COMPARISON OF POTENTIATING EFFECT ON GUSTATORY RESPONSE BY DISODIUM 2-METHYL MERCAPTO-5'-INOSINATE WITH THAT BY 5'-IMP

Masayasu Sato, Hisashi Ogawa and Satoru Yamashita*

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

Summary The potentiating effect of disodium 2-methyl mercapto-5'-inosinate (Mes-IMP) on gustatory responses to monosodium glutamate (MSG) was examined and compared with those of sodium 5'-inosinate (5'-IMP) and sodium 5'-guanylate (5'-GMP) by recording responses in the whole chorda tympani nerve and in single gustatory fibers in rats. Mes-IMP had a lower threshold for response than 5'-IMP. Addition of 0.0001% Mes-IMP to 0.3% MSG yielded significant enhancement in the response. The enhancement produced by Mes-IMP was nearly equal to that shown by 5'-IMP of an amount ten times greater. The enhancement was predominantly observed in fibers responsive to sucrose. The magnitude of the enhancement, produced by the addition of a fixed amount of Mes-IMP to MSG of varying concentrations, was greater at MSG concentrations below 0.03 M than at higher concentrations.

Since the strong synergistic effect between monosodium glutamate and 5'-ribonucleotides such as sodium 5'-inosinate (5'-IMP) and sodium 5'-guanylate (5'-GMP) was demonstrated by Kuninaka (1960), results of experiments dealing with the potentiating effect of 5'-ribonucleotides on the chorda tympani response to MSG in rats have been reported by Sato and Akaike (1965); Sato and Yamashita (1965); and Sato et al. (1970). These experiments revealed that the addition of a minute amount of 5'-GMP or 5'-IMP to MSG yielded a marked enhancement of the magnitude of the chorda tympani response in rats, 5'-GMP showing a stronger potentiating effect than 5'-IMP. The existence of such a synergistic effect between MSG and various derivatives of 5'-GMP or 5'-IMP was also investigated by Yamaguchi et al. (1968), using psychophysical methods, and by Kasahara et al. (1970) with neurophysiological techniques. Among the various substances examined by Yamaguchi et al. (1968), disodium 2-methylmercapto-
5'-inosinate (Mes-IMP) has been shown to possess a low threshold (0.0025%) in comparison with 5'-IMP (0.025%), and to have such a strong synergistic effect when added to MSG that its potentiating effect is similar to that of a ten times greater amount of 5'-IMP.

In the present experiment, the effect of combined Mes-IMP and MSG on the chorda tympani response in rats was compared with that of MSG and 5'-IMP or 5'-GMP.

MATERIALS AND METHODS

Female albino rats of Sprague-Dawley stock, weighing 100–250 g, were used in the experiments. They were anesthetized by intraperitoneal injection of sodium amobarbital (70 mg/kg body weight). The chorda tympani was surgically exposed at the submandibular region and centrally cut.

Responses of the chorda tympani to gustatory stimuli were monitored by an oscilloscope and recorded with a pen-writing recorder, using a pair of Ag-AgCl electrodes, an amplifier, and an integrator circuit. The magnitude of response 10 sec after stimulation was measured and used as an index for quantitative comparison. For obtaining impulse discharges in single fibers in response to gustatory stimuli, the chorda tympani was dissected with a pair of needles. Spikes of uniform size were the criterion for single unit response. Impulse discharges in response to gustatory stimuli were recorded with a kymograph camera.

For stimulation of gustatory receptors, the tongue was enclosed in a flow chamber made of glass, through which a fixed amount of test solution was passed at a constant rate. The tongue was rinsed with water after stimulation. In order to avoid the effect of the preceding stimulus, the interval between successive stimulations was made about 3 min. In order to avoid thermal responses, the temperature of both test solution and water was kept nearly the same as that of the surface of the tongue.

The following test solutions were employed for the experiments on the whole chorda tympani response: (1) 0.3% (0.0177 M) MSG, 0.3% MSG in 0.0003–0.01% Mes-IMP, and 0.3% MSG in 0.003–0.1% 5'-IMP or 5'-GMP. (2) 0.0003–1.0 M MSG, to which a given amount of Mes-IMP, 5'-IMP or 5'-GMP was added. For the experiments on single fibers, 0.5 M sucrose, 0.1 M NaCl, 0.01 N HCl, and 0.02 M quinine hydrochloride were employed first in order to determine their response characteristics. Then, 0.3% MSG, a part of which had been replaced by 5'-IMP, 5'-GMP, or Mes-IMP, was used as a test stimuli.

Mes-IMP, 5'-IMP, and 5'-GMP were kindly supplied by Takeda Chemical Industries, Ltd.
RESULTS

Potentiation of chorda tympani response by addition of Mes-IMP, 5'-IMP, and 5'-GMP to MSG

As reported by SATO and AKAIKE (1965), a marked enhancement of the chorda tympani response to MSG was obtained by the addition of a small amount of 5'-IMP or 5'-GMP to MSG, but the addition of Mes-IMP produced an even greater enhancement than either 5'-IMP or 5'-GMP. An example of enhanced responses in the chorda tympani is shown in Fig. 1, where the effects of the addition of 0.0001 or 0.001% Mes-IMP to 0.3% MSG are compared with those produced by the addition of 0.001 or 0.01% 5'-GMP and 5'-IMP. As seen in this figure, 0.3% MSG in 0.0001% Mes-IMP produced an increased response similar to that produced by 0.3% MSG in 0.001% 5'-GMP or 5'-IMP, and 0.3% MSG in 0.001% Mes-IMP enhanced the response magnitude to an extent comparable to that produced by 0.3% MSG in 0.01% 5'-GMP or 5'-IMP.

Fig. 1. Enhancement of the chorda tympani response to MSG by addition of Mes-IMP, 5'-IMP, and 5'-GMP. Test stimuli: 0.3% MSG, 0.3% MSG in 0.0001% Mes-IMP, 0.3% MSG in 0.001% 5'-IMP, 0.3% MSG in 0.001% 5'-GMP, and 0.0001% Mes-IMP (from left to right in upper row); 0.3% MSG, 0.3% MSG in 0.001% Mes-IMP, 0.3% MSG in 0.01% 5'-IMP, 0.3% MSG in 0.01% 5'-GMP, and 0.001% Mes-IMP (from left to right in lower row).

The results of four experiments are summarized in Fig. 2, where the magnitude of responses to mixtures of 0.3% MSG with ribonucleotides of several concentrations is expressed relative to that for 0.3% MSG alone. As indicated in the figure, addition of 0.001% 5'-IMP and 5'-GMP to 0.3% MSG yielded relative response magnitudes of 1.3 and 1.8, respectively, and the relative response magnitude increased gradually with increases in the amount of 5'-IMP and 5'-GMP (empty and filled circles in the figure). Enhancement of the response attained a nearly saturated value of 4.5 when 0.1% 5'-IMP and 5'-GMP were added to MSG. However, significant enhancement was obtained by the addition of 0.0001% Mes-
IMP to MSG, which is only 1/3,000 of the amount of MSG and which by itself does not produce any response in the chorda tympani. The relative response magnitudes obtained by the addition to 0.3% MSG of 0.0001, 0.0003, 0.001, 0.003, and 0.01% Mes-IMP were 1.4, 2.1, 3.0, 3.9, and 4.6 respectively (crosses in Fig. 2), while the relative response magnitudes produced by 0.0003, 0.001, 0.003, and 0.01% Mes-IMP were 0, 0.1, 0.25 and 0.6 (triangles). These results indicate the existence of a marked synergism between Mes-IMP and MSG.

The ratios of the amount of 5'-IMP, 5'-GMP, and Mes-IMP that had to be added to 0.3% MSG to enhance the response magnitude to two, three, and four times the original level were 0.0027:0.0014:0.00026, 0.0082:0.0044:0.00095, and 0.0305:0.0145:0.0035, respectively. Therefore, the average ratio of the amount of 5'-IMP, 5'-GMP, and Mes-IMP necessary for producing an equal potentiating magnitude of response is approximately 9.2:4.7:1. This indicates that Mes-IMP possesses a potentiating effect similar to that produced by a ten times greater amount of 5'-IMP.

Fig. 2. Relationship between the magnitude of chorda tympani response and the concentration of ribonucleotides in 0.3% MSG. In the figure, relative magnitudes of responses produced by Mes-IMP in 0.3% MSG (×), by 5'-GMP in 0.3% MSG (○), by 5'-IMP in 0.3% MSG (○), and by Mes-IMP alone (△) are plotted as ordinates against concentrations of the three kinds of ribonucleotides as abscissae.

Potentiation of chorda tympani response by addition of a given amount of Mes-IMP, 5'-IMP, and 5'-GMP to MSG of varying concentrations

Figure 3 indicates relationships between response magnitude and MSG concentration when 0.0001 M Mes-IMP, 5'-IMP, or 5'-GMP was added to varying MSG concentrations. Addition of any of the three kinds of ribonucleotides
markedly lowered the threshold for neural response and significantly enhanced the magnitude of response at nearly all MSG concentrations. The enhancement is stronger at MSG concentrations below 0.03 M than at the concentrations above it. The ability to enhance the response is on the order of Mes-IMP > 5'-GMP > 5'-IMP.

Figures 4 and 5 illustrate the potentiating effect of addition of Mes-IMP and 5'-IMP of two different concentrations. In these figures, the response magnitude-concentration relationship for MSG shows a characteristic pattern, i.e., the magnitude reaches a step at 0.03 M and then increases steeply with the increase in concentration, attaining a saturated value at about 1 M. Addition to MSG of Mes-IMP in as low a concentration as 0.00001 M lowered the threshold and enhanced the response magnitude. Enhancement of the response is marked at MSG concentrations below 0.03 M compared with that occurring at concentrations above it. In Fig. 4, the relative response magnitudes obtained by the addition of 0.00001 M Mes-IMP to 0.003, 0.01, 0.03, 0.1, 0.3, and 1 M MSG were 0.37, 0.89, 1.30, 1.97, 3.24, and 3.55, respectively, while, as shown in Fig. 5, those attained by the addition of 0.0001 M and 0.001 M 5'-IMP to similar concentrations of MSG were 0.31 and 0.71, 0.60 and 1.03, 0.89 and 1.32, 1.31 and 1.80, 2.21 and 2.59, and 2.90 and 3.09, respectively. Although these data were obtained from two different sets of experiments and cannot be accurately compared with each
Fig. 4. Relationship between the relative magnitude of chorda tympani responses and MSG concentration. Crosses indicate the dose-response curve for MSG. Filled circles represent the response magnitude obtained when 0.00001 M Mes-IMP was added to MSG, while empty circles represent that obtained by addition of 0.0001 M Mes-IMP.

other, the addition of 0.00001 M Mes-IMP seems to yield a greater response magnitude than that produced by the addition of 0.0001 M 5'-IMP and a response magnitude nearly equal to that produced by 0.001 M 5'-IMP.

Comparison of the potentiating effect of Mes-IMP with that of 5'-IMP can be made more quantitatively in Fig. 6, where the ratio of the response produced by the mixture of MSG and the ribonucleotide \((R_{M+N})\) to the sum of responses induced by MSG \((R_M)\) and by ribonucleotide \((R_N)\) is plotted against MSG concentration. The potentiation ratio of Mes-IMP shown at the top in Fig. 6 is 2.5–3.0 at MSG concentrations ranging from 0.003 M to 0.03 M and is decreased gradually with an increase in concentration, reaching unity at approximately 1 M. On the other hand, the potentiation ratio of 5'-IMP is 1.5–2.5 at MSG concentrations between 0.003 M and 0.03 M and is decreased with increasing concentration, reaching unity at 0.3 M. Therefore, the figure indicates further that Mes-IMP possesses a stronger MSG response potentiating action than 5'-IMP.

Potentiation of response observed in single chorda tympani fibers

Impulse discharges in response to MSG alone, as well as mixtures of MSG with one of the ribonucleotides, were recorded successfully in nine single chorda tympani fibers. Response profiles of these nine units are illustrated in Fig. 7,
in which fibers are arranged in order of responsiveness to 0.5 M sucrose. Among
the nine units, five responded well to sucrose, while the remaining four scarcely
responded to sucrose but yielded responses to NaCl alone or NaCl, HCl, and
quinine. As seen in the figure and in Table 1, there is a significant difference
between the two groups of units. In general, the former responded well to 0.3%
MSG and showed a marked enhancement of response magnitude by replacement
of one-tenth of the MSG with the ribonucleotide, while the latter responded poorly
to 0.3% MSG and showed little enhancement. This is in agreement with the re-
sults reported by SATO et al. (1970) on the potentiation produced by 5'-GMP and
5'-IMP. The enhancement of the response to MSG produced by addition of
the ribonucleotide was more marked during the steady phase of the impulse dis-
charge than during the initial phasic response, as indicated in Fig. 8, where the
average number of impulses discharged every second is plotted against time after
the onset of response. In this figure, it is also clearly demonstrated that no signifi-
cant enhancement of the response magnitude was produced by replacement of
one-hundredth or one-tenth of the MSG by the ribonucleotides in the units insen-
sitive to sucrose, whereas in those responsive to sucrose, the number of im-
pulses discharged during the steady phase of the response was augmented four to
five times. As seen in Figs. 7 and 8, the magnitude of response to 0.297% MSG
in 0.003% Mes-IMP during the steady phase is nearly the same as that for 0.27%
MSG in 0.03% 5'-IMP. Therefore, these results further indicate that the poten-
Fig. 6. Magnitudes of potentiation produced in the chorda tympani response by addition of a given amount of Mes-IMP (upper figure) and of 5'-IMP (lower figure) to MSG of varying concentrations. Ordinate: ratio of the magnitude of response to a mixture of MSG with ribonucleotide \( (R_{M+R}) \) to the sum of the magnitude of response to MSG alone \( (R_M) \) and to that for ribonucleotide alone \( (R_R) \). Abscissa: MSG concentration. The ratios in the figure were calculated from the data shown in Figs. 4 and 5. Symbols used in the upper figure correspond to those in Fig. 4, while symbols in the lower figure are the same as those in Fig. 5.

Fig. 7. Response profiles of nine chorda tympani fibers for six kinds of test stimuli. Fibers were arranged in the order of responsiveness to 0.5 M sucrose in the abscissa \((A, B, \ldots, I)\), while the ordinate gives the number of impulses discharged in the first 5 sec after stimulation. Test solutions employed are indicated above each profile.
Fig. 8. Enhanced responses to MSG by addition of Mes-IMP and 5'-IMP in five fibers responsive to sucrose (top), in four fibers insensitive to sucrose (middle), and in all nine fibers (bottom). Average number of impulses discharged in each second was plotted successively against time. ×, 0.3% MSG; ○, 0.27% MSG in 0.03% 5'-IMP; ●, 0.297% MSG in 0.003% Mes-IMP; +, 0.27% MSG in 0.03% Mes-IMP.

Table 1. Enhancement of the MSG response by 5'-GMP, 5'-IMP, and Mes-IMP. Numerals outside and inside parentheses indicate, respectively, the average number of impulses discharged in the first 5 sec after stimulation and in the next 5 sec.

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<th>0.1 M NaCl</th>
<th>0.5 M sucrose</th>
<th>0.3% MSG</th>
<th>0.03% 5'-GMP</th>
<th>0.27% MSG</th>
<th>0.27% 5'-IMP</th>
<th>0.297% MSG</th>
<th>0.297% 5'-IMP</th>
<th>Mes-IMP</th>
<th>0.003% MSG</th>
<th>0.003% Mes-IMP</th>
<th>0.03% MSG</th>
<th>0.03% Mes-IMP</th>
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<td>(45.4)</td>
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<td>(45.6)</td>
<td>(6.2)</td>
<td>(24.4)</td>
<td>(27.8)</td>
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<td>(9.3)</td>
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<td>Units insensitive</td>
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<td>(3.8)</td>
<td>(3.0)</td>
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<td>to sucrose (n=4)</td>
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<td>(1.0)</td>
<td>(1.8)</td>
<td>(2.0)</td>
<td>(3.8)</td>
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<td>Average of 9 units</td>
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<td>(13.6)</td>
<td>(25.5)</td>
<td>(27.9)</td>
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tiating effect of Mes-IMP on MSG response is nearly equal to that of 5'-IMP of an amount ten times greater.

DISCUSSION

The results of the present experiments, demonstrating that Mes-IMP possesses a very marked potentiating effect, confirmed the results of the psychophysical experiments by Yamaguchi et al. (1968). The threshold concentration of Mes-IMP for eliciting chorda tympani response was found to lie at about 0.0003% (Fig. 2), in comparison with 0.001% for 5'-IMP (Sato and Akaike, 1965). The magnitude of response produced by the addition of Mes-IMP to MSG is nearly equal to that elicited by the addition of a ten times greater amount of 5'-IMP. This was further confirmed by the experiments analyzing responses of single chorda tympani fibers. Replacement of one-hundredth of the MSG by Mes-IMP yielded nearly the same magnitude of enhancement as that produced by substituting one-tenth of the MSG with 5'-IMP. Analysis of single nerve fiber responses also indicated that the enhancement of MSG response by Mes-IMP occurs only in fibers responsive to sucrose. Those that do not possess sensitivity to sucrose do not show enhancement of MSG response by Mes-IMP. This fact is in agreement with the results reported earlier (Sato et al., 1970).

It has been found that the relationship between response magnitude and MSG concentration shows a characteristic curve, the magnitude increasing gradually up to 0.03 M and rising steeply from 0.03 M to 1.0 M. The characteristic shape of the curve may be explained by the assumption that the chorda tympani nerve consists of two groups of fibers having different response characteristics for MSG. As demonstrated by Sato et al. (1970), and also in the present experiment, a marked difference in sensitivity to 0.3% MSG exists between units responsive to sucrose and those sensitive to NaCl but not sucrose. In addition, Sato et al. (1970) proposed that, when gustatory receptors are stimulated by MSG, units responsive to sucrose give rise to impulses in response to glutamate ions, while those sensitive to NaCl respond to Na ions. Based on this idea, one may assume that units responsive to sucrose are predominantly stimulated by MSG at concentrations below 0.03 M, whereas responses in units sensitive to NaCl contribute to a steep increase in the response magnitude for MSG above 0.03 M. This assumption may be supported by the fact that the enhancement in the magnitude of response to MSG by the addition of 5'-GMP, 5'-IMP, and Mes-IMP is more marked at MSG concentrations below 0.03 M.

REFERENCES


