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The preliminary results of a study on the neuromuscular blocking effect of monosodium glutamate(MSG) are reported. Four concentrations of MSG (6, 12, 17 and 23 mg/ml) were tested on the rat phrenic nerve-hemidiaphragm preparations, and the neurally-evoked twitch of the diaphragm was recorded. The neuromuscular blocking effect was found to be dose-dependent. All of the drug concentrations used could produce a complete (100%) blockade provided the exposure time was sufficient. However, the higher drug concentrations required a shorter time to produce such complete blockade. Moreover, muscle fasciculations were also observed at higher concentrations of MSG. The possible mechanisms of action as well as the approach of further study is discussed.

Key words : Monosodium Glutamate, Neuromuscular Blockade, Chinese Restaurant Syndrome

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Monosodium glutamate(MSG) is the monosodium salt of the naturally occurring L-form of glutamic acid which is one of the dicarboxylic amino acids.[1,11] Glutamate is found at a very high concentration in the mammalian brain, and it has an extremely powerful excitatory effect on neurons in virtually every region of the central nervous system (CNS).[1] It has been reported that glutamate is the neurotransmitter at the neuromuscular junction of insects.[9] MSG is widely used as a flavoring for food. It is responsible for the Chinese restaurant syndrome which is usually characterized by flushing and a faint feeling, pain in the neck and other vasomotor phenomena.[6] Most studies on the pharmacological action of glutamate have focused on the CNS; its effect on the peripheral nervous system has received less attention.[4,5,10] The aim of this study is to observe the in-vitro effect of MSG on the neuromuscular junction of rat diaphragm muscle.

Materials and Methods

Experimental Animals

Adult white Wistar rats of either sex weighing between 150-250 g, obtained from Animal House Unit, Faculty of Science,

Prince of Songkla University, Hat Yai Campus, were used in this study.

Drugs and Chemicals

Monosodium glutamate was purchased from Ajinomoto Co., (Thailand) Ltd. All other chemicals and compounds used in this investigation are of analytical grade.

Preparation of the isolated rat phrenic nerve-hemidiaphragm for recording neurally-evoked twitch

The rat phrenic nerve-hemidiaphragm preparation was isolated and set up for recording neurally-evoked twitch based on the technique of Bulbring.[2] A rat was killed by cutting its head and left to bleed as much as possible. The chest was opened by cutting the ribs alongside the joints at the sternum. The diaphragm was cut into fan-shaped segments and the phrenic nerve attached to each hemidiaphragm was cleared from surrounding tissues until to reach the thymus gland and the end of phrenic nerve was immediately cut. Then the isolated rat phrenic nerve-hemidiaphragm preparation was obtained and it was transfered to a glass dish containing Krebs solution(g/litre : NaCl 6.92, KCl 0.35, $CaCl_2$ 0.28, $MgSO_4.7H_20$ 0.29, $NaHCO_3$ 2.1, KH_2PO_4 0.16, glucose 2.0, ascorbic acid 0.02 and EDTA 0.025) aerated with 5% carbon dioxide in oxygen. The base of the hemidiaphragm was tied with a cotton thread in order to be hooked at the bottom of the water-jacketed organ bath. Another thread was tied firmly to the apex of the fan-shaped hemidiaphragm and connected to a force displacement transducer(Grass FT 0.3C). The rat phrenic nerve-hemidiaphragm preparation was suspended in a water-jacketed organ bath containing 100 ml Krebs solution aerated with 5% carbon dioxide in oxygen and the temperature was kept constant at 37° C. The pH of the solution was adjusted to 7.4. The preparation was allowed to equilibrate for 30 minutes under 1 g tension before the beginning of the experiment. The phrenic nerve was continuously stimulated with a square-wave pulse of 0.2 ms duration at a frequency of 0.1 Hz with a supramaximal voltage using a Grass Model S88 stimulator (Grass International Co., Quincy, Mass., U.S.A.). The isometric contraction of the diaphragm was measured with a force displacement transducer (Grass FT 0.3C) and recorded on a polygraph (Grass International Co., Quincy, Mass., U.S.A.).

Preparation of MSG solution

Four concentrations of MSG, 6, 12, 17 and 23 mg/ml (final concentration in 100 ml organ bath) were used to investigate the neuromuscular blocking effect.

Data presentation

The twitch tension produced by each concentration of MSG was measured at 10, 20 and 30 minutes after addition of MSG solution to the bath. The results were expressed as percentage of depression of the twitch tension.

In all experiments when 100 per cent depression of the twitch tension had been established, the preparations were washed out with MSG-free Krebs solution at least 3 times. Recovery was expressed as a percentage of the controlled twitch tension.

Results

1. The effect of MSG on the twitch tension of the rat phrenic nerve-hemidiaphragm preparation

From this experiment it was found that all of the concentrations of MSG produced neuromuscular depression and the effect was dose-dependent. Complete depression of twitch tension was obtained with all of the concentrations used. However, the higher concentrations required a shorter time to produce 100 per cent depression (figure 1). The per cent twitch depression produced by MSG in different concentrations measured at 10, 20 and 30 minutes was shown in Table 1 and figure 2. It was also noted that muscle fasciculations occurred at higher concentrations (12, 17 and 23 mg/ml) of MSG (figure 1).

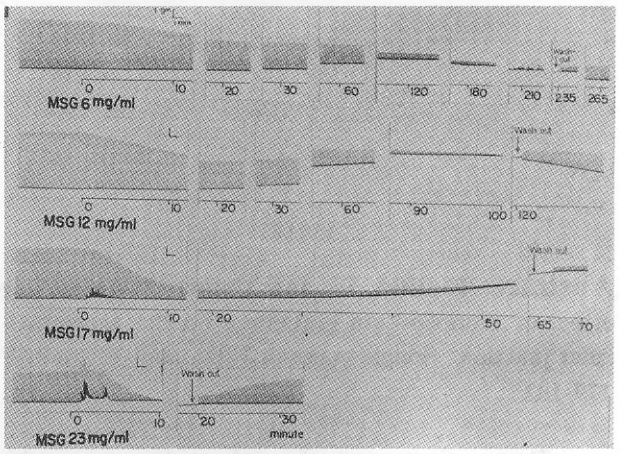

Figure 1. **The effect of MSG (6, 12, 17 and 23 mg/ml) on the twitch tension of the rat phrenic nerve-hemidiaphragm preparations. Twitch depression produced by MSG was shown at different times, e.g. 10, 20, 30 minutes and etc. Muscle fasciculations were also observed at concentrations of 12, 17 and 23 mg/ml.**

2. The effect of MSG on baseline shift and the wash-out recovery of the rat phrenic nerve-hemidiaphragm preparation

After the addition of each concentration of MSG (6, 12, 17 and 23 mg/ml), a shift of the baseline, caused by an apparent muscle contracture, was observed. The percentage of recovery of the twitch tension after washing out the bath with fresh Krebs solution at least 3 times is shown in Table 1.

Discussion

The results demonstrated that MSG had a neuromuscular blocking effect on the twitch tension

Table 1. The effects of MSG on twitch tension, baseline shift and the wash-out recovery in rat phrenic nerve-hemidiaphragm.

Concentration of MSG (mg/ml)	*Per cent twitch depression produced by MSG at			Time for complete twitch depression (minutes)	Baseline shift	wash-out recovery
	10 min.	20 min.	30 min.			
6	17.81 ± 5.03	29.66 ± 8.67	35.77 ± 10.11	242.49 ± 10.25	yes	20-50%
12	27.98 ± 3.01	42.96 ± 4.00	51.38 ± 4.34	135.34 ± 7.42	yes	20-60%
17	63.94 ± 2.93	78.72 ± 2.78	84.30 ± 2.32	78.68 ± 9.36	yes	10-50%
23	89.42 ± 4.77	100	100	12.72 ± 3.27	yes	60-100%

*Mean ± S.E., n = 6. Twitch depression measured at 10, 20 and 30 minutes.

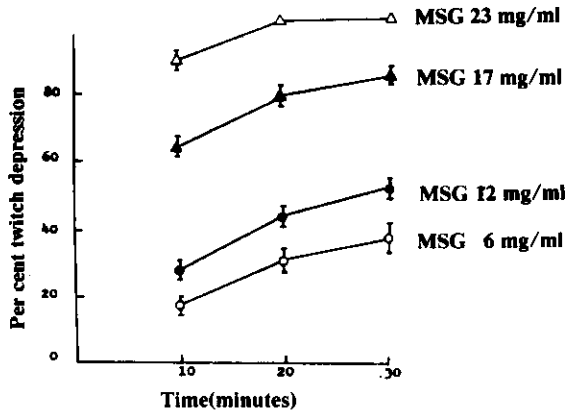

Figure 2. Relationship between exposure time and per cent twitch depression produced by MSG (6, 12, 17 and Each point is the mean of 6 observations ± S.E.

of the isolated rat phrenic nerve-hemidiaphragm preparation. All of the concentration of MSG used produced complete neuromuscular depression, but higher concentrations of MSG required a shorter time to produce complete twitch depression. The concentrations necessary to produce neuromuscular impairment were rather high, and at higher concentrations, MSG produced fasciculations.

Neuromuscular blocking agents are classified into 2 groups. 1) Non-depolarizing neuromuscular blocking agents, e.g., d-tubocurarine; drugs in this group produce neuromuscular depression by competing with acetylcholine(ACh) for reacting with cholinergic receptors on the postjunctional membrane[8]. 2) Depolarizing neuromuscular blocking agents; ACh and succinylcholine are EXAMPLES OF THIS GROUP. They act by producing an immediate and persisting depolarization at the motor end-plate and the adjacent area, i.e. the sarcoplasmic membrane[8].

In the present studies, it was found that fasciculation was produced by MSG in much the same manner as by high doses of ACh, succinylcholine and other depolarizing agents which have an initial action by depolarizing the membrane. This results in a brief period of repetitive excitation, which may be manifested by transient muscular fasciculation. This phase is followed by a blockade of neuromuscular transmission.

Therefore, one of the possible mechanisms of action of MSG in producing twitch depression may be similar to that of depolarizing agents. Such a depolarizing effect of MSG is in agreement with the works of many investigators [4,5,6]. Neurophysiological effects of glutamic acid have been studied in several preparations. Those studies suggest that glutamate causes a consistent and long-lasting depolarization of the excitable neurons, which persists for a considerable length of time after glutamate is removed from the superfusing medium[3,5]. Similar results have been found with kainic acid but the depolarization induced by this amino acid is much longer than that of glutamate and at high concentration the effect is irreversible[3]. The results of other electrophysiological experiments suggest that the membrane depolarization following the application of gluatamate as characterized by a change in the potassium conductance, resulting in large extracellular potassium concentrations which further depolarize the cell and disturb its ionic regulatory mechanisms[4,10].

In the present investigation, it is possible to conclude that the neuromuscular depression produced by MSG may be similar to that of depolarizing agents because both MSG and depolarizing agents can produce fasciculations. However, in order to obtain more information on the neuromuscular blocking effect of MSG, further studies of MSG and some

neuromuscular blocking agents should be made both in in-vivo and in-vitro.

In term of human toxicity, it is well known that MSG induces the Chinese restaurant syndrome when orally administered.[6] It was reported that the Chinese restaurant syndrome occurred in sensitive persons after taking of 5 g (approximate 1 tea-spoonful) MSG.[7] Estimated acceptable daily intake of MSG for adults, and children over 12 weeks is up to 120 mg per Kg body weight (approximate 7 g or 1-2 teaspoonful in 60 Kg person), as glutamic acid, additional to glutamic acid from all nature sources.[7] From our experiment the lowest concentration of MSG which produced twitch depression was 6 mg/ml, comparable to 12 g/Kg in 60 Kg person (base on concentration in extracellular water which is about 20 per cent of body weight). This dose of MSG is much higher than the oral dose that causes the Chinese restaurant syndrome in human. The relationship between twitch depression and symtoms of the syndrome has not been established. If they are related, however, it might suggest that rats are more resistant to MSG than the sensitive human. Accordingly it is implied that the rat phrenic nerve-diaphragm preparation is not a good model for the study of MSG toxicity.

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บทคัดย่อ. วิบูลย์ ฤทธิทิศ และ พีรรัชต์ ไทยนะ 2528. ผลของผงชูรสต่อบริเวณปลายประสาทและกล้ามเนื้อ. ว.สงขลานครินทร์ 7: 179-182

ได้ทำการศึกษาผลของผงชูรสต่อบริเวณปลายประสาทและกล้ามเนื้อโดยใช้กล้ามเนื้อกระบังลม ที่มีเส้นประสาทฟรีนิคของหนูขาว (isolated rat phrenic nerve-hemidiaphragm preparation) เป็นแบบ (model) ในการศึกษา ผงชูรสในขนาดความเข้มข้น 6,12,17 และ 23 มก./มล. สามารถกดการหดตัวของกล้ามเนื้อกระบังลมได้ ถ้าความเข้มข้นของผงชูรสมากขึ้น กล้ามเนื้อกระบังลมจะถูกกดเร็วขึ้น จนกระทั่งในที่สุดกล้ามเนื้อกระบังลมไม่หดตัว นอกจากนี้แล้วยังพบว่า ผงชูรสในขนาดความเข้มข้นที่สูงจะทำให้กล้ามเนื้อกระบังลมกระตุกได้เองเป็นระยะ ๆ (fasciculations) ในช่วงขณะหนึ่งนำมาก่อนที่จะกดการหดตัวของกล้ามเนื้อกระบังลม ส่วนกลไกการออกฤทธิ์ที่กดการทำงานที่รอยต่อปลายประสาทและกล้ามเนื้อนี้ที่อาจจะเป็นไปได้จะได้กล่าวในรายละเอียดต่อไป

ภาควิชาเภสัชวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ วิทยาเขตหาดใหญ่