Emetic stimulation inhibits swallowing reflex in decerebrate rats

Key words: swallowing reflex, hypoxia, gastric distension, emetic drug

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SUMMARY

1. INTRODUCTION

The nucleus of the solitary tract (NST) is the first relay for afferent fibers contained principally in the glossopharyngeal and vagal nerves (Kalia and Mesulam, 1980; Contreras et al., 1982). The NST plays important roles as the relay station for cardiovascular (Palkovits and Zaborszky, 1977; Van Giersbergen et al., 1992), digestive (Car et al., 1975; Ootani et al., 1995) and respiratory regulation (Cohen, 1979; Donoghue et al., 1982; Finley and Katz, 1992). The NST is further known to relay afferents for swallowing and vomiting reflexes.

Swallowing is triggered by sensory signals from the pharyngeal and laryngeal mucosa, which are sent to the NST. Afferents for the swallowing reflex run mainly in the superior laryngeal nerve (SLN). The SLN stimulation activates the NST, which contains neurons involved in the generation of the swallowing motor pattern (Jean and Car, 1979; Kessler and Jean, 1985; Ezure et al., 1993).

Vomiting can be elicited by a wide spectrum of emetic stimulation, e.g., visceral afferent activation (Andrews et al., 1990), hypoxia (Fukuda and Koga, 1993; Anand et al., 2006) and emetic drug (Wang and Borison, 1952; Parker et al., 2004). The common relay nucleus of these stimuli is the NST, and its excitation sequentially activates medullary nucleus to induce autonomic signs and somatic movements of vomiting (Koga and Fukuda, 1992; Koga et al., 1998; Fukuda et al., 1999).

The swallowing of a meal and the vomiting of gastric contents are antagonistic acts which produce opposite movement of the bolus within the upper alimentary canal. Thus, it may be possible that the emetic stimulation influences on NTS neurons contributing for swallowing reflex. We investigated effects of emetic stimulation on swallowing reflex elicited by SLN stimulation in decerebrated rats.

2. MATERIALS AND METHODS

The experiment procedures described here were carried out in accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Science (Physiological Society of Japan).

2.1 Surgical procedure

Experiments were performed on 43 Sprague Dawley rats, weighing from 300 to 400g, anesthetized with intravenous application of α-chloralose (60mg/kg) and sodium barbiturate (16mg/kg). When necessary, supplementary doses of sodium barbiturate (5 mg/kg, i.v.) were given before decerebration. Rectal temperature was maintained at 36-38 C with a heating pad.

The rats were fixed in a stereotaxic frame in the prone position. Precollicular decerebration was performed after craniotomy. No further anesthetics were given after decerebration. The animals were then rotated to the supine position. A midline incision was made on the ventral side of the neck. Polyethylene cannulae was inserted to trachea to maintain respiration. Blood pressure of the femoral artery was continuously monitored. Continuous infusion of normal saline (1.5ml/h) and/or drug administration was performed through the femoral vein. Each superior laryngeal nerve (SLN) was dissected free from surrounding tissues and placed on bipolar electrodes.

2.2 Stimulation and recording

Swallowing reflex was elicited by electrical stimulation of the SLN at 20Hz (3-5V, 0.3ms duration). Swallowing reflex was identified by the electromyographic activity (EMG) of suprahyoid muscles. The EMG of diaphragm and abdominal muscle were recorded to distinguish swallowing reflex from gag reflex. All data were converted to digital data using an analog-to-digital converter (Power Lab, AD Instruments), and stored in a computer (Macintosh G3, Apple). The digitalized data were analyzed using a software program (Chart ver 5.0, AD Instruments).

2.3 Effect of hypoxia on swallowing reflex

2.3.1 Effect of hypoxia by control of artificial ventilation

Effect of tidal volume reduction on number of swallows was investigated in 7 rats. The cannulae inserted to trachea was connected to a ventilator (SAR-830, CWE). Tidal volume was adjusted 3.4 ml at rate of 40/min for normal respiration. Then tidal volume was reduced to 2.2 ml at same rate for producing condition of hypoxia. Number of swallows elicited by SLN was measured under each condition.

We examined how hypoxia affected swallowing reflex induced electrical stimulation of the SLN. Hypoxia was induced by nitrogen gas inhalation or tidal volume reduction.

2.3.2 Effect of hypoxia using nitrogen gas

In 9 rats, nitrogen gas inhalation was performed to investigate the effects of hypoxia without influence of pulmonary afferent activity. Thus, tidal volume was kept constant for normal respiration. To produce hypoxia condition, nitrogen gas was inhaled for 20 sec through artificial ventilator. Number of swallows elicited by SLN was measured under inhalation of nitrogen gas and normal air.

Partial pressure of oxygen (PO_2), partial pressure of carbon dioxide (PCO_2), and pH were analyzed using blood gas analyzer (Rapid lab850). Blood sample was collected from femoral artery. Oxygen saturation (SpO_2) was continuously measured by standard pulse oxymeter (OLV-3000, Nihon Kohden). PO_2, PCO_2, pH and SpO_2 were measured during normal respiration and 40 sec after nitrogen gas inhalation using additional 6 rats.

2.4 Effect of gastric distention

Effect of gastric distension on swallowing was investigated in 9 rats. The stomach was exposed after midline incision and latex rubber balloon connected to a polyethylene tube was inserted into the stomach. The stomach was distended by injection of 3 ml warm saline.

2.5 Effect of emetic drug

We examined effect of emetic drug on swallowing reflex. Intravenous administration of LiCl (100mg/kg) was performed in 12 rats. Number of swallows was counted 2 min after the injection. Then, changes of swallow number was investigated every 5 min up to 22 min.

2.6 Statistical analysis

Statistical analysis was performed using paired t-test and ANOVA where appropriate. Significance level was taken as $p<0.05$.

3. RESULT

3.1 SLN-induced swallowing

Successive swallowing reflex was elicited by electrical stimulation (20Hz, 3-5V, 0.3ms duration) of the SLN for 20sec in all experiments (Fig.1). Swallowing reflex was identified by rhythmic activities of suprahyoid muscles. Interval of swallow gradually elongated. Diaphragmatic activity associated with respiration was initially suppressed and became irregular during stimulation (Fig.1). Abdominal muscle activity was silent during stimulation.

3.2 Effect of hypoxia on swallowing reflex

3.2.1 Effect of hypoxia by control of artificial ventilation

Tidal volume was adjusted 3.4 ml at rate of 40 times per minutes for normal respiration. Under normal respiration, the number of swallows was 16.4 ± 9.0 during 20-sec stimulation (n=7, not shown). When tidal volume was reduced to 2.2 ml, the number of swallows decreased to 12.4 ± 5.4 (n=7). The number of swallows recovered to 14.7 ± 6.9, when tidal volume was readjusted 3.4 ml. However, there was no significant difference between these values.

3.2.2 Effect of hypoxia using nitrogen gas

To examine effect of hypoxia, nitrogen gas was inhaled under artificial ventilation. Before the inhalation, the number of swallows was 17.6 ± 6.7 (n=9). The nitrogen gas inhalation was conducted for 40sec. The SLN stimulation was started at 20 sec after the onset of inhalation. The number of swallows during nitrogen gas inhalation was 14.9 ± 5.5 (n=9), and it was significantly lower than that during air ventilation ($p<0.05$). Moreover, burst activity of abdominal muscle, which was presumably associated with gag reflex (Fig. 2, indicated by "G"), was elicited during the SLN stimulation in 5 of 9 rats. Air ventilation was resumed after nitrogen gas inhalation, the number of swallows recovered to 18.2 ± 6.7 (n=9), and burst activity of abdominal muscle was not observed during the SLN stimulation.

To confirm blood gas change by nitrogen gas inhalation, changes of PO_2, PCO_2, pH and SpO_2 were analyzed in additional 6 rats (Table 1). The nitrogen gas inhalation was conducted for 40sec. These values were measured before the inhalation (control), at the end of the inhalation (N_2 inhalation) and 10 min after discontinuance the inhalation (recovery). During N_2 inhalation, PO_2 was significantly lower than each value of control and recovery (n=6, $p<0.01$). Similarly, SpO_2 was significantly lower than each value of control and recovery (n=6, $p<0.05$). However, no significant change was recognized in values of PCO_2 and pH during N_2 inhalation.

3.3 Effect of gastric distention on swallowing reflex

To examine how gastric vagal afferent activities affect on swallowing reflex, gastric distention were performed during swallowing reflex. The number of swallows was 12.9 ± 5.0 before gastric distention (n=9, Fig. 3A). The gastric distention was started 10sec before the SLN stimulation, and continued for 30sec. Respiratory cycle was reduced and tonic abdominal muscle activity appeared by the distension (Fig. 3B). The number of swallows was 10.4 ± 4.5, this number was significantly lower than that before distension (n=9, $p<0.05$, Fig. 3B). After discontinuance of the distension, respiratory cycle and abdominal muscle activity returned as control level. Further, the

number of swallows recovered to 13.0 ± 4.6 after the distension (Fig. 3C).

3.4 Effect of emetic drug on swallowing reflex

To examine effect of emetic drug, intravenous administration of LiCl was conducted. The number of swallows was measured at 2, 7, 12, 17 and 22 min after LiCl injection. The number of swallows was 20.5 ± 5.6 before injection and it gradually decreased (n=12, Fig. 4). The number was minimum (15.6 ± 5.3) at 12 min after the injection, and gradually recovered. The number measured at 7, 12 and 17 min was significantly lower than that before the injection (n=12, $p<0.05$, Fig. 4). In 5 of 12 rats, burst activity of abdominal muscles presumably associated with gag reflex was observed in 2-17 min after the injection.

4. DISCUSSION

4.1 Effect of hypoxia and/or hypercapnia

Prevalence of acute mountain sickness including vomiting is known to frequently appear with altitude (Sutton JR, 1976; Maggiorini M et al., 1990; Anand et al., 2006). Fukuda and Koga (1993) reported that hypoxia and/or hypercapnia facilitate the transition from retching to expulsion (vomiting). They further indicated that the transition is induced by activation of afferent fibers from carotid body (Fukuda and Koga, 1995). In this study, we first found that reduction of tidal volume tended to decrease number of swallows. Reduction of tidal volume could produce two effects, one is hypoxia and/or hypercapnia and the other is lung deflation. However, lung inflation has an inhibitory influence on the swallowing reflex (Kijima et al., 2000). Thus, inhibitory effect on swallowing reflex by reduction of tidal volume might be due to hypoxia and/or hypercapnia.

We further investigated effect of hypoxia on swallowing reflex using nitrogen gas inhalation without tidal volume change. In this study, severe hypoxia but not hypercapnia was induced by nitrogen gas inhalation (Table 1), and the swallowing reflex was inhibited in the period of hypoxia. Afferent fibers from chemoreceptor of carotid body enter medulla and make synapse on the NST neurons (Finley and Katz, 1992). Thus, chemoreceptor afferent inputs inhibit swallowing reflex arc between the NST and swallowing pattern generator in the medulla oblongata. These results are supported by Nishino et al. (1986), who reported that hypoxia depresses the swallowing reflex, whereas hypercapnia has no effect in cats. In human, hypercapnia decrease the frequency of water induced swallowing (Nishino et al., 1998). On the contrary, hypercapnia significantly increases the swallowing frequency, whereas hypoxia tended

to decrease the swallowing frequency in newborn lamb (Duvareille et al., 2007). Taken together, hypoxia seems to depress the swallowing reflex but effect of hypercapnia on swallowing reflex should be further investigated.

4.2 Effect of gastric afferent

Gastric distension is well known to stimulate 5-HT release from the enterochromaffin cells and the released 5-HT trigger vomiting reflex in carnivore (Andrews and Horn, 2006). Mazda et al. (2004) suggested that the released 5-HT by gastric distension activates 5-HT_3 receptors located on the vagal afferent nerve terminals in specific brain nuclei including the NST in rat. In this study, 3ml-distension of stomach did not induce vomiting like movement of rat, whereas significantly inhibited the swallowing reflex in this study. Thus, neurons in the NTS may inhibit the swallowing reflex arc via 5-HT_3 receptors.

On the other hand, 20-Hz SLN stimulation for 20 sec elicited 20.5 ± 5.6 swallows under spontaneous respiration (Fig. 4). Interestingly, stimulation of same parameters elicited only 12.9 ± 5.0 swallows in balloon inserted rats for gastric distension, even when balloon was not inflated. Surgery for balloon insertion could activate gastric vagal afferent, as the result, the number of swallows might decrease before distension. Recent study indicates that microinjection of leptin in the NST inhibits swallowing in rats (Felix, 2006). Neurons in the NST are activated by both gastric distension and leptin (Huo et al., 2007). It may be speculated that stimulation of gastric vagal afferent due to the surgery activate NST neurons sensitive to leptin, and that these neurons inhibited the swallowing reflex.

4.3 Effect of emetic drug

Lithium chloride (LiCl) is the most commonly employed unconditioned stimulus for taste avoidance in rodents (Ossenkopp and Eckel, 1995). Administration of LiCl is reported to induce vomiting in *Suncus murinus* (Parker et al., 2004). The number of swallows was significantly inhibited by intraveneous application of LiCl in this study. Effect of apomorphine, one of the emetic drug, on the swallowing reflex is well investigated in rats and cats (Bieger et al., 1972, 1978; Weerasuriya et al., 1979; Kessler and Jean, 1986). It may be assumed by their study that apomorphine applied to forebrain exerted a facilitatory effect on swallowing, whereas it applied to hindbrain induced an inhibition of the reflex. In this study, the action site of LiCl was considered to be hindbrain, because all rats were decerebrated at midbrain level. It is well known that one of the action sites of LiCl and apomorphine is the area postrema of hindbrain. Moreover, area postrema neurons are reported to project to the NST (Shapiro and Miselis, 1985). Therefore, LiCl may inhibit the swallowing reflex via the NST neurons

by excitation of area postrema.

4.4 Gag like movement during SLN stimulation

Pharyngeal irritation induces gag reflex in dog, cat and human (Chaffee et al., 1970; Sieck and Fournier, 1989; Ashida and Koga, 2006). The main power for gag reflex is increase of abdominal pressure due to abdominal muscle contraction. In this study, each stimulus of nitrogen gas inhalation, gastric distension and emetic drug application did not induce burst activity of abdominal muscle. However, combination of these emetic stimuli and SLN stimulation occasionally produced burst activity of abdominal muscle (Fig. 2). Rats are known to have no ability of vomiting, and no report described gag reflex using rats has been found. In this study, we applied both emetic stimuli and electrical stimulation of the SLN in the decerebrated rat. The combination stimulation of glossopharyngeal and abdominal vagal afferents facilitates gag reflex (Fukuda and Koga, 1995). In addition, decrease of arterial oxygen saturation facilitate gag reflex in human (Ashida and Koga, 2006). The burst activity of abdominal muscle observed in this study, therefore, may be associated with gag reflex.

The NST mediates both gag and swallowing reflexes. However, physiological role of gag reflex and that of swallowing reflex are considered to be reciprocal. Therefore, emetic stimulation might inhibit swallowing pattern generator via the NST, in turn, facilitate gag reflex.

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Figure legends

Fig.1. Swallowing reflex in a decerebrated rat preparation. The EMG of swallowing is induced by electrical stimulation of the superior laryngeal nerve (SLN) at 20Hz (3-5V, 0.3ms duration). A stimulus period (20 s) is indicated by a thick line. SH: suprahyoid muscles, DIA: diaphragm, ABD: abdominal muscles. These abbreviations applied to Fig. 2-3.

Fig.2. Effect on swallowing reflex of hypoxia induced by nitrogen gas inhalation. A: The EMG of SLN-induced swallowing started at 20sec after the onset of the inhalation. Note that burst activity of abdominal muscle, which is presumably associated with gag reflex, is indicated by “G”. B: Effect of hypoxia to the number of swallows induced by SLN stimulation. Each bar represents mean values ± SE (n=9) of swallows before the

inhalation (control), at 20sec after the onset of the inhalation (N_2) and after resumption of air ventilation (recovery). Hypoxia significantly inhibits the number of swallows induced by SLN stimulation (*$p<0.05$).

Fig.3. Effect on swallowing reflex of gastric distention. A, B, C: Each figure indicates EMG of swallowing before the distention (A), during the distention (B) and after discontinuance of the distension (C). Note that tonic abdominal muscle activity appeared by the distension. D: Effect of gastric distention to the number of swallows induced by SLN stimulation. Each bar represents mean values ± SE (n=9) of swallows during control, gastric distention and recovery. Gastric distention significantly inhibits the number of swallows induced by SLN stimulation (*$p<0.05$).

Fig.4. Effect on swallowing reflex of intravenous injection of LiCl. The ordinate is the number of swallows induced by SLN stimulation. The abscissa indicates the time after LiCl injection. Values are means ± SE (n=12). The number of swallows at 7, 12 and 17 min was significantly lower than that before the injection (*$p<0.05$).

Table1. The change of PCO_2, PO_2, SpO_2 and pH by nitrogen gas inhalation. These values were measured before the inhalation (Control), at the end of the inhalation (N_2 inhalation) and 10 min after discontinuance the inhalation (Recovery). Values are means ± SE (n=6). N_2 inhalation significantly inhibits PO_2 and SpO_2 (**$p<0.01$, * $p<0.05$).