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ORIGINAL

Taste effectiveness of glutamate compounds with different cations in rat

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Abstract : A role of a cation of glutamate compounds for their taste has not been well elucidated yet. In this experiment, the difference of taste quality of glutamate compounds which have a different cation was compared to reveal the role of cations in the glutamate compounds on their characteristics of taste. Monosodium L-glutamate (MSG), monopotassium glutamate (MPG) and monocalcium glutamate (MCG) were used. The preference test in rats revealed that the rate of drinking volume per day was not statistically different among MSG, MPG and MCG. In the next experiment, the conditioned taste aversion test was performed in rats, and rats were conditioned to hate to drink MSG, MPG and MCG respectively by pairing the intraperitoneal injection of LiCl. In the MSG conditioned animals, the drinking rate of MSG decreased, and that of MPG significantly increased, but that of MCG did not change. In the MPG conditioned animals, drinking rate of MPG was strongly depressed, but that of MCG tended to increase, and that of MSG did not change at all. In the MCG conditioned animals, the drinking rate of MCG decreased, but MSG and MPG did not change. The chorda tympani responses to 0.1 M MSG applied to the tongue surface was larger than those to 0.1 M MCG, and the responses to 0.1 M MPG were the smallest out of these three glutamate compounds. The nerve responses of the glossopharyngeal nerve to MSG, MCG and MPG were very similar to each other. These results suggest that rats may discriminate the taste of MSG, MCG and MPG mainly through the taste information via the chorda tympani nerve which innervates the frontal parts of the tongue. The cation of glutamate compounds play an important role in taste discrimination.

Intoroduction

Generally, anion and/or cation of a chemical compound have been known to play an important part of its taste. Hiji[1] reported on role of the anion for the taste of sodium salts, by the response of the chorda tympani nerve in rats. Nerve responses to various salt solutions were evaluated by Beidler[2] and Fishman[3] through the chorda tympani nerve responses, and by Ogawa[4] through the glossopharyngeal nerve responses in rats. However, a role of cations for the taste quality of glutamate, one of the well known flavor enhancers, has not been well studied yet.

In this experiment, in order to elucidate the role of the cations of glutamate compounds on its taste, the taste of three different glutamates compounds, that is, monosodium L-glutamate (MSG), monopotassium L-glutamate (MPG) and monocalcium di-L-glutamate (MCG), was compared and evaluated through the three-bottle preference test in rats. The conditioned taste aversion test and recording of chorda tympani nerve and glossopharyngeal nerve responses to the test taste solutions were also analysed in rats.

Materials and Method

1. Behavioral preference test

The three bottle preference test was performed in free choice situations in eight male Wistar albino rats, weighing 230-310 g. The animals took food ad libitum throughout the experiment. The drinking volumes of 0.1 M MSG, 0.1 M MPG and 0.1 M MCG were measured everday at 10 : 00 a.m. for 8 days. To prevent the development of position preferences, the position of the water bottles was changed on a random basis from day to day.

2. Conditioned taste aversion test

At the conditioned taste aversion test, twenty-eight mice Wistar rats (250-300 g) were used. Rats were conditioned to avoid drinking MSG (N=8), MPG (N=5) and MCG (N=7) respectively by pairing the intraperitoneal injection of 0.15 M LiCl (2 % of body weight). Rats were exposed to glutamate solutions for 30 min preceding LiCl injections. Using a cafeteria method, the drinking volumes of these three liquids were measured, respectively. The cafeteria method means that the rat may have access to drink these 3 glutamate solutions freely. The rats (N=8) with distilled water as a conditioning liquid, provided the standard. Responses of the rats to glutamate solutions were tested for 5 days after LiCl injections. The drinking volume of 0.1 M MSG, 0.1 M MPG and 0.1 M MCG were measured everyday at 10 : 00 a.m.. The position of the bottles was changed everyday at random. The suppression of the drinking of a test liquid, was expressed as a suppression ratio according to the formula[5].

Suppression ratio = 1 − (drinking rate / E)/(drinking rate/C)

When E is for the experimental group, which received sapid solutions as the conditioning stimuli, and C is for the control group in which distilled water was a conditioning stimulus. Suppression ratio multiplied by 100 gives percent suppression ratio.

3. Neurophysiological test

Male Wistar rats (N=27) were deeply anesthetized by intraperitoneal injection of sodium pentobarbital and the trachea was cannulated. The chorda tympani or glossopharyngeal nerve was dissected free from the adjacent connective tissue, and the electrical activities from multi fibers or functionally single fibers, were recorded when a test taste solution was applied to the tongue surface at room temperature 24±2°C. Each stimulation was about 3 m*l* of a test solution passed by gravity flow (10 m*l*/s) from a burette held approximately 5 mm above the center of the exposed tongue surface and was followed by a distilled water rinse. Electrical activities were recorded by a Nihon-Kohden AB632J differential amplifier monitored by CRO (Nihon Kohden VT 612J). Multi fiber activities were discriminated by their amplitudes (100-500 μV, S/N ratio 1.5-10.0), and the spike numbers beyond a certain level of amplitudes were counted using a Nihon Kohden FN601 J window slicer and a Nihon Kohden ET612 J pulse counter. The number of nerve impulses was counted per 1-sec for a pre-stimulus period of 10 sec and post-stimulus period of 10 sec. When the magnitude of the mean response per second in a unit to a stimulus exceeded the 1 sec spontaneous rate±SD of this unit in more than half of the 10 sec post-stimulus rates, the units were considered to be responsive to a given stimulus[6]. As the control liquids, 1 M sucrose, 0.1 M NaCl, 0.1 M tartaric acid, 0.001 M quinine-HCl, 0.1 M MSG, 0.1 M MPG, 0.1 M MCG were used.

Results

1. Behavioral preference test

As shown in Fig. 1 the rates of drinking volumes of 0.1 M MSG, 0.1 M MPG and 0.1 M MCG which have different cations were 38.6±3.5 % (mean±SE), 32.8±2.5 % and 28.6±2.2 % respectively, in the three bottle preference test for 8 days. The daily mean intakes of MSG, MPG and MCG were 24.9 m*l*, 21.1 m*l* and 17.4 m*l*, respectively, and the rates of drinking volumes were not so different statistically among MSG, MPG and MCG.

2. Conditioned taste aversion test

The result of taste aversion was shown in Fig. 2. Under the conditions to avoid MSG, the rate of drinking volume decreased by 77.2 % compared with the control ($p<0.01$). However, the rate of drink of MPG highly increased by 55.8 % ($p<0.05$) and the rate of MCG was unchanged. In the case of those conditioned to MPG, the drinking rate of MPG decreased by 34.9 % ($p<0.1$), but the

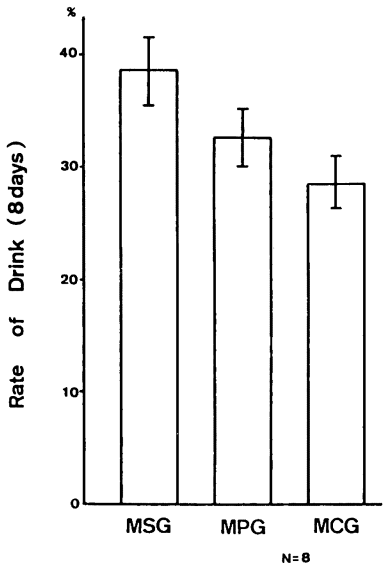

Fig. 1 The rate of drinking volumes among 0.1M MSG, 0.1M MPG and 0.1M MCG for eight days in case of the preference test. Values are mean±SE of eight rats. The rates of MSG, MPG and MCG were not different statistically.

drinking rate of MCG tended to increase, and that of MSG did not change statistically. Under conditioning of MCG, the rate of drinking volume of MCG decreased by 57.9 % ($p < 0.1$), but the drinking rates of MSG and MPG were almost unchanged.

3. Neurophysiological test

The response of the chorda tympani multi fibers to 0.1 M NaCl applied to the tongue surface was the largest of all used taste stimuli. Responses of chorda tympani multi fibers to other taste solutions compared with those of 0.1 M NaCl (Fig. 3). Response rates to 0.1 M tartaric acid, 1 M sucrose, 0.001 M quinine-HCl compared with that of 0.1 M NaCl were 74.0 %, 11.8 % and 18.6 % respectively. The rate of 0.1 M MSG was 39.9 % and it was larger than 0.1 M MCG of 19.6 %. The 0.1 M MPG response which was the smallest of the three glutamate compounds was 10.9 %. In addition, to examine the effect of different anions on taste, the responses of the chorda tympani nerve to sodium salts, calcium salts and potassium salts were compared. The response magnitude was in order of 0.1 M NaCl > 0.1 M $NaNO_3$ > 0.1 M MSG, in order of 0.1 M $CaCl_2$ > 0.1 M $Ca(NO_3)_2$ > 0.1 M MCG, and 0.1 M KCl > 0.1 M KNO_3 > 0.1 M MPG. The lower concentrations of MSG, MCG and MPG induced the smaller responses. The magnitudes of responses to MSG, MCG and MPG were dependent on their concentration.

As shown in Fig. 4, the NaCl best single fiber in the chorda tympani nerve showed higher responses to MSG than those of MCG and MPG. As shown in Fig. 5 and Fig. 6, the responses of sucrose best fiber and of quinine-HCl best fiber to MSG, MCG and MPG

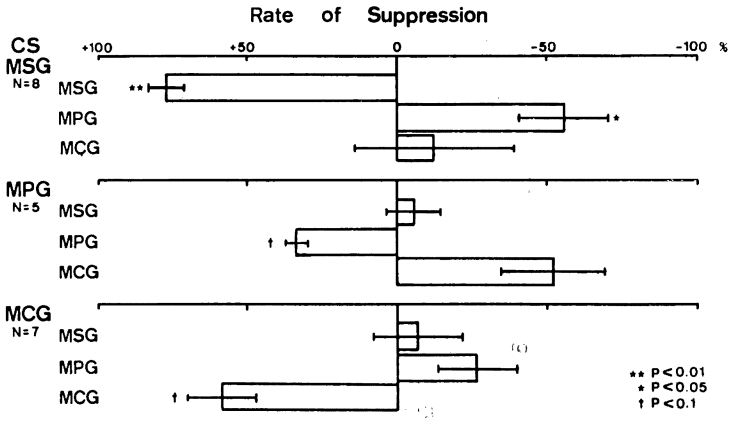

Fig. 2 The suppression ratio of drinks using conditioned taste aversion test. Values are mean±SE. In case of conditioned to MSG, the rate of MPG highly increased, but that of MCG was not changed.

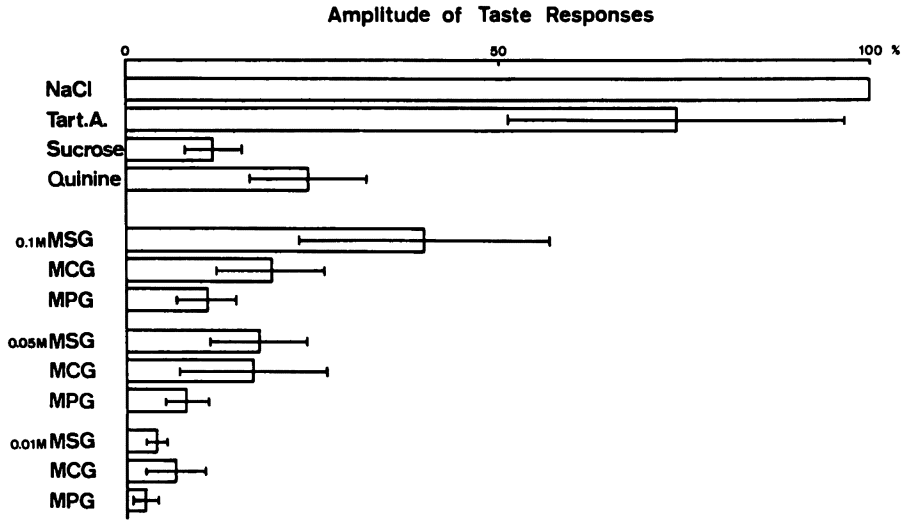

Fig. 3 The relative amplitude response to each liquid in the chorda tympani multi fibers compared with that to 0.1M NaCl. Values are mean±SE of 15 rats. The rate of MSG was higher than that of MCG, followed by MPG. As the control liquids, 1 M sucrose, 0.1 M NaCl, 0.1 M tartaric acid and 0.001 M quinine-HCl were used.

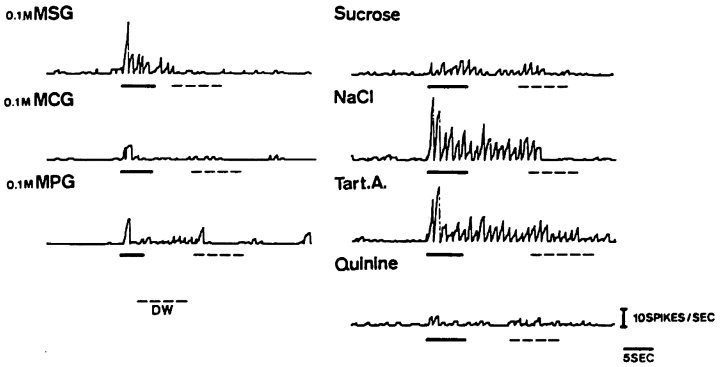

Fig. 4 The response of NaCl best fiber of chorda tympani nerve. The magnitude of response to MSG was higher than those to MCG and MPG. As the control liquids, 1M sucrose, 0.1M NaCl, 0.1M tartaric acid and 0.001M quinine-HCl were used.

solutions were small.

The responses of the glossopharyngeal nerve to MSG, MCG and MPG were rather small in comparison with those of the chorda tympani, and they were similar each other.

Discussion

In this experiment, a statistically significant difference was not recognized in drinking volumes of MSG, MPG and MCG at the behavioral preference test of rats. This fact suggests that the taste effect of MSG, MPG and MCG in preference is similar in rats, and they like the taste of these glutamate solutions. Ohara and Naim (1977)[7] and Ohara *et al.* (1979)[8] reported that the solutions conitaning MSG were preferred over deionized water in rat. MSG may be recognized as a favorable solution.

Using conditioned taste aversion test, rats

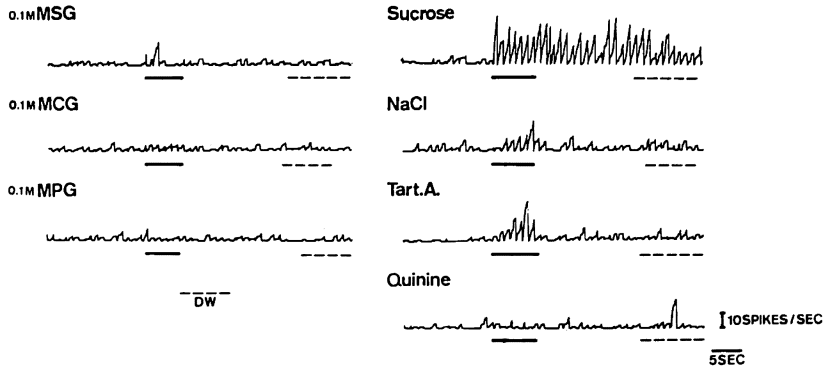

Fig. 5 The response of sucrose best fiber of chorda tympani nerve. As the control liquids, 1M sucrose, 0.1M NaCl, 0.1M tartaric acid and 0.001M quinine-HCl were used.

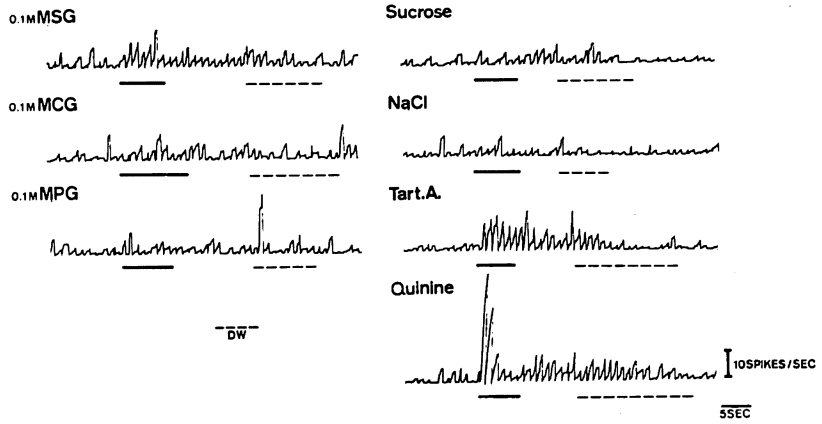

Fig. 6 The response of quinine best fiber of chorda tympani nerve. As the control liquids, 1M sucrose, 0.1M NaCl, 0.1M tartaric acid and 0.001M quinine-HCl were used.

were found to be able to discriminate these substances. MSG, MCG and MPG may have different taste effects.

The response of the chorda tympani nerve to NaCl was the largest within the used taste liquids. The response of the nerve to MSG was larger than those to MCG and MPG. Response of the chorda tympani was larger in MCG than in MPG. These results suggest that the information about the difference of taste among MSG, MCG and MPG might be mainly transmitted through the chorda tympani nerve.

The difference of responsiveness to MSG, MCG and MPG in taste aversion test might imply the taste difference of glutamate compounds with different cations. However, the response magnitude was in the order of $NaCl > NaNO_3 > MSG$, in order of $CaCl_2 > Ca(NO_3)_2 > MCG$, and $KCl > KNO_3 > MPG$, and this result is the same as reported by Hiji[1]. The total responsiveness to MSG, MCG and MPG might be related not only with cation but also with anion.

In NaCl best fiber of chorda tympani nerve, the responses to these umami substanses was also recognized. The same data has been reported in mice[9] and hamster[10].

Sato *et al.*[11] reported that a highly significant correlation was observed between the

magnitude of response to the mixture MSG with 5'-ribonucleotide and that for sucrose in the chorda tympani nerve fibers in the rat. However, they also found that a highly significant correlation exists between the amount of response to 0.1 % NaCl and that for 0.3 % MSG.

Yamamoto *et al.* (1988)[10] reported that no responses were observed to 0.3 M MSG, and 0.3 M IMP in hamster glossopharyngeal nerve. However Ogawa (1972)[4] in rats, and Ninomiya and Funakoshi (1987)[9] in mice, recognized the responses in the glossopharyngeal nerve to MSG. In this experiment, the responses of the glossopharyngeal nerve to MSG, MCG and MPG was rather small, however, taste information of umami substances such as MSG might be sent through the glossopharyngeal nerve as well as the chorda tympani nerve.

Acknowledgement

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抄録：グルタミン酸化合物の味覚効果に対する陽イオンの役割を明らかにするために，グルタミン酸ナトリウム（MSG），グルタミン酸カリウム（MPG），グルタミン酸カルシウム（MCG）の味覚特性をラットを用いて比較した。嗜好テストでは MSG, MPG, MCG の3者に有意な差は見られなかった。MSG を条件刺激で味覚性嫌悪条件づけを行うと，MPG の飲水量が対照群に比べ有意に増加し，MCG の飲水量に変化はなかった。MPG で条件づけを行うと，MPG の飲水量は減少し，MCG の飲水量は増加する傾向があり，MSG の飲水量に有意な変化は認められなかった。MCG で条件づけを行うと MCG の飲水量は減少し，MSG, MPG の飲水量に変化はなかった。各溶液に対する鼓索神経の反応では，MSG, MCG, MPG の順に小さくなった。舌咽神経の反応は3者とも小さかったが，反応の大きさにほとんど差はみられなかった。これらの結果より，MSG, MCG, MPG に対するラットの嗜好性に相違はなく，少なくとも鼓索神経を介して3者を識別していることが示唆された。

References

1) Hiji, Y.: Preference for and neural gustatory response to sodium salts in rats. Kumamoto Med. J. **20**: 129-138, 1967.
2) Beidler, L. M.: Properties of chemreceptors of tongue of rat. J. Neurophysiol. **16**: 595-607, 1953.
3) Fishman, I. Y.: Single fiber gustatory impulses in rat and hamster. J. Cell Comp. Physiol. **49**: 319-334, 1957.
4) Ogawa, H.: Taste response characteristics in the glossopharyngeal nerve of the rat. Kumamoto Med. J. **25**: 137-147, 1972.
5) Yamamoto, T., Yuyama, N., Kato, Y. and Kawamura, Y.: Gustatory responses of cortical neurons in rats. III. Neural and behavioral measures compared. J. Neurophysiol. **53**: 1370-1386, 1985.
6) Yamamoto, T., Yuyama, N., Kato, T. and Kawamura, Y.: Gustatory responses of cortical neurons in rats. I. Response characteristics. J. Neurophysiol. **53**: 616-635, 1985.
7) Ohara, I. and Naim, M.: Effects of monosodium glutamate on eating and drinking behavior in rats. Physiol. Behav. **19**: 627-634, 1977.
8) Ohara, I., Tanaka, Y. and Otsuka, S.: Discrimination of monosodium glutamate and sodium chloride solutions by rats. Physiol. Behav. **22**: 877-882, 1979.
9) Ninomiya, Y. and Funakoshi, M.: Qualitative discrimination among "umami" and the four basic taste substances in mice. In: Umami: A Basic Taste. Y. Kawamura and M.R. Kare (Eds). New York: Marcel Dekker, pp.365-385, 1987.
10) Yamamoto, T., Matsuo, R., Kiyomitsu, Y. and Kitamura, R.: Taste effects of "umami" substances in hamsters as studied by electrophysiological and conditioned taste aversion techniques. Brain Res. **451**: 147-162, 1988.
11) Sato, M., Yamashita, S. and Ogawa, H.: Potentiation of gustatory response to monosodium glutamate in rat chorda tympani fibers by addition of 5'-ribonucleotides. Jpn. J. Physiol. **20**: 444-464, 1970.