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Behavioral Suppression Induced by Oral Administration of Monosodium L-glutamate in Rats

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TADOKORO, S., Y. HIGUCHI, H. KURIBARA AND K. OKUIZUMI. *Behavioral suppression induced by oral administration of monosodium L-glutamate.* PHARMAC. BIOCHEM. BEHAV. 2(5) 619–625, 1974. – Adult male rats, trained under a fixed ratio 30 schedule of food reinforcement, were observed for behavioral changes after oral administration of monosodium L-glutamate (MSG). Following the administration of more than 0.5 g/kg of MSG, transient suppression was observed as a function of dose level. Thus, responses were inhibited immediately after MSG, attained the minimum at 20–25 min, and then abruptly returned to the initial level. When oral MSG 2.0 g/kg was concurrently accompanied by subcutaneous diazepam 0.25–1.0 mg/kg or oral caffeine 5–20 mg/kg, the MSG-induced suppression was markedly modified. Thus, the several minutes suppression observed immediately after MSG was significantly antagonized by these drugs. However, the suppression observed at 15–30 min was significantly enhanced by diazepam in proportion with the dose, and markedly antagonized by caffeine. There were indications that MSG's central action might be involved in the development of behavioral suppression induced by it. The exact nature of the toxic hazards of MSG remain obscure, but nevertheless present indications are that one should refrain from adding a large dose of MSG to food.

Monosodium L-glutamate Behavioral suppression Diazepam Caffeine Synergism and antagonism

MONOSODIUM L-glutamate (MSG) is a food additive and flavor enhancer, which is frequently and extensively used in daily diets. Recently it has been employed by food makers evidently for the purpose of increasing the weight of foods such as *kobumaki* (Japanese food made of sea weed), pickled vegetables and instant foods, since it can now be obtained at low wholesale prices. It is said that as much as 20–45% of MSG is occasionally added to food [16]. As a consequence, systematic studies on the massive intake of MSG from the pharmacological and toxicological viewpoints are required. There are many reports on central nervous toxic effects produced by large doses of MSG [3, 5, 6, 18, 19, 20, 21, 22, 25]. There are, however, divergent opinions concerning the toxicity of MSG and, moreover, the work cited above was concerned with neonatal or infant animals, mostly from the morphological viewpoint, and rarely from the functional.

It has recently been pointed out that the so-called Chinese restaurant syndrome, which frequently occurs after taking Chinese food, with a burning sensation and facial pressure as the chief symptoms, may be ascribed to a large amount of MSG contained in the food [16,28].

In the present work, a relatively large dose of MSG was given orally to mature rats, and a characteristic transient behavioral suppression was manifested.

METHOD

Animals

Experimental animals were Wistar strain, adult, male rats which were inbred for more than 20 years by brother-sister mating in the Department of Pharmacology. They were divided into 4 groups of 6 animals each. Their ages ranged from 120–150 days. Body weights were decreased to 85–80% by food deprivation when free-feeding weights had attained 300–350 g. They were trained in a Skinner-box for a 1 hr session each day, and after 20–30 sessions, a stable behavioral baseline was established. Namely, they had learned to obtain a food pellet by pressing the lever under the fixed ratio 30 schedule (FR 30). Two groups (N = 12) selected at random from the four were used for each experiment.

Procedure

Monosodium L-glutamate was a pure, crystaline powder (Ajinomoto Co.). According to the dose to be given, a 1.25–20% aqueous solution was prepared, and the volume of the given solution was fixed at 10 ml/kg, which was given orally by gastric catheter immediately before placing the animal into the box. On repeating the MSG adminis-

tration, the responses during the non-treatment were always observed for more than 5 sessions before and after the administration, as a check on baseline stability. Moreover, saline solution was administered orally to animals on the days before MSG administration as a control procedure. The interval between MSG administrations was at least one week.

Diazepam was an injectable preparation (CERCIN-inj. Takeda Co.). Before use, it was adequately diluted with a 20% propylene glycol solution, and given subcutaneously into the back at the time of MSG or saline administration. An aqueous solution of caffeine was prepared so that a 10 ml/kg solution contained the desired dose of caffeine. When caffeine was used in combination with MSG, the mixed aqueous solution was prepared in the same way immediately before administration, and this was always given orally.

Gross behaviors were observed on a TV monitor in another room connected to a TV camera which was fixed on the animal's sound-attenuated chamber. Temperature was controlled to 25 ± 2° C.

RESULTS

Behavioral Change Elicited by Administration of MSG Alone

There was scarcely any change in FR responding after the administration of 0.125–0.25 g/kg of MSG, nor was any gross behavioral abnormality observed. After the administration of 0.5 g/kg, however, some animals manifested transient response suppression at 15–30 min. After 1.0 g/kg, responding was suppressed immediately in most of the animals, though there were individual differences in the degree. At 5–15 min some showed a transient recovery, but at 20 min responding again decreased reaching a minimum level at 25 min. At 40–45 min, the responding returned rapidly to the control levels. After 2 g/kg, the suppression became more marked, and conspicuous changes also were observed in gross behaviors. Thus, when more than 1.0 g/kg of MSG was given, rats did not commence the lever pressing immediately, and many remained restless for 2–3 min. Even when they began to press the lever, the postreinforcement pause was longer than in controls, and at 15–20 min after MSG administration they often departed from the site of the lever and wandered about in the box. At 20–25 min, they stopped locomoting, stretched their bodies and lay down with their abdomens flat on the grid. In this state, they of course stopped lever-pressing completely. They generally breathed irregularly, closed their eyes, and sometimes showed tremor, facial spasm or salivation. But when the experimental box was tapped from outside, they soon stood up, and if food pellets were manually presented in a tray they promptly ate them.

Figure 1 shows sample cumulative records of typical FR responding after the administration of 0.5–2.0 g/kg of MSG. In order to clarify the pattern of change, the response pen was reset every 5 min. In this case, slight behavioral suppression appeared after the administration of 0.5 g/kg. Figure 2 presents mean response rates for 30 min after the administration of various doses of MSG. After 1.0 g/kg, the response rate was significantly suppressed compared with that after saline. Figure 3 presents changes in the response rate at 5 min intervals after the administration of 0.5, 1.0 and 2.0 g/kg of MSG. In all the cases significant change was

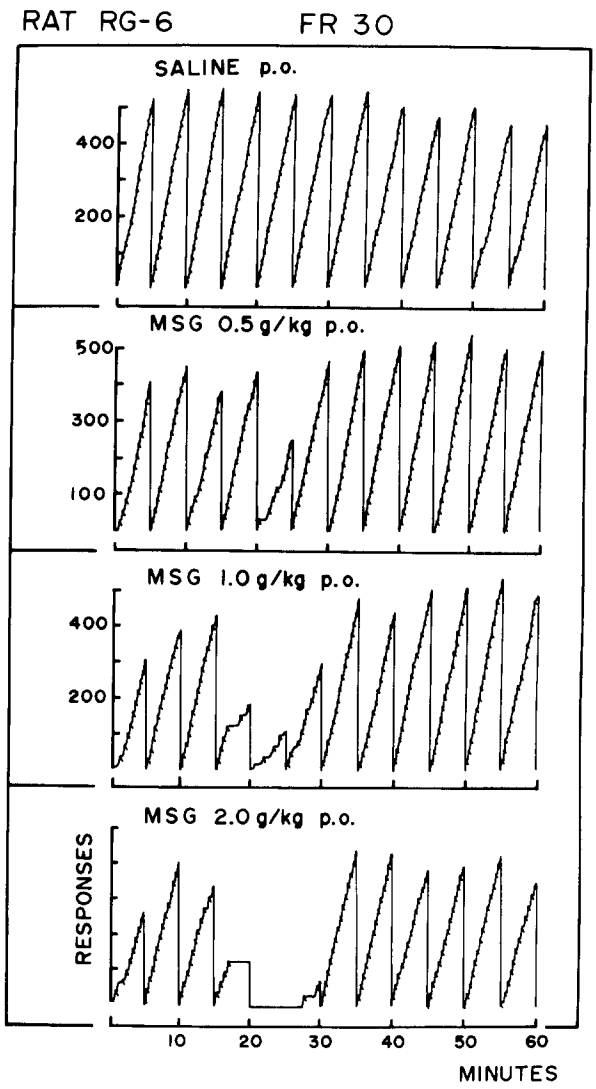

FIG. 1. Sample cumulative records after oral administrations of 0.5, 1.0 and 2.0 g/kg of MSG. The response pen was reset every 5 min.

manifested within 35 min, and thereafter responding returned to the control level. Thus, the change elicited by MSG was of short duration and responding on the following day was not affected.

Effect of Diazepam on MSG-induced Suppression

After the subcutaneous (SC) administration of 0.25, 0.5 and 1.0 mg/kg of diazepam alone, no significant change was observed in the FR-response. But at 15–30 min after 1.0 mg/kg, a few rats exhibited ataxia and slight suppression. On the whole, however, little change was observed (see Fig. 4).

When the same SC doses of diazepam were given concurrently with oral administration of 2.0 g/kg of MSG, marked changes were elicited. They can be divided into two patterns. One is the complete antagonism exerted by all the diazepam doses against the 5-min behavioral suppression which was observed immediately after the administration of

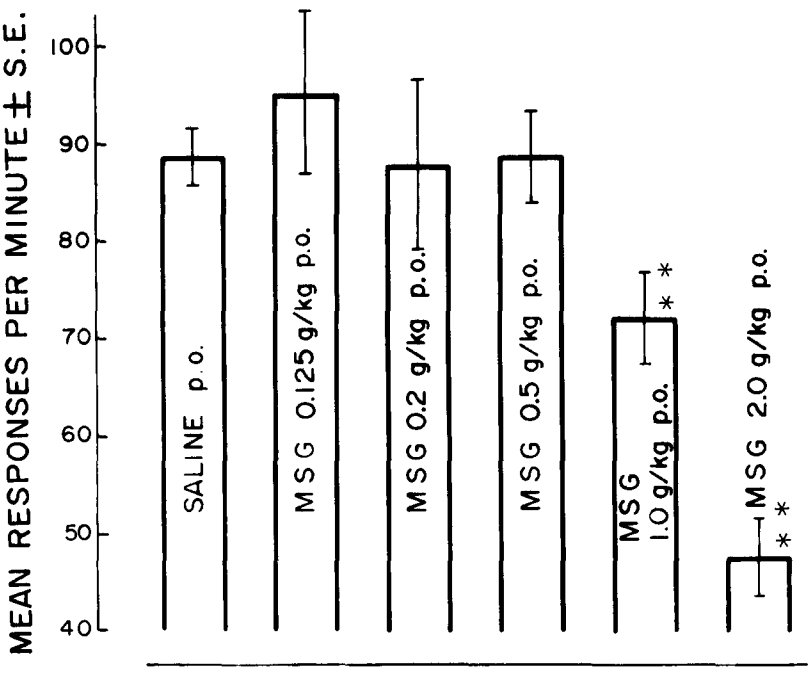

FIG. 2. Comparison of mean response rates for 30 min after oral administrations of 0.125–2.0 g/kg of MSG. ‡ Significantly different from the value for the saline-given control ($p<0.01$).

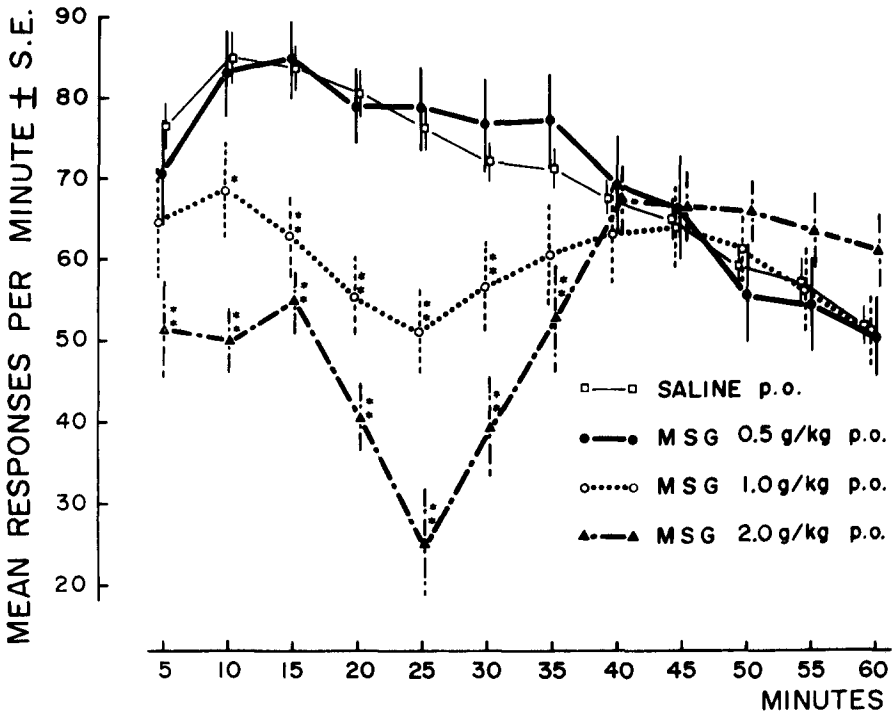

FIG. 3. Temporal change in mean response rates after MSG administration. * Significantly different from the control value after administration of saline alone ($p<0.05$). ‡$p<0.01$.

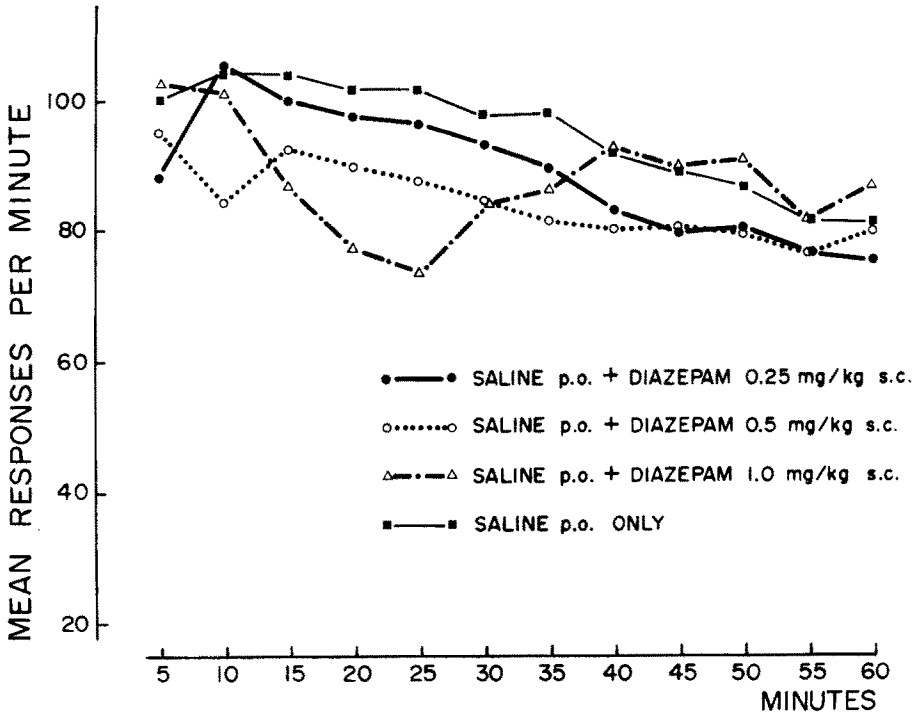

FIG. 4. Temporal changes in mean response rates after 0.25, 0.5 and 2.0 mg/kg of diazepam.

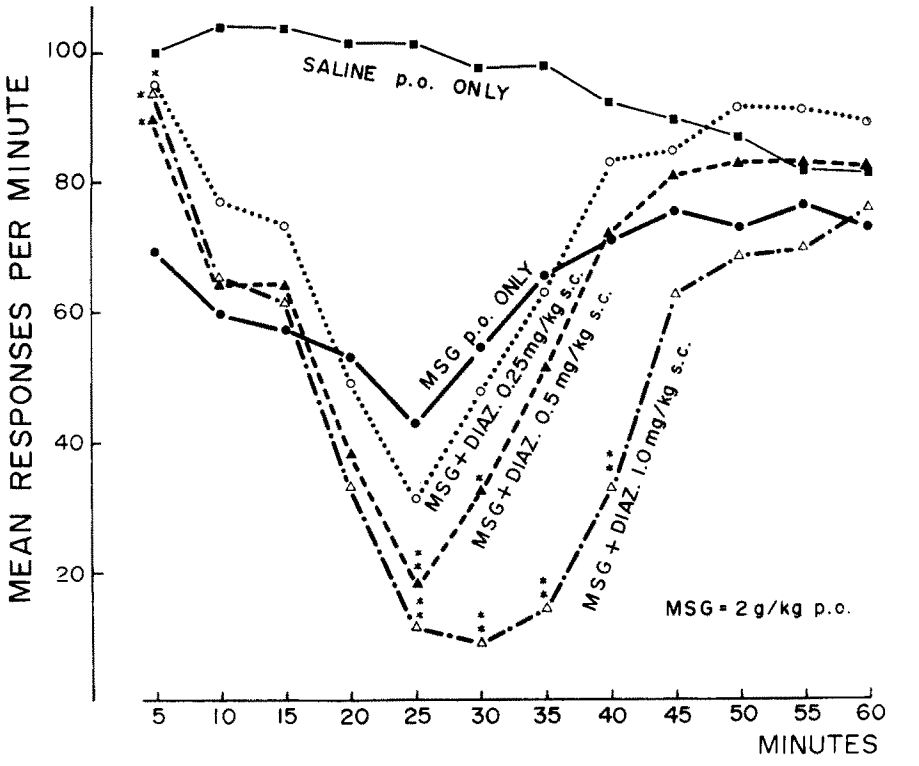

FIG. 5. Temporal changes in mean response rates after concurrent administration of MSG 2.0 g/kg and diazepam 0.25, 0.5 and 1.0 mg/kg. * Significantly different from the value after MSG 2.0 g/kg alone ($p<0.05$). ‡$p<0.01$.

MSG alone. The other is the graded enhancement of the suppression which was proportional to the diazepam dose which was seen at 20–30 min after MSG. This enhancement was especially marked after 1.0 mg/kg of diazepam given concurrently with MSG. Also, the gross behaviors of rats were markedly altered, and at 15–30 min after concurrent administration, many animals lay completely immobile on their sides. Figure 5 shows the beginning antagonism and subsequent enhancement of the MSG-induced suppression seen after the combined administration of diazepam and MSG.

Effect of Caffeine on MSG-induced Suppression

After the administration of 5, 10 and 20 mg/kg of caffeine alone, no significant change was observed as

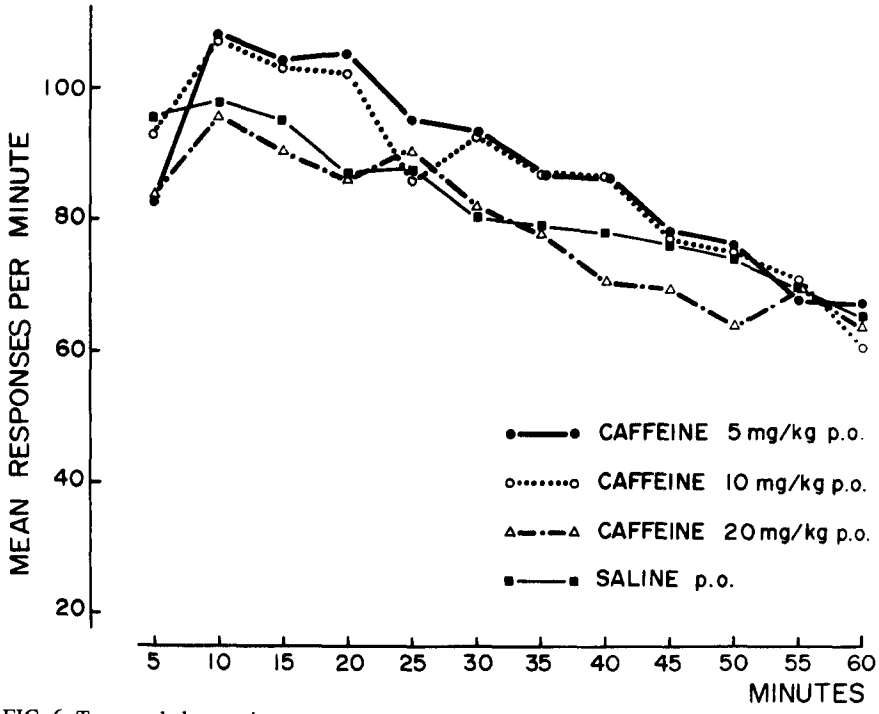

FIG. 6. Temporal changes in mean response rates after administration of 5, 10 and 20 mg/kg of caffeine.

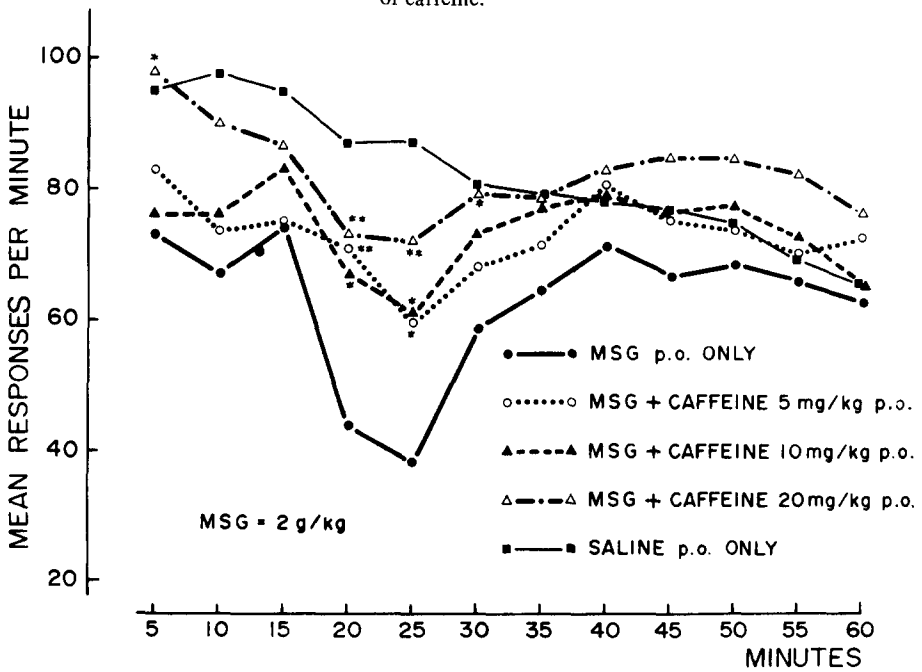

FIG. 7. Temporal changes in mean response rates after concurrent administration of 2.0 g/kg of MSG and 5, 10 and 20 mg/kg of caffeine. * Significantly different from the value after administration of MSG 2.0 g/kg alone ($p<0.05$). ‡$p<0.01$.

compared with the saline control (see Fig. 6). When, however, the same doses of caffeine were orally given in combination with 2.0 g/kg of MSG, the suppression by MSG was antagonized by caffeine in proportion with the dose. Figure 7 was obtained by plotting changes at 5-min intervals after combined administrations of 5, 10 and 20 mg/kg of caffeine with MSG, in comparison with changes after the administration of 2.0 g/kg of MSG alone. At 20–30 min, the responding was at a significantly higher level than that after administration of MSG alone. When 20 mg/kg of caffeine was given in combination with MSG, the response was significantly higher immediately after the administration. Clearly the suppression by MSG is antagonized by caffeine. The suppression of gross behavior also was antagonized by caffeine, since the animals rarely stretched or lay quietly, although they often departed from the lever at 20–25 min after the combined administration.

DISCUSSION

Relatively large oral doses of MSG produced marked behavioral suppression in rats. This is difficult to detect in rats trained under the discriminated avoidance schedule or fixed interval schedule of food reinforcement, but is evident on the FR schedule with high baseline response rates. MSG is different from general central depressants since it does not attenuate the conditioned emotional response [30]. Also gross behavioral changes observed after MSG were not prominent, and rats easily recovered from them when given some stimulus. These effects of MSG are evidently transient, attaining the maximum at 20–25 min after the administration, and being rapidly abolished in 40 min. On account of this, definite changes elicited by MSG may be overlooked unless the experiment is performed under strict control. The minimal effective dose of MSG in this case is assumed to be 0.5 g/kg. Considering the report that some cases of Chinese restaurant syndrome in Tokyo in 1972 each took 13–14 g of MSG [16], the doses in the present experiment do not seem too extravagant.

When infant mice or pigs were given 1.0–2.0 g/kg of MSG either SC or orally, plasma glutamate level was said to attain the maximum in 15–20 min [24,29]. It was also reported that when about 80 mg/kg of MSG was orally given as an aqueous solution to human subjects, plasma glutamate level attained the maximum at 20–30 min, then abruptly dropping [17]. The behavioral change observed in the present experiment after MSG was assumed to develop in agreement with plasma glutamate concentration. Furthermore, combined administration of MSG with diazepam and with caffeine suggested that there might be two factors involved in the suppression of the FR response. One is the several-min suppression immediately after the combined administration, which was evidently antagonized by 0.125–1.0 mg/kg of diazepam, or by 20 mg/kg of caffeine; the other is the suppression seen at 15–30 min after the administration, which was conversely accelerated by diazepam and antagonized by caffeine. The former may be a non-specific effect exerted by physical irritation of high MSG concentration, which can be antagonized by antianxiety agents. The true, systemic pharmacological action of MSG must doubtlessly be the latter effect.

According to the authors' unpublished data, not only MSG but also sodium L-aspartate has behavior-suppressing effects, which can not therefore be considered specific to MSG. Since diazepam and caffeine, which clearly possess central action, exerted synergistic or antagonistic effects on the suppressive action of MSG, it is plausible that this suppression is exerted through the mediation of the central action. Besides diazepam, ethanol was observed to have similar synergistic action [31].

Glutamic acid content of the brain outweighs other amino acids, and it is even assumed to have significance as a neurotransmitter [7,9]. Moreover, it is known that when animals are given glutamic acid either intracerebrally or intraventricularly, they manifest marked clonic or tonic convulsions [12,33]. Usually blood amino acid is not considered to pass through the blood-brain barrier, and this was confirmed in many reports [7,9]. But under the condition in which blood glutamate level is abruptly elevated, it is possible that glutamic acid may pass through the barrier into the central nervous system to produce imbalance in intracerebral amino acids, thus exerting some central action through variation in metabolic enzymes, either directly or indirectly. Perez and Olney [24] reported that when 2.0 g/kg of MSG(SC) was given to infant mice, glutamate level was abnormally elevated in the hypothalamus, especially in the arcuate nucleus after as long as 3 hr. It was also reported that after the oral or IP administration of 1.0–3.0 g/kg of MSG to adult rats, acceleration of spontaneous motor activity, marked fall in convulsion threshold, frequent development of facial spasm and manifestation of tonic-clonic convulsions were more or less observed in proportion with the dose [4,10]. All these observations indicate the possibility that MSG may penetrate the brain under certain conditions to exert its excitatory effect. Creasey and Malawista [8] stated that when adult mice were injected IP with 0.3–0.6 g/kg of MSG, glucose uptake by the brain was transiently deterred at 10–20 min more or less significantly in proportion with the dose. It is of interest that there is temporal correspondence between their results and development of suppression observed in the present experiment.

On the other hand, emetic action was often observed in men and dogs accompanied by pulse rate drop, salivation and nausea after the oral or IV administration of MSG [13, 27, 32]. It is not clear whether this is produced by peripheral or the central effects of MSG. One possibility is that MSG may induce aversive effects which, in turn, may suppress behavior.

It was suggested that when 0.5–0.8 g/kg of MSG were given to mice, rats or monkeys either orally or SC, retinotoxic effects might be produced [6, 19, 25], or the hypothalamus, especially the arcuate nucleus might be damaged [3, 5, 11, 18, 20, 22]. Olney [18] reported that mice, which were treated with MSG in infancy, became obese and neuroendocrinologically abnormal after maturation. Some workers, however, failed to confirm the neurotoxic effects of MSG [2, 15, 23, 26]. Furthermore, marked species difference is reported in this effect [1], mice being especially susceptible to it. The present experiments do not give any positive evidence indicating brain damage or irreversible alterations.

The aversive reaction, manifested in human beings after MSG intake, is exemplified by the so-called Chinese restaurant syndrome [28]. It includes facial pressure, burning sensation and chest strangulation feeling, and develops at 5–30 min after the intake of high MSG food, and disappears at 1–2 hr. Its pathogenic mechanism is not yet elucidated. But its development course and subtle symptoms closely resemble the behavioral suppression observed

in the present experiment. If the same mechanism really underlies these two, the central factor may be involved in the etiology of Chinese restaurant syndrome. It is probably wise to refrain from adding large amounts of MSG to food since it has been corroborated that the intake of a relatively large dose of MSG produces definite, though transient, symptoms.

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