THE ENHANCING ACTION OF 5'-RIBONUCLEOTIDE ON RAT GUSTATORY NERVE FIBER RESPONSE TO MONOSODIUM GLUTAMATE

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Summary Responses of single chorda tympani fibers of rats to gustatory stimulations by monosodium glutamate (MSG) of varying concentrations with or without the presence of a fixed amount of sodium 5'-guanylate (5'-GMP) were recorded. The effect of addition of 5'-GMP to MSG differs according to the response characteristics of the unit. In units predominantly responsive to NaCl, little enhancement was observed in the response magnitude with the addition of 5'-GMP, but in units responding well to sucrose a marked enhancement occurred. In a majority of these units, not only a synergistic enhancement in the response magnitude but also a marked lowering of threshold was obtained after the addition of 5'-GMP. The relative magnitude of enhancement was greater at a lower concentration of MSG, and decreased with increasing concentration. Analysis of the concentration-response magnitude relationships suggests that the synergistic action of MSG and 5'-GMP on gustatory receptors is a mechanism known as 'competitive and noncompetitive' sensitization.

A number of electrophysiological experiments on the chorda tympani response of the rat (SATO and AKAIKE, 1965; SATO and YAMASHITA, 1965; ADACHI et al., 1967; HUI and SATO, 1967; KASAHARA et al., 1970) demonstrated a marked enhancement in the magnitude of neural response to chemical stimulation of the tongue by sodium glutamate (MSG) after the addition of a small amount of 5'-ribonucleotides, such as sodium 5'-inosinate (5'-IMP), sodium 5'-guanylate (5'-GMP), and sodium 5'-adenylate (5'-AMP). Further experiments on single chorda tympani fiber responses in the rat have revealed that the enhancement of the MSG response by 5'-ribonucleotides occurs mostly in units responsive to sucrose, not in those predominantly sensitive to NaCl (SATO et al., 1970, 1971).

However, the mechanism of this enhancement is not yet clearly understood.
though it has been suggested by Sato et al. (1970) that the addition of 5'-ribo-
nucleotides to MSG would probably facilitate binding of glutamate ions with
sucrose-sensitive receptor molecules. Therefore, in the present study, further
analysis was made by recording impulse discharges in single chorda tympani
fibers of rats produced by MSG solutions of varying concentrations with or
without the addition of a fixed amount of 5'-ribonucleotides. Since the enhancing
effects of 5'-IMP, 5'-GMP, and 5'-AMP on the rat chorda tympani response
are qualitatively similar but differ quantitatively (Sato et al., 1970), only the effect
of 5'-GMP was examined in the present study.

MATERIALS AND METHODS

Female albino rats of Sprague-Dawley stock, weighing 150–250 g, were
anesthetized by intraperitoneal injection of sodium amobarbital, 70 mg/kg body
weight. The chorda tympani was surgically exposed at the submandibular region
and cut centrally. Subsequently, the chorda tympani was dissected with a pair
of needles under a binocular microscope to obtain single units. Spikes of uniform
size were the criterion for single-unit response. Units examined in this study were
not randomly obtained but were selected either because of their characteristic
response pattern or because of relatively high sensitivity to sucrose.

Gustatory receptors were stimulated by passing 100 ml of a test solution at a
constant rate through a flow chamber in which the tongue had been enclosed.
The tongue was rinsed with tap water after each stimulation. The interval
between successive stimulations was set at about 3 min, during which period
evoked impulse discharges ceased. The temperature of both test solutions and
tap water used for rinse and preadaptation was kept at about 25°C.

Test solutions used were varying concentrations of MSG dissolved in de-
ionized water and in 0.001 M 5'-GMP (courtesy of Takeda Chemical Industries,
Ltd). The concentration of 5'-GMP was fixed at 0.001 M in the experiment,
because 5'-GMP at this concentration scarcely produced a response in a majority
of chorda tympani fibers of rats but yielded a significant enhancement of the
response when added to MSG (Sato et al., 1970).

Since the enhancement of the chorda tympani fiber response to MSG by 5'-
GMP differs according to responsiveness of fibers to sucrose and NaCl, response
characteristics of each unit were examined at the beginning of an experiment by
applying to the tongue the four standard test solutions (0.1 M NaCl, 0.5 M sucrose,
0.01 N HCl, and 0.02 M quinine hydrochloride).

As in the earlier investigation (Ogawa et al., 1969), the magnitude of the
chorda tympani fiber response to any stimulus was in most cases expressed by
number of impulses discharged during the initial 5 sec after stimulation, that is,
the dynamic phase of the discharge. However, for applying the method of
analysis of the dose-response curves for drugs to the results obtained in the present
experiments, the number of impulses between 5 and 10 sec after stimulation, that is, the steady discharge phase, were used, because the method of analysis of the dose-response curves was based essentially on the equilibrium state of chemical reactions.

RESULTS

Responses of chorda tympani fibers to MSG of varying concentrations

Responses of 12 chorda tympani fibers to MSG of varying concentrations were recorded. All the units responded to MSG, though in different degrees. Among 12 units, 1 was predominantly responsive to NaCl (G in Table 1), and 2 were sensitive to NaCl, HCl, and quinine (A and D), but they rarely responded to sucrose. One unit among the remaining 9 was predominantly sensitive to sucrose (L), and the other 8 responded to sucrose and NaCl. Among these 8 units, 2 showed a very high sensitivity to both MSG and 0.001 M 5'-GMP and were more broadly tuned to the four basic stimuli than the other units (B and C in Table 1).

Table 1. Response characteristics of 12 chorda tympani fibers to various chemicals.

<table>
<thead>
<tr>
<th>Fiber No.</th>
<th>0.1 M NaCl</th>
<th>0.5 M Sucrose</th>
<th>0.01 N HCl</th>
<th>0.02 M Quinine</th>
<th>0.1 M MSG</th>
<th>0.001 M 5'-GMP</th>
<th>0.1 M MSG + 0.001 M 5'-GMP</th>
<th>R_{M+G}/R_M</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>155</td>
<td>0</td>
<td>21</td>
<td>16</td>
<td>43</td>
<td>0</td>
<td>54</td>
<td>1.3</td>
</tr>
<tr>
<td>B</td>
<td>103</td>
<td>35</td>
<td>11</td>
<td>6</td>
<td>105</td>
<td>58</td>
<td>149</td>
<td>1.4</td>
</tr>
<tr>
<td>C</td>
<td>76</td>
<td>41</td>
<td>61</td>
<td>4</td>
<td>100</td>
<td>52</td>
<td>194</td>
<td>1.9</td>
</tr>
<tr>
<td>D</td>
<td>64</td>
<td>5</td>
<td>18</td>
<td>13</td>
<td>18</td>
<td>0</td>
<td>17</td>
<td>0.9</td>
</tr>
<tr>
<td>E</td>
<td>40</td>
<td>75</td>
<td>20</td>
<td>0</td>
<td>46</td>
<td>9</td>
<td>118</td>
<td>2.6</td>
</tr>
<tr>
<td>F</td>
<td>36</td>
<td>38</td>
<td>4</td>
<td>2</td>
<td>7</td>
<td>0</td>
<td>54</td>
<td>7.7</td>
</tr>
<tr>
<td>G</td>
<td>27</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>22</td>
<td>0</td>
<td>20</td>
<td>0.9</td>
</tr>
<tr>
<td>H</td>
<td>25</td>
<td>75</td>
<td>3</td>
<td>1</td>
<td>34</td>
<td>—</td>
<td>84</td>
<td>2.5</td>
</tr>
<tr>
<td>I</td>
<td>17</td>
<td>16</td>
<td>2</td>
<td>0</td>
<td>8</td>
<td>7</td>
<td>51</td>
<td>6.4</td>
</tr>
<tr>
<td>J</td>
<td>15</td>
<td>90</td>
<td>6</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>46</td>
<td>5.1</td>
</tr>
<tr>
<td>K</td>
<td>11</td>
<td>51</td>
<td>16</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>73</td>
<td>6.6</td>
</tr>
<tr>
<td>L</td>
<td>5</td>
<td>109</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>28</td>
<td>81</td>
<td>7.4</td>
</tr>
</tbody>
</table>

$R_{M+G}$: Magnitude of response to 0.1 M MSG in 0.001 M 5'-GMP

$R_M$: Magnitude of response to 0.1 M MSG in deionized water

The high sensitivity of these 2 units to MSG solutions is also clearly demonstrated in Fig. 1, showing the relationships between the MSG concentration and number of impulses elicited during the initial 5 sec after stimulation for the units. As seen in this figure, the threshold for MSG in units B and C was low (about 0.001 M) in comparison with that in other units, and their responses showed high saturated magnitudes at 1–2 M. In the remaining units, the threshold ranged
from 0.001 M to 0.1 M, and the maximum response magnitude varied from one unit to another.

Enhancement in the magnitude of response after addition of a fixed amount of 5'-GMP

As already demonstrated by SATO et al. (1970), the enhancement in the magnitude of response to MSG after the addition of 5'-GMP was observed only in units responsive to sucrose, not in those predominantly responsive to NaCl. As shown in Fig. 2A, in the 3 units insensitive to sucrose (A, D, and G in Table 1) the magnitude of responses to MSG solutions of various concentrations did change slightly after the addition of 5'-GMP. In 2 units, which showed high sensitivity to MSG as well as 5'-GMP (B and C in Table 1), the enhancement of the magnitude of response to MSG by the addition of 5'-GMP was additive rather than synergistic. This is demonstrated in the concentration-response magnitude curve shown in Fig. 2B. The ratio $R_{M+G}/R_M$ ($R_{M+G}$ is the magnitude...
of response to MSG in 0.001 M 5'-GMP and \( R_s \), that for MSG alone) was not large in these 2 units compared with the ratio found in the other 7 units (Table 1).

Of the remaining 7 sucrose-sensitive units, 3 units that did not respond to 5'-GMP alone showed a marked enhancement in the response magnitude after the addition of 5'-GMP (F, J, and K in Table 1). This is clearly demonstrated in Fig. 2C.

Fig. 2. Relationships between MSG concentration and number of impulses discharged during the initial 5 sec after chemical stimulation of the tongue in 4 units before and after the addition of 0.001 M 5'-GMP to MSG solutions. A: Unit predominantly responsive to NaCl (unit A). B: Unit highly sensitive to MSG and 5'-GMP (unit B). C and D: Units highly sensitive to sucrose (units K and E, respectively). Empty circles represent the relationship for MSG alone, and filled circles that for MSG in 0.001 M 5'-GMP. Dotted lines in B and D indicate number of impulses discharged in response to 0.001 M 5'-GMP alone. In B stimulations by 5'-GMP were carried out twice.

The threshold for MSG in these units was at about 0.01–0.03 M. The addition of 5'-GMP to MSG lowered their threshold by about 1 to 2 log-units and produced a significant enhancement in the magnitude of response at all MSG concentrations, indicating the presence of synergism between the two chemicals. The synergism observed here was not a parallel shift of the magnitude toward a lower concentration along the x axis, but a general enhancement of the magnitude at all concentrations (Fig. 2C). Three sucrose-sensitive units, which were moderately responsive to both MSG and 5'-GMP (E, I, and L in Table 1), yielded concentration-response magnitude curves also showing a synergistic effect between the two.
In order to demonstrate this enhancement produced by 5'-GMP in a quantitative manner, the ratio of the magnitude of response to mixtures of MSG with 5'-GMP \((R_{M+G})\) against the sum of the magnitude of response to MSG \((R_M)\) and that to 5'-GMP \((R_G)\) in 11 units was calculated. In Fig. 3 the ratio obtained from the number of impulses during the initial dynamic phase was plotted against the MSG concentration. As indicated in this figure, the ratio in 5 units was nearly unity at MSG concentrations higher than 0.1 M, because the addition of 5'-GMP to MSG was either ineffective (A, D, and G in Table 1) or additive (B and C).

In 4 of these units (A, D, G, and C), the ratio was about 2 at 0.01 M MSG. This could be attributed either to the presence of a minute amount of synergistic action or to the effect of an increase in the amount of sodium ions resulting from added 5'-GMP, which is a disodium salt. In the remaining 6 units, the ratio ranged from 36 to 2 for 0.01 M MSG and from 62 to 2 for 0.03 M MSG, but it decreased gradually with increasing MSG concentration. When calculations of the ratio \(R_{M+G}/(R_M + R_G)\) were carried out on numbers of impulses during the steady discharge phase, essentially similar results were obtained, although the ratio was slightly higher. These results are essentially in agreement with those obtained...
Analysis of the enhancing action of 5'-GMP on the interaction between MSG and receptors

As shown above, the concentration-response magnitude curve for MSG shifted toward a higher magnitude of response in the presence of 5'-GMP. This indicates an increase in intrinsic activity of MSG to evoke the response. The method of analysis of the dose-response curves for drugs employed by Matsumoto and Kumoi (1958) and Ariëns et al. (1964) was applied to investigate the nature of the enhancing effect of 5'-GMP on MSG response. Relationships between the MSG concentration and number of impulses during the steady discharge phase in 3 units showing synergistic enhancement of response were plotted in Fig. 4: log C–R, log C–log \( \frac{R}{(R_m-R)} \), and \( \frac{1}{C} - \frac{1}{R} \), where \( R \) represents the magnitude of response to MSG of varying concentrations (C) without and with 5'-GMP, and \( R_m \) is the maximum magnitude of response obtained at high MSG concentrations. The latter two relationships could be fitted approximately with a straight line. Deviation of a few points from the straight line relationship...
in the figure may be attributed to the facts that the number of impulses discharged by a single fiber in response to a given stimulus fluctuated widely (OGAWA et al., 1969), especially when concentrations of stimuli were low, and when the stimulus concentration was high, adaptation of the response or depression occurred. Figure 4 demonstrates that after the addition of 5'-GMP R shifts toward a higher value and leftwards (A), log \([R/(Rm-R)]\) values shift toward the left side along the x axis (B), and \(1/R-1/C\) relationships intercept the y axis at a point lower than that obtained before the addition of 5'-GMP. Therefore, compared with the model by MATSUMOTO and KUMOI (1958) and ARIENS et al. (1964), the synergism shown in Fig. 4 can probably be classified as competitive and noncompetitive sensitization (see ARIENS et al., p. 297).

DISCUSSION

In agreement with the earlier reports (SATOH et al., 1970, 1971), the results in the present study have indicated that synergism between MSG and 5'-GMP occurred only in units responsive to sucrose and that those insensitive to sucrose scarcely showed any enhancement in the magnitude of response to MSG by the addition of 5'-GMP. The results have further revealed that a significant response occurred in the chorda tympani fibers to MSG solution of otherwise subthreshold concentration by the addition of 5'-GMP, which in itself did not yield any response in a majority of fibers, and that the enhancement in the magnitude of response was greater for MSG below 0.03 M, decreasing gradually with increasing MSG concentration. However, among 9 sucrose-sensitive units 2 responded massively to 5'-GMP as well as MSG. They showed a threshold for MSG as low as 0.001 M, and the addition of 5'-GMP produced an additive effect rather than a synergistic one.

In an earlier paper by SATOH et al. (1971) it was demonstrated that the concentration-response magnitude relationship for MSG obtained from the chorda tympani nerve showed a characteristic curve: The magnitude of response attains first a plateau at about 0.01–0.03 M, but it increases steeply with increasing concentration above 0.1 M, reaching a saturated value at 1–2 M (see Figs. 4 and 5 in SATOH et al., 1971). In the preceding paper this characteristic shape of the relationship was assumed to arise from summation of activities in two groups of fibers, one responsive to sucrose and the other predominantly sensitive to NaCl and not to sucrose. The concentration-response magnitude curves for MSG obtained from single units in the present study reveal that the threshold in individual units varies from 0.001 M to 0.03 M and that the response magnitude increases monotonously with increasing concentration. Consequently, the characteristic shape of the concentration-response magnitude curve for MSG obtained from the whole chorda tympani nerve could be attributed to summation of responses from different types of units with varying response properties.
Deviation from the sigmoid curve in the concentration-response magnitude curve of the whole chorda tympani nerve for mixtures of MSG of varying concentrations with a fixed amount of 5'-ribonucleotides was also reported by SATO et al. (1971), and this was especially noticeable around 0.01 M MSG (see Figs. 3, 4, and 5 in that paper). This may also be explained as the summation of activities of two groups of units, one showing a marked enhancement of response to 0.01–0.03 M MSG after the addition of 5'-GMP, the enhancement gradually decreasing in amount with increasing concentration of MSG, and the other yielding little enhancement (Fig. 3).

In an earlier paper (SATO et al., 1970), responses to MSG in units predominantly sensitive to NaCl were attributed to the effect of sodium ions on the Na+-receptor molecules, and those of units sensitive to sucrose were attributed to the formation of a complex between glutamate ions and sucrose-receptor molecules. The latter assumption was based on the following facts: (i) The magnitude of response to MSG in sucrose-sensitive units is greater than that expected from the effect by Na ions; (ii) enhancement in the response to MSG due to addition of 5'-ribonucleotides appears only in units responsive to sucrose; and (iii) application of gymnemic acid A1, which suppresses response to sugars, changes the unique taste of MSG to a taste much like NaCl (KURIHARA, 1969).

The results in the present study further indicate that the mode of action of 5'-GMP on the reaction between glutamate ions and receptor molecules appears to be a type of 'competitive and noncompetitive synergism,' that is, 5'-GMP enhances the formation of the glutamate-receptor complex by interacting with receptors different from those for glutamate ions. Such a mechanism has also been demonstrated by OZEKI and SATO (1970) in the crayfish muscle, where potentiation of excitatory junctional potentials and glutamate-induced responses occurs in the presence of various kinds of 5'-ribonucleotides.

In the present study the effects of 5'-GMP in combination with MSG were additive in 2 units, which were highly sensitive to both chemicals. The mechanism of 5'-GMP action on gustatory receptors in these receptors appears to be quite different from that discussed in the preceding paragraph.

REFERENCES


