO and SATO, 1958).

Some units of 3(c) type, which responded massively to quinine, produced only a few impulses to water, and the discharge frequency to both 1/4 M sucrose in water and in Ringer was almost the same as that to distilled water (Fig. 4 C, D and E). These units responded well to a high concentration of \( \text{NaCl} \), \( \text{NH}_4\text{Cl} \), \( \text{BaCl}_2 \), \( \text{MgCl}_2 \) together with the massive discharges to quinine, but these units hardly responded to 1/64 N acetic acid. The units sensitive to 1/64 N acetic acid and belonging to 3(c), discharged impulses in response to water, but did not respond to quinine (Fig. 4 B).

4. Units responding mainly to monovalent salts; Eight examples of the units responding mainly or exclusively to monovalent salts were found except the units of 3(b) type. They hardly responded to any other taste solutions. These units can be called M-units (SATO and KUSANO, 1960). The response of these two units is illustrated in Fig. 6. The photographs in Fig. 6 and the graph in Fig. 5 A show the concentration effects of \( \text{NaCl} \), \( \text{LiCl} \), \( \text{CsCl} \) and \( \text{KCl} \). The properties of these units agree with those of the so-called “salt-fibre” described by COHEN et al. They have generally a smaller spike amplitude than D-units. In Fig. 6 B a few impulses were produced by application of water, but, contrary to the water response obtained in ordinary D-units, these units did not produce any impulses in response to successive application of water (Fig. 6 C). However, threshold of these units to \( \text{NaCl} \) or ordinary monovalent salts were lowered distinctly below that of the normal state immediately after application of water (compare Fig. 6 A with D) (KUSANO, 1958b; GORDON et al., 1959).

5. Units responding mainly to quinine; Nine examples of the units responding mainly to quinine were observed. These units can be called Q-units (SATO and KUSANO, 1960). As described before 5 units responded to quinine alone; the other 4 units responded not only to quinine but also to one of 1/8 M \( \text{MgCl}_2 \), 1/64 N acetic acid and 1/4 M saccharin to some extent. The sensitivity of these units to quinine was extremely high; sometimes they responded to a quinine solution of less than 1/10000 M. The rate of adaptation to quinine was very fast and the discharges ceased within 2 sec after the application of the quinine solution.
Fig. 6. Gustatory impulses from a few glossopharyngeal nerve fibres responding mainly to monovalent salts. Test solutions applied in each record is; A: Ringer, B: water, C: water, D: Ringer, E: 1/4 M NaCl, F: 1/8 M NaCl, G: 1/16 M NaCl, H: 1/2 M LiCl, I: 1/4 M LiCl, J: 1/4 M CsCl, K: 1/8 M CsCl, L: 1/16 M CsCl, M: 1/4 M KCl, N: 1/8 M KCl, O: 1/16 M KCl, P: 1/128 M CaCl₂, Q: 1/4 M sucrose in water, R: 1/256 M quinine hydrochloride, S: 1/64 N acetic acid. Time signal: 1 sec. Lower figures show the number of impulses in 2 sec immediately after the application of each solution.

In these Q-units, when the tongue was washed with normal Ringer solution after a test by quinine, the pattern of discharge similar to the quinine discharge was usually seen as observed on the whole nerve of winter frog (KUSANO, 1958a). In general, these units have smaller amplitude of impulses than those of other units and discharge at a relatively low frequency. Units producing the smallest spike height are involved in these Q-units.

6. Units responding mainly to acetic acid; Seven examples of units sensitive to acetic acid were obtained. Among them 1 unit responded only to acetic acid;
TABLE 1
Relative effectiveness of cations and anions, as expressed by number of impulses per 2 sec.

<table>
<thead>
<tr>
<th>Salts</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/4 M NaCl</td>
<td>0</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>1/4 M LiCl</td>
<td>1</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>1/4 M KCl</td>
<td>0</td>
<td>70</td>
<td>0</td>
</tr>
<tr>
<td>1/4 M CaCl</td>
<td>4</td>
<td>30</td>
<td>18</td>
</tr>
<tr>
<td>1/4 M NH₃Cl</td>
<td>67</td>
<td>35</td>
<td>6</td>
</tr>
<tr>
<td>1/8 M NaCl</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>1/8 M LiCl</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>1/8 M KCl</td>
<td>0</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>1/16 M MgCl₂</td>
<td>1</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>1/16 M SrCl₂</td>
<td>4</td>
<td>10</td>
<td>28</td>
</tr>
<tr>
<td>1/128 M CaCl₂</td>
<td>39</td>
<td>19</td>
<td>0</td>
</tr>
</tbody>
</table>

* I, II and III indicate different preparations, and A, B, C, etc. different units in the same nerve.

3 units responded not only to acetic acid but also to water; 2 units also responded to 1/8 M KCl and 1 unit also responded to quinine. These units can be called A-units (SATO and KUSANO, 1960); The discharge rate of these units was lower than that of the Q-units. If the impulse frequencies of these particular units with those of units in 3(c) type which respond to acetic acid are compared, the latter is larger than the former. This A-unit may correspond to the “acid-fibre” in the cat (COHEN et al., 1955) and monkey (GORDON et al., 1959).

7. Units responding to sucrose; The unit exclusively sensitive to 1/4 M sucrose in water or to that in Ringer was not observed. In many cases, units sensitive to sucrose responded also to water, CaCl₂ and MgCl₂ to the same degree. Therefore, all the units sensitive to sucrose belong to D-units. The impulse number of 4 units discharged per 2 sec to distilled water and to two kinds of sucrose solution is presented in Table 2. Comparing the responses to 1/4 M sucrose in water with those to distilled water, the former is greater than the latter except a few cases. In a few units response to 1/4 M sucrose in Ringer elicited a more intense discharge than 1/4 M sucrose in water (Unit Da). In some of D-units (b, c, d) the number of impulses elicited by 1/4 M sucrose dissolved in Ringer is less than that by water.
discharge. Therefore one cannot say that the response to sucrose in water is algebraic sum of the response to water and that to sucrose in Ringer; but one can simply say that some units respond to sucrose and that they belong mostly to D-units.

DISCUSSION

From the analyses of the single unit activity of gustatory receptors, it has now become clear that single gustatory receptor-units of the frog responding to several kinds of chemicals are more numerous than the units responding to a particular quality of chemicals and that each unit has complicated response properties. But rough classification of these units into four groups was made possible according to their response patterns to various taste stimuli.

Although, in the present experiments, units responding to monovalent and divalent salts were divided into M- and D-units according to a different sensitivity to these salts, many units responded to both monovalent and divalent salts. In addition, some units produced massive discharges in response to NaCl, but not to KCl solution of similar or higher concentration; and some units responded to 1/128 M CaCl₂ and 1/8 M MgCl₂, but not to 1/8 M BaCl₂. This suggests that classification of these two units is not absolute, but rather of statistical nature. However, the units responding to monovalent salts such as NaCl, KCl and LiCl (8 units of M-units) are quite different from those sensitive to divalent salts such as CaCl₂, MgCl₂ and SrCl₂ (27 units of 3(a) type). This supports the suggestion proposed by KUSANO and SATO (1958) based on the experimental results on the effects of various anions and of narcotics upon the gustatory receptors of frogs (KUSANO, 1959b), in which two different receptor mechanisms for monovalent salts and divalent salts were presented.

Although ZOTTERMAN (1949) and COHEN et al. (1955) have found receptors specifically sensitive to water in the tongue of frogs and cats, such a specific unit has not been found in the present experiments except for a few cases. Three units have very similar properties to those of “water-fibre” in frogs and cats.

### Table 2

Response of five receptor-units to four kinds of taste solutions, as expressed by number of impulses per 2 sec. (a, b, c, d and e indicate different units)

<table>
<thead>
<tr>
<th>Taste solution</th>
<th>Unit type</th>
<th>D</th>
<th></th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>a</td>
<td>57</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>M/4 Suc-w.</td>
<td>b</td>
<td>47</td>
<td>23</td>
<td>41</td>
</tr>
<tr>
<td>M/4 Suc-R.</td>
<td>c</td>
<td>66</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>M/4 NaCl</td>
<td>d</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
observed by Zotterman (1949) and Cohen et al. (1955). However, it seems necessary to repeat more accurate experiments on the units responding specifically to water whether they are strictly specific units or not. In many cases the receptors sensitive to water also responded to divalent salts and sucrose. It was frequently observed in the present experiments that some D-units did not respond to 1/256 M quinine hydrochloride and 1/64 N acetic acid, both of which were dissolved in distilled water. This result indicates that not only ordinary monovalent salts but also quinine and acetic acid have inhibitory effect on the water response. It is also known that in some insects (Calliphora vomitoria), the labellar chemoreceptor response to sucrose is depressed by quinine (Morita, 1959). As previously proposed, the inhibitory effect of ordinary monovalent salts on water discharge may be attributed to suppression of ionic fluxes through receptor membranes; and also the inhibitory effect of quinine and acetic acid on water discharge of the D-units may have a similar mechanism. However, there is at the moment no definite evidence indicating that water response is produced by movements of ions through the membrane by diffusion, but on the contrary the present experiments may supply evidences against the above hypothesis on the mechanism of water response; and it inhibition by various salts and chemicals should be investigated further.

It is known that dogs and rats have sweet receptors, but that cats have no sweet receptors (Anderson et al., 1950, Cohen et al., 1955). However, Frings (1951) has reported that cats can distinguish “sweet” things from “non-sweet”, and according to Pfaffmann (1955) cats have receptors responding to sweet substances. The latter has also found sugar-sensitive units in the rat. On the behavioral experiments, it is not yet known whether frogs can distinguish sweet substances or not. Early investigators have denied the existence of receptors for sweet substances in the frogs (Pumphrey, 1935, Koketsu, 1951, Zotterman, 1949), but the receptors responding to sucrose were demonstrated in the experiments in which response to 1/4 M sucrose dissolved in distilled water remained after complete depression of the response to water and divalent salts (Kusano, 1959a). However in the present experiments no units, which specifically respond to sucrose, were found. Most units sensitive to sucrose dissolved either in Ringer or in water, responded to divalent salts and therefore they should belong to the D-units. Beidler (1953) and Fishman (1957) have found in the rat's tongue that saccharin produces a response in the units sensitive to sucrose as if it was a sweet substances like sucrose, while saccharin acts on the units sensitive to salts as if it was a salt; Gordon et al. (1959) have found that saccharin produces a response in the tongue of Macacus rhesus almost always as a sweet substance. But on the frog's tongue saccharin has an effect similar to monovalent salts (Kusano and Sato, 1957).

In the present experiments, so called D-units are most commonly observed
and M-, Q-, A- units are found less frequently. This will not suggest that majority of the units of gustatory receptors in the frog tongue have the properties of D-units, but it may merely suggest that during the isolating procedure of nerve fibres D-units remain uninjured while other units are killed or injured, because the fibre diameter of D-units is larger than that of M-, Q-, and A-units.

Histological evidences on the gustatory end organs of frogs (GAUPP, 1896, KUSANO and SATO, 1957) and of mammals (FISHMAN, 1957), suggest that a single gustatory receptor unit is likely to consist of an axon with several gustatory cells. PFaffmann (1941) has mentioned, from his data on the cat's single gustatory receptor units, the possibility that receptors of different properties may be connected to the same fibre. Therefore in the frog's tongue, it is probable that the unit responding to only one kind of taste solution may consist of receptors of similar properties and that one responding to several kinds of taste solutions may consist of several receptors of different properties. However, the possibility that one gustatory cell responds to several kinds of taste solutions and that each unit possesses several of these receptors, but to a different degree, cannot be ruled out.

**SUMMARY**

Properties of single units of gustatory receptors in the frog tongue were investigated by recording the impulse discharge of single glossopharyngeal nerve fibres to various taste solutions.

1. The great majority of the units observed in the present experiment responded to more than one kind of taste solution (94 units among 105), but the units responding to all taste stimuli employed were only 3 cases found. The specific units responding to a particular taste solution were observed in 11 examples; 5 units to 1/256 M quinine hydrochloride, 2 units to 1/64 N acetic acid, 3 units to distilled water and 1 unit to 1/4 M saccharin sodium. Receptor-units were divided into four main types.

2. The D-units responded to divalent salts and in most cases they also responded to distilled water and to sucrose dissolved in water or in Ringer. Sometimes, they responded also to monovalent salts, quinine and acetic acid. This type occupied the great majority of the receptor-units observed in the present experiments (67 units among 105).

3. The M-units responded mainly to monovalent salts such as NaCl, KCl and LiCl, but not to other kinds of taste solutions (8 units among 105).

4. The Q-units and A-units responded to quinine and acetic acid respectively (9 and 7 units among 105).

5. No receptor-units which specifically responded to sucrose were found; but the units responding to sucrose belonged to the D-units.
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