Gastric relaxation induced by electrical and chemical stimulation of the area postrema in the rat

Masanao Kawachi[1,3], Nobuaki Hori[1], Mineo Takei[3], Tadashi Kurimoto[3,4], Norio Akaike[2] and Yushi Ito[1,2]

[1] *Department of Pharmacology, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan*
[2] *Research Division for Life Sciences, Kumamoto Health Science University, Izumi 324, Kumamoto 861-5598, Japan*
[3] *Central Research Laboratories, ZERIA Pharmaceutical Co., Ltd., 2515-1 Numagami, Oshikiri, Kumagaya-shi, Saitama 360-0111, Japan*
[4] *Technology Transfer Support Division, Department of Technology Transfer Promotion Office of Technology Transfer and Innovation, Japan Science and Technology Agency, 5-3, Yonbancho Chiyoda-ku, Tokyo 102-8666, Japan*

Abstract. Area postrema (AP) is considered to be an important neural center for emesis in carnivores. However, it is also known that AP mediates motor responses induced by apomorphine in rats which do not have an emetic reflex. To shed more light on the possible role of AP in the control of gastric motility in physiological or pathophysiological conditions, we observed the effects of electrical or chemical (apomorphine) stimulation of AP neurons on intragastric pressure (IGP) or intragastric volume (IGV) in rat. We found that electrical stimulation (ES) reduces IGP, and this is sensitive to hexamethonium or L-NAME, and apomorphine also reduces IGP and increases IGV. In slice preparations, apomorphine (10 μmol/l) increased the frequency of spontaneous single unit discharges of AP neurons recorded extracellularly. We also succeeded retrograde labeling of AP neurons by DiI applied into the gastric corpus, for the first time. These observations indicate that rat stomach receives efferent neural input from AP and the excitation of AP neurons relaxes the stomach in rat, suggesting some functional roles of AP neurons in the regulation of gastric motility.

Key words: Area postrema (AP) — Gastric motility — Retrograde labeling of AP neurons — Electrical stimulation of AP — Apomorphine

Introduction

It is generally considered that gastric motility is controlled mainly by the parasympathetic neurons in the dorsal motor nucleus of the vagus (DMV) through their efferent projections into the vagus nerves. In addition, it is also well documented that the nucleus tractus solitarius (NTS) is the major recipient of visceral afferent information arising from various regions of the gastro-intestinal tract (Blessing 1997), and that the NTS neurons provide direct inhibitory and excitatory inputs to preganglionic parasympathetic neurons in the DMV (Rogers et al. 1999). Thus, the DMV and NTS comprise a functionally integrated structure, termed the dorsal vagal complex, serving as the synaptic circuitry responsible for the coordination of classic vago-vagal gastrointestinal reflexes.

On the other hand, in the unusual gastric motility involved in emesis, the area postrema (AP) is thought to be an important neural center for its trigger in carnivores (Borison 1989), and excitation of AP neurons was hypothesized to activate the central pattern generator for emesis *via* dorsal vagal complex (Koga and Fukuda 1992). AP is in the wall of the obex of the fourth ventricle, and is a highly vascular structure with a very weak blood-brain diffusion barrier, allowing most substances to penetrate into the interstitial space between the neurons, and it was generally recognized that it acts as a chemoreceptor trigger zone for the

Correspondence to: Yushi Ito, Research Division for Life Sciences, Kumamoto Health Science University, Izumi 324, Kumamoto 861-5598, Japan
E-mail: itou@kumamoto-hsu.ac.jp

induction of emesis (Borison and Wang 1953; Carpenter et al. 1988). So far, however, there have been few studies in which the effects of electrical and chemical stimulation of AP on gastric motility were investigated at the same time. To shed more light on the possible role of AP in the control of gastric motility in physiological or pathophysiological conditions, we applied electrical and chemical (apomorphine) stimulation to the AP and recorded changes in the intragastric pressure (IGP) and intragastric volume (IGV). In addition, we performed retrograde labeling of the AP neuron by applying 1.1'-dioctadecyl-3,3,3'3-tetramethyl indocarbocyanine perchlorate (DiI) into the gastric corpus according to procedures described elsewhere (Browning et al. 1999), since there have been no such study till now.

Materials and Methods

Animal preparation for recording the IGP and IGV

Experiments were performed on adult male Sprague Dawley rats (270–350 g), purchased from Charles River Laboratories Japan (Yokohama, Japan), in accordance with "Guiding Principles for the Care and Use of Animals" approved by the Japanese Pharmacological Society and all efforts were made to minimize both the number of animals used and their suffering.

Prior to experimentation, animals were fasted overnight with ad libitum access to water. Anesthesia was induced in the animals with urethane (1.5 g/kg, s.c.) and its depth monitored by assessing for the absence of limb withdrawal to a noxious toe pinch. Since urethane is known as suitable general anesthesia for studying neural function, and one shot of urethane provides long period anesthesia with minimal cardiopulmonary depression. Rats were intubated via the trachea to maintain an open airway. The femoral vein was cannulated with polyethylene tubing (O.D. 1.0 mm, I.D. 0.5 mm) for systemic infusion of drugs. A laparotomy was then performed to expose the stomach. In some experiments, the vagus nerves were carefully exposed along their esophageal extent, and ligatures were tied around both nerves and then bilateral vagotomy was performed.

An intragastric thin latex rubber balloon was tied around polyethylene tubing (O.D. 2.3 mm, I.D. 1.3 mm) and inserted into the stomach *via* a small incision made in the forestomach, and placed in the gastric corpus to obtain measurements of IGP. The balloon was secured with a running suture to prevent movement and the incision made in the forestomach was closed. The tubing was connected to a pressure transducer TP-400T (Nihon Koden Kogyo, Tokyo, Japan), interfaced to a bridge amplifier AP-610J (Nihon Koden Kogyo), the signal of which was fed into an analog-digital converter (MacLab system, AD Instruments, Grand Junction, CO) and the data stored on a personal computer for subsequent analysis. The stomach was inflated by introducing 0.4–1.0 ml of water into the intragastric balloon to achieve a baseline pressure of ~10 cm H_2O.

The barostat (Distender series II R; G&J Electronics Inc., Toronto, Canada) was also employed to record changes in IGV, which utilizes a rigid cylinder piston-type pump (20 ml) with a maximum flow rate of 5 ml/min. The data was also stored in a personal computer through software (EightStar version 6.0; Star Medical Inc., Tokyo, Japan) for subsequent analysis. A rigid, non-compliant polyethylene tubing connected the barostat to the balloon. The balloon was constructed from thin walled non-compliant polyethylene material. This balloon was then tied to the polyethylene tubing with a silk suture to yield a spherical shape with a maximum volume at least 2 ml. A deflated balloon was inserted into the stomach via a small incision into the forestomach, and placed in the gastric corpus to obtain measurement of IGV. Pressure was measured internally from the barostat. The reported volume in the present experiments is the IGV at 6 ± 0.3 mm Hg relative to the IGV at atmospheric pressure (set to 0 mm Hg); that is, the IGV is not the absolute volume of the stomach.

The animal was positioned in a stereotaxic apparatus SR-5 (Narishige, Tokyo, Japan), and a partial craniotomy was performed to expose the dorsal surface of the medulla. The cerebellum was pushed up gently so that the fourth ventricle and the rostral-most pole of the AP, could be viewed from the dorsal aspect and used as a point of reference for the stereotaxic placement of stimulating bipolar coaxial electrodes or push-pull cannula for the application of apomorphine to the AP.

The bipolar coaxial electrode (diameter of 250 μm for the shaft, and 80 μm for the tip) was stereotaxically placed in the AP (coordinates: 0.2 ~ 0.6 mm rostral to the obex and ~0.4 mm below the surface of the AP), according to the atlas of Paxinos and Watson (2005). To decide the minimum stimulus intensity, which selectively activates the neurons in AP, electrical stimulation (ES) with square wave pulses of 0.5 ms was applied with various stimulus voltage (1–20 V) for a period of 10 s at a constant frequency of 1 Hz, while the IGP was monitored. It was found that the minimum stimulus intensity to evoke changes in IGP was 5 V (data not shown). Thus, ES at a fixed stimulus intensity (5 V) at various frequencies (0.1–20 Hz) was applied through the electrode from an electronic stimulator SEN-7103 (Nihon Koden Kogyo, Tokyo, Japan).

To investigate the effects of hexamethonium or N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME) on ES-induced decrease in IGP, the responses induced by ES (2 Hz) were compared before and 5 min after administration of the drugs. To prevent the muscarinic antagonistic action of L-NAME (Buxton et al. 1993), intravenous infusion of carbachol (50 μg/kg/h) was performed during the experi-

ments in which the effects of L-NAME were observed on ES-induced decrease in IGP.

Apomorphine was applied to the localized region of AP by use of push-pull perfusion method (Myers et al. 1998). Briefly, concentric push-pull cannula (outer diameter ~500 μm for the tip), prepared from glass capillary, was attached to silicon tubing and then the cannula tip was placed tightly on the surface of AP. Physiological saline or apomorphine dissolved in saline, was perfused at a rate of 150 μl/min using a peristaltic pump MP-1000D (Tokyo Rikakikai, Tokyo, Japan).

Retrograde labeling of neurons in AP and DMV

Male rats were deeply anaesthetized by pentobarbital (50 mg/kg) before abdominal surgery was performed. The abdominal area was cleaned with 70% ethanol and the stomach was exposed after laparotomy. The retrograde tracer DiI was dissolved in dimethyl sulfoxide (0.4%), and 30–50 μl of the solution were injected into the gastric wall from the serosal surface of the ventral or dorsal part of the gastric corpus by use of a micro-syringe. The surgical area was washed with warm Krebs-Ringer solution and blotted dry with cotton swabs. Then the wound was closed using 3/0 silk sutures, and allowed to recover. After 30–40 days the rats were anesthetized and perfused transcardially with 4% paraformaldehyde in 0.1 mol/l phosphate buffer, pH 7.4. Brainstems were removed and placed in ice-cold saline. Coronal brain stem sections containing the AP, DMV and NTS at a thickness of 200 μm were obtained by use of a slicer. Retrogradely labeled neurons were identified with a Leica DM LFSA microscope equipped with a RITC epifluorescence filter.

Single unit discharge measurement from AP neurons in slice preparation

Spontaneous single unit discharges were recorded extracellularly with glass microelectrodes filled with Krebs-Ringer solution from AP neurons in the slice preparation to observe the effects of apomorphine. Under ether anesthesia, rats were euthanized by cervical dislocation, and the brain stem and cerebellum were rapidly removed to cold Krebs-Ringer solution. The brain was transected at the inferior collicular level, the cut caudal part was fixed on a stage in the chamber of a slicer using cyanoacrylated glue and coronal sections including AP, NTS and DMV at a thickness of 300 μm were sliced. Each slice was incubated at 33°C for at least 2 h before the start of the experiments in Krebs-Ringer solution bubbled with 95% O_2 and 5% CO_2. The slices were then placed on a plexiglas mesh in a submerged recording chamber perfused with oxygenated Krebs-Ringer solution at 33°C at a flow rate of 3 ml/min. The pipettes were made from borosilicate capillary glass (O.D. 1.5 mm, I.D. 0.9 mm) by use of a vertical pipette puller PE-2 (Narishige, Tokyo, Japan). Voltage was continuously monitored on an oscilloscope VC-6023 (Hitachi, Tokyo, Japan) and fed into an analog-digital converter (PowerLab system; AD Instruments, Grand Junction, CO, USA) and data stored on a personal computer for subsequent analysis. We used Krebs-Ringer solution containing (in mmol/l): 126 NaCl, 5 KCl, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 1.26 KH_2PO_4, 26 $NaHCO_3$ and 10 D-glucose.

Data and statistical analysis

To standardize the changes in IGP induced by electrical and chemical stimulation of AP, the minimum IGP observed after the administration of sodium nitroprusside dihydrate (SNP; 100 μg/kg, i.v.) was used as the zero level of IGP. Concerning the effects of apomorphine, the mean values of IGP and IGV before and during application of apomorphine for 5 min were calculated.

Data were expressed as mean ± standard error of the mean. Statistical analyses of the data were performed by *t*-test or paired *t*-test. $p < 0.05$ was considered to be statistically significant.

Drugs

DiI was obtained from Molecular Probes (Engene, OR, USA). SNP, hexamethonium chloride, L-NAME, carbamoylcholine chloride (carbachol), apomorphine hydrochloride hemihydrate and all other chemicals were purchased from Sigma-Aldrich-RBI (St. Louis, MO, USA).

Results

Retrograde labeling of neurons in DMV and AP

When DiI was applied into the gastric wall of the ventral and dorsal part of the gastric corpus, retrogradely labeled neurons were identified in the left and right DMV, respectively (Fig. 1). In addition, neurons in AP, the somata of which were much smaller than those in DMV, were also labeled by DiI applied to both parts of the gastric corpus (Fig. 1B,D and E). The labeled neurons were diffusely distributed in AP. However, no neurons in the NTS were labeled, thereby indicating that DiI labeled neurons run retrogradely.

Effects of ES applied to AP on IGP

Microelectrodes were inserted into the AP of 6 rats, and ES at a constant stimulus intensity (5 V) and various frequencies (0.1–20 Hz) were applied to the AP neurons. Decrease in IGP was observed in all the rats examined in response to ES at

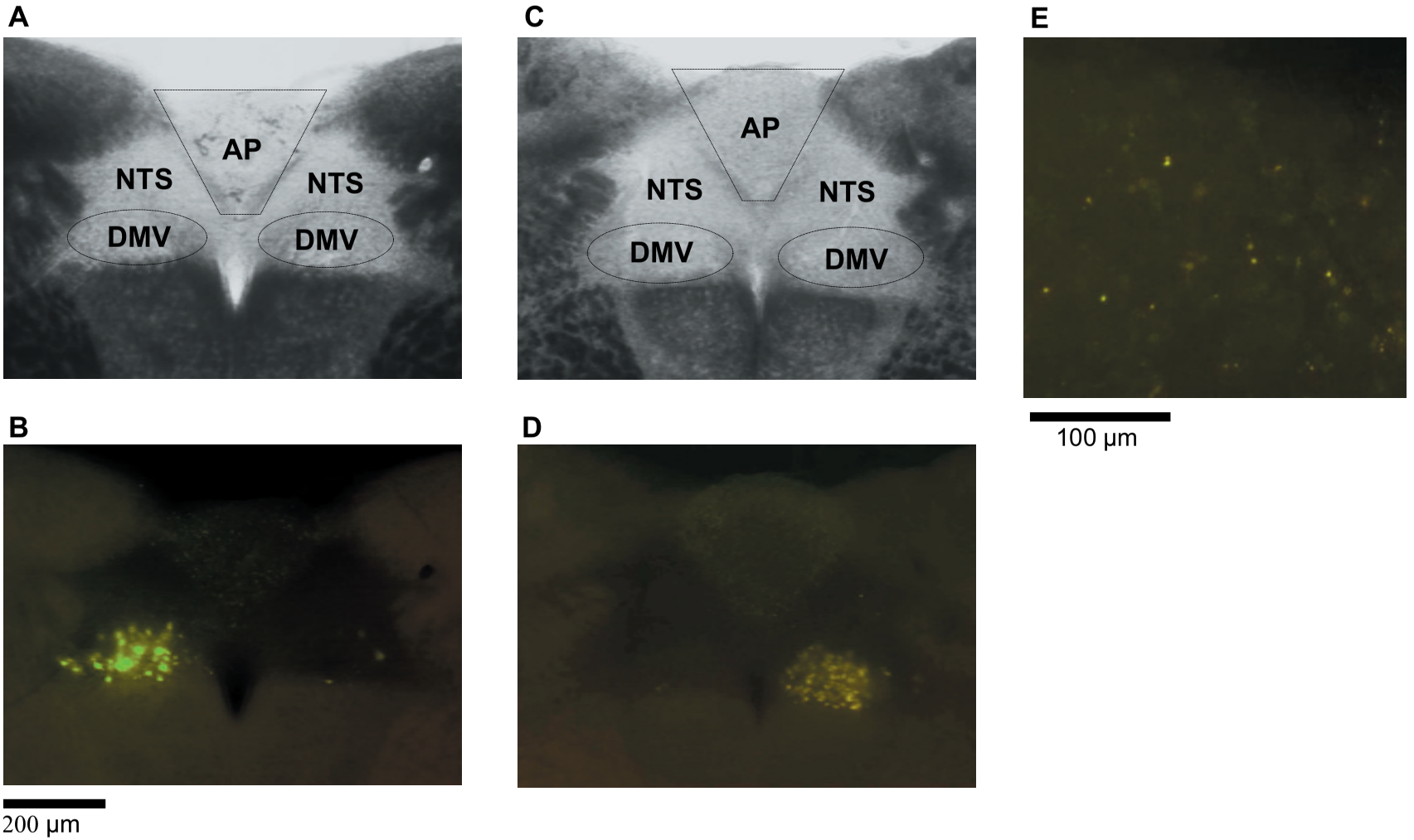

Figure 1. A. and **C.** – transverse microscopic sections of the brain stem 0.4–0.6 mm rostral to the obex, sowing area postrema (AP), nucleus tractus solitarius (NTS) and dorsal motor nucleus of the vagus (DMV). **B.** and **D.** – fluorescence photomicrographs showing the fluorescent neurons in the left (B) and right (D) DMV or AP approximately 1 month after the injection of DiI into the gastric wall from the serosal surface of the ventral or dorsal part of the gastric corpus, respectively. A, B or C, D were sections prepared from rats injected with DiI into the ventral or dorsal part of the gastric corpus, respectively. **E.** Fluorescence photomicrographs of AP in expanded scale.

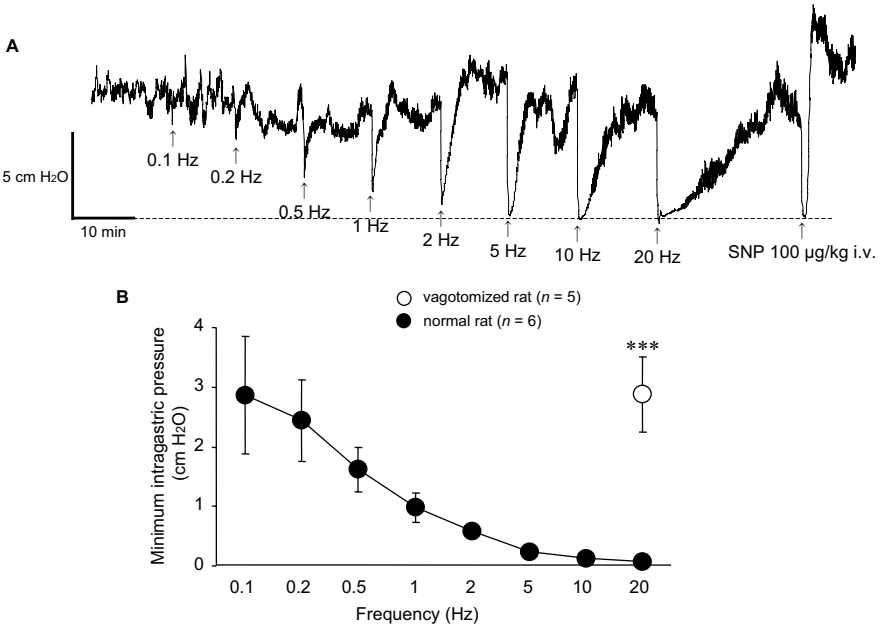

Figure 2. Effects of electrical stimulation (ES) of area postrema (AP) on the intragastric pressure (IGP). **A.** An actual trace of the effects of ES at a constant stimulus intensity (5 V) and various frequencies (0.1–20 Hz) on IGP. Sodium nitroprusside dihydrate (SNP; 100 μg/kg, i.v.) was applied and the level of IGP induced by SNP was used as the zero level of IGP. **B.** Closed circles: the relationship between the various frequency of ES and relative change in the IGP ($n = 6$). Open circle: the effects of bilateral vagotomy on the relationship between the IGP and stimulus frequency, observed at a stimulus frequency of 20 Hz ($n = 5$). *** $p < 0.001$ (t-test).

frequencies more than 0.2 Hz, and the degree of the decrease in IGP was greater with increase in frequency (Fig. 2).

Effects of bilateral vagotomy, L-NAME and hexamethonium on the ES-induced decrease in IGP

To determine whether the ES-induced decrease in IGP was due to activation of preganglionic motoneurons projecting to the stomach through the vagus nerve, bilateral vagotomies were performed in 5 rats. The decrease in IGP induced by ES (20 Hz) of AP was almost completely suppressed by bilateral vagotomy (Fig. 2B, open circle).

Then we observed the effects of intravenously applied hexamethonium and L-NAME on the ES-induced decrease in IGP (Fig. 3A and C). Hexamethonium greatly suppressed the reduction in IGP induced by ES (2 Hz) applied to the AP. Similarly, L-NAME dose-dependently suppressed the reduction in IGP induced by ES applied to the AP. Fig. 3B and D show the mean values of the minimum IGP evoked by ES before and after the application of hexamethonium and L-NAME, respectively.

Effects on IGP of local application of apomorphine into AP

Apomorphine (0.1–100 μmol/l) was locally applied to the exposed AP by use of the push-pull perfusion method to observe the effects on IGP. Apomorphine (>1 μmol/l) applied to the AP reduced the frequency of spontaneous phasic contractions and reduced the level of IGP in a concentration-dependent manner (Fig. 4A,B). Upon removal of apomorphine, IGP gradually recovered to the original level with marked increase in the frequency of spontaneous contractions. The mean values of IGP for 5 min before and during application of apomorphine were calculated to evaluate the effects of apomorpine on IGP. As shown in Fig. 4B, apomorphine (>1 μmol/l) reduced the mean IGP concentration-dependently.

Effects on IGV of apomorphine applied to AP

Although ES and apomorophine applied to AP decreased IGP, Koga et al. (2003) reported that apomorphine inhibits the phasic gastric contractions but does not induce relaxation in either the proximal or distal stomach. Therefore, it was of interest to observe the effects of apomorphine applied to AP on the IGV by use of the barostat method. We found that during application of apomorphine (>1 μmol/l) for 5 min to the AP, the IGV increases in a concentration-dependent manner (Fig. 5A,B), thereby indicating that apomorphine induces relaxation of the stomach.

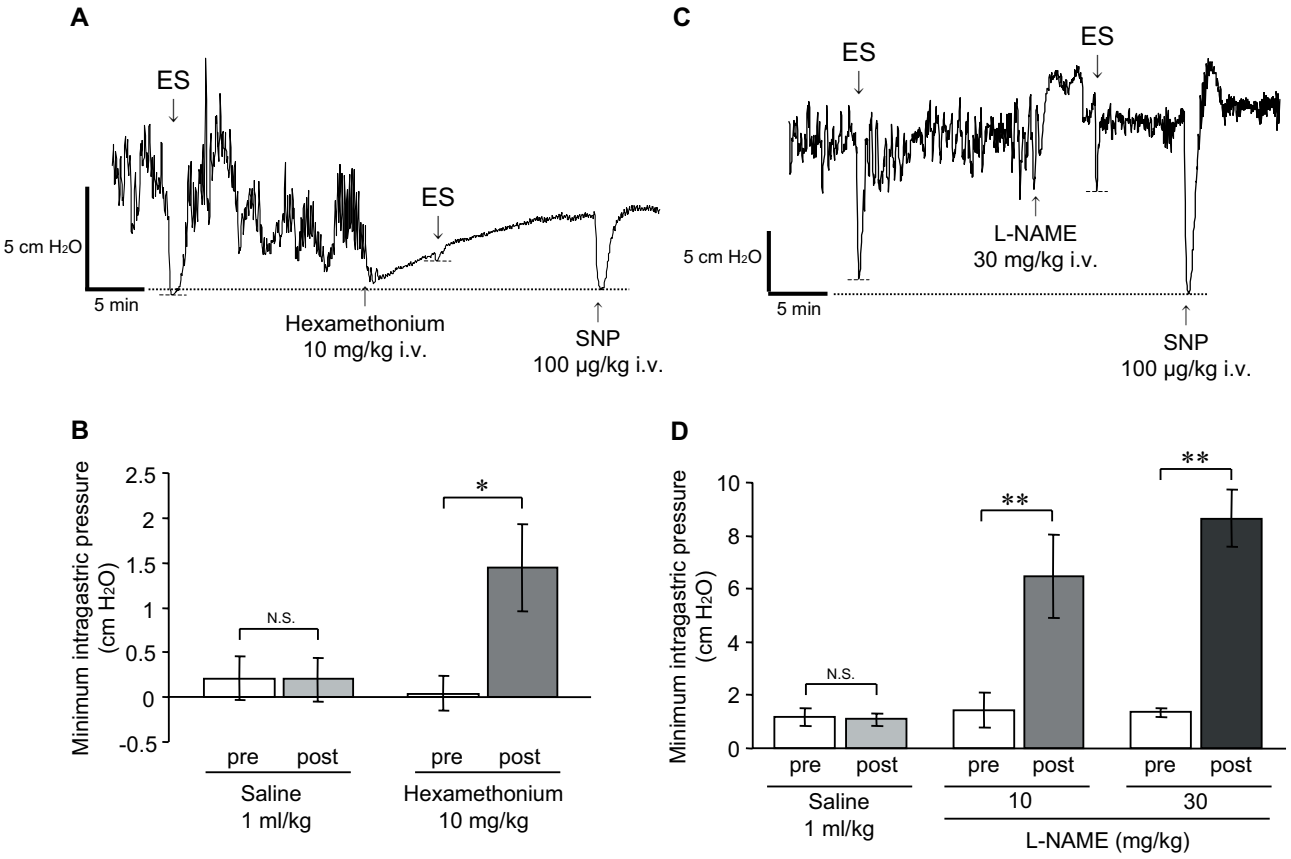

Figure 3. Effects of hexamethonium (10 mg/kg, i.v.) and L-NAME (10 or 30 mg/kg, i.v.) on the change in the intragastric pressure (IGP) induced by electrical stimulation (ES) at 2 Hz. **A.** and **C.** – actual traces. **B.** and **D.** – mean values of the action of hexamethonium (10 mg/kg, i.v.) and L-NAME (10 and 30 mg/kg, i.v.) obtained from 5 experiments. SNP, sodium nitroprusside dihydrate; * $p < 0.05$; ** $p < 0.01$ (paired *t*-test).

Effects of apomorphine on the spontaneous activity of AP neurons in slice preparations

To observe the effects of apomorphine on the spontaneous activity of AP neurons, single unit discharges were recorded extracellularly from AP neurons in slice preparations. Single unit discharges showed firing rates ranging between 0.37 to 1.85 unit discharges/s (1.22 ± 0.20, $n = 8$). As shown in Fig. 6, apomorphine (10 μmol/l) significantly enhanced the frequency of spontaneous single unit discharges, and the effects lasted for prolonged periods (more than 1 h) after the wash out of apomorphine.

Discussion

It has been reported that i.v. administration of apomorphine, an emetic considered to act directly on the AP neurons through dopamine receptors (Andrews et al. 1990, 2001), into the conscious dog (Lefebvre et al. 1981) or cat (Abrahamsson et al. 1973) evokes gastric relaxation and vomiting. It has also been reported that microinjections of DL-homocystic acid, which is thought to excite cell bodies and not axons (Ziegygansberger and Puil 1973; Goodchild et al. 1982), into the AP of anesthetized rabbits provoked gastric relaxation with small changes in blood pressure but marked excitatory effects on respiration (Bongianni et al. 1994). On the other hand, a recent study indicates that i.v. administration of apomorphine evokes two distinctive gastric motor responses in the rat (Koga et al. 2003). These are inhibition of the phasic contraction of the stomach followed by an increase in the frequency of small phasic contractions accompanied by an increase in gastric tone. These appear with relatively short and long delay, respectively, with no gastric relaxation in either the proximal or distal stomach (Koga et al. 2003). In the present experiments, however, we found that both ES and apomorphine (1–100 μmol/l) applied to AP neurons decrease IGP. In addition we also found that apomorphine applied to the AP increases IGV concentration-dependently. Taken together, these observa-

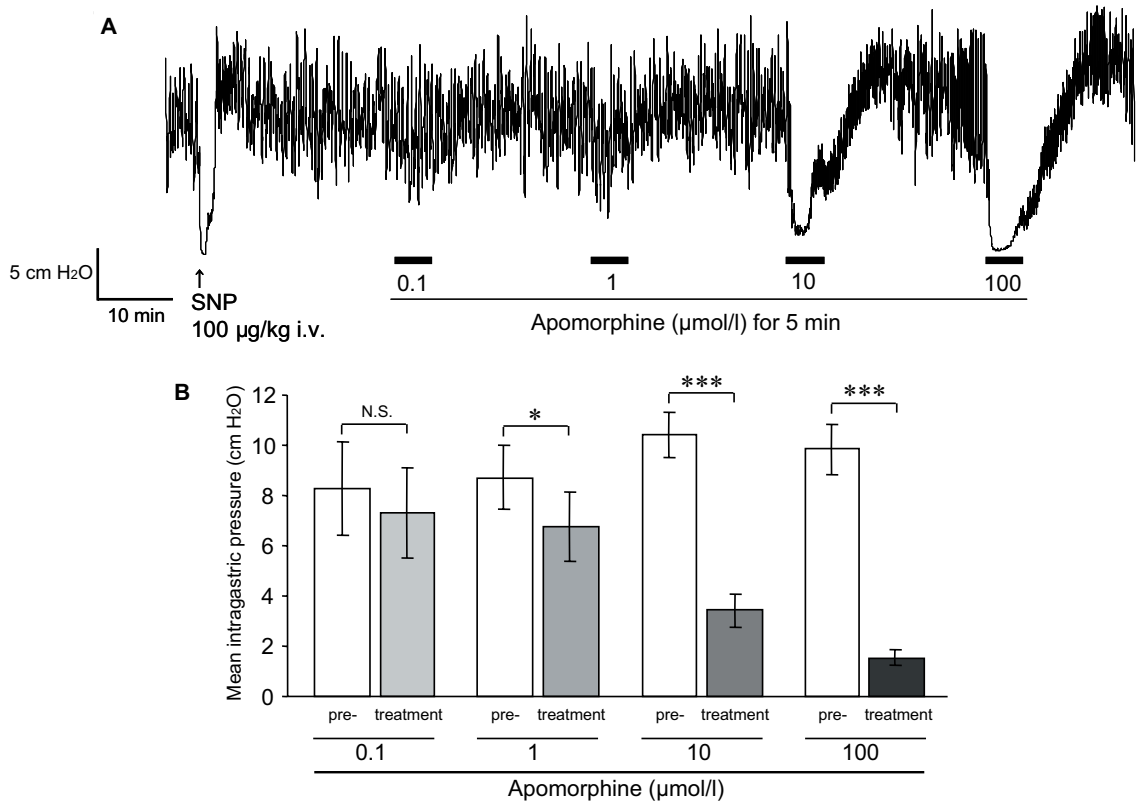

Figure 4. Effects on intragastric pressure (IGP) of local application of apomorphine to area postrema (AP). **A.** Actual trace showing the effects of local application of apomorphine (0.1–100 µmol/l) on IGP. The mean values of IGP for 5 min before and during application of apomorphine were used. Apomorphine reduced the IGP concentration-dependently. **B.** The relationship between the concentration of apomorphine and relative change in IGP ($n = 5$). SNP, sodium nitroprusside dihydrate; N.S., non significant; * $p < 0.05$; *** $p < 0.001$ (paired t-test).

tions strongly indicate that gastric relaxation occurs when the AP neurons are excited by ES or apomorphine in the rat. Therefore, it seems reasonable to conclude that electrical or chemical (apomorphine) stimulations of AP neurons relax the stomach in animals with (e.g. the dog or cat) or without (e.g. the rabbit or rat) an emetic reflex, and AP neurons play some physiological role in the regulation of stomach motility. The present results provide the first morphological evidence that rat stomach receives efferent inputs from neurons in the AP area, and the evidence that apomorphine directly acts on the AP neurons and increases the frequency of spontaneous discharges in the slice preparations.

For several decades, the primary function of the AP had been considered to be the chemoreceptor trigger zone for emesis (Borison and Wang 1953). However, subsequent investigations revealed some functional roles of AP in regulation of cardiovascular (Gatti et al. 1985; Sun and Spyer 1996), respiratory (Srinivasan et al. 1993) and gastric (Bongianni et al. 1994; Tsukamoto and Adachi 1994; Sabbatini et al. 2004) systems and the control of food intake (Contreras et al. 1984; van der Kooy 1984). Bilateral vagotomy abolished the gastric and cardiovascular effects, and comparable gastric relaxation was also induced after the treatment of the animals with atropine and guanethidine. These observations, therefore, indicate that AP plays a role in the control of gastric motility *via* vagus nerves and non-adrenergic non-cholinergic intramural inhibitory neurons (Bongianni et al. 1994). In the present experiments, intravenously applied hexamethonium or L-NAME greatly suppressed the gastric relaxation evoked by ES applied to AP, confirming that the gastric relaxation is due to the activation of non-adrenergic non-cholinergic intramural inhibitory neurons (Bongianni et al. 1994). It should be stressed again that this role of AP is being exhibited in animal species with or without the emetic reflex (Lefebvre et al. 1981; Carpenter et al. 1988), thereby indicating a possible inhibitory role of AP neurons in the control of gastric motility in addition to the emetic reflex.

In the previous and present experiments, apomorphine induced an increase in the frequency of phasic contraction accompanied by an increase in the gastric tone, and this phenomenon was considered to be induced by the action

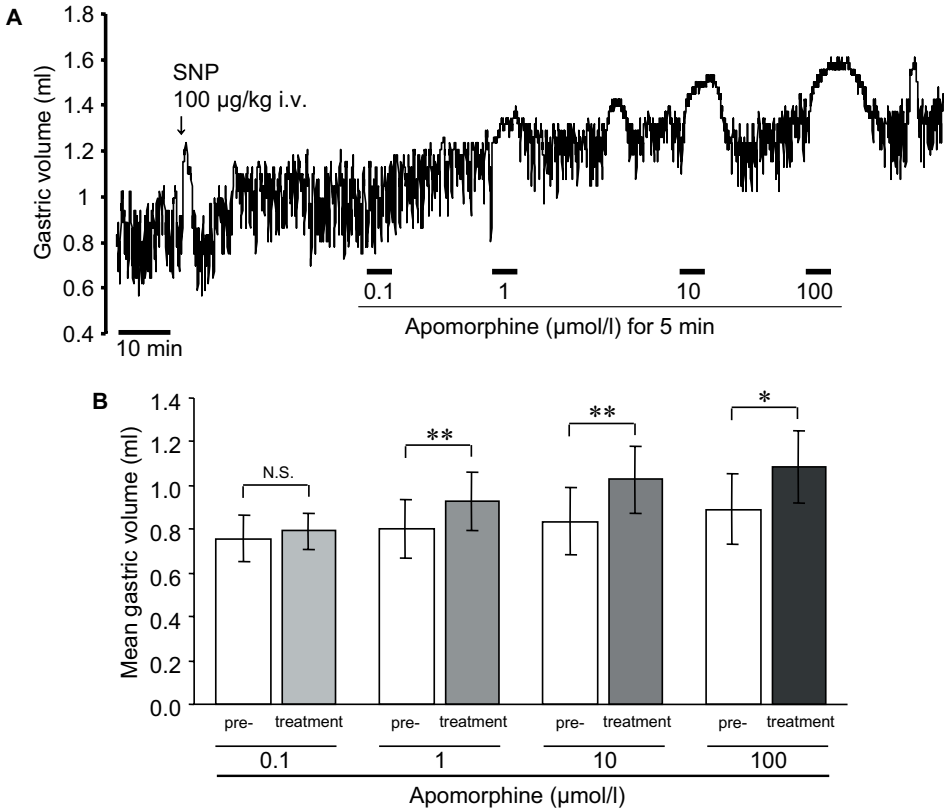

Figure 5. Effects of local application of apomorphine (0.1–100 µmol/l) on the intragastric volume (IGV) measured by barostat method. **A.** An actual trace of the effects of apomorphine on the IGV. **B.** The relationship of the concentration of apomorphine and relative change in IGV ($n = 5$). The mean values of IGV for 5 min before and during application of apomorphine were measured. SNP, sodium nitroprusside dihydrate; N.S., non significant; * $p < 0.05$; ** $p < 0.01$ (paired t-test).

of apomorphine on the AP with longer delay (Koga et al. 2003). Similar increase in the frequency of phasic contraction with an increase in the gastric tone was also observed by i.v. administration of SNP in the present experiments (see for example Figs. 2A and 4A), thereby indicating that this might be due to the reflex after the reduction in the IGP. Bilateral vagotomy abolished the increase in frequency of contraction after application of SNP, or ES in the present experiments. These observations strongly indicate the possible involvement of the vago-vagal reflex in the increase in frequency of the phasic contraction and gastric tone after the application of SNP or apomorphine, and would explain the longer delay reported in the previous study (Koga et al. 2003).

Neuroanatomical studies performed mainly by use of horseradish peroxidase during the last three decades indicate that the AP receives vagal afferent inputs (Kalia and Mesulam 1980; Ciriello et al. 1981; Kalia and Sullivan 1982). In addition, ES of the ventral and dorsal gastric vagal branches elicited orthodromic unitary responses in the AP neurons, providing functional evidence that AP receives vagal afferent inputs from the stomach and suggests a possible role of the AP in the regulation of food intake (Yuan and Barker 1993). On the other hand, in the present experiments, we found, for the first time, retrogradely labeled neurons in the AP on application of DiI to the gastric corpus. The neurons in NTS, which receive visceral afferent projections, were not labeled by DiI applied to the stomach, thereby indicating the presence of efferent inputs from the AP to the gastric corpus. Taken together, it seems reasonable to conclude that afferent and efferent inputs exist between the AP and gastric corpus in rat. Whether the gastric relaxation obtained by electrical or chemical stimulation of the AP in this study, is effectively mediated via the direct efferent pathway between the AP and the stomach shown morphologically in this study, can not yet be concluded with certainty and requires additional experiments. Indeed, the efferent pathway activated by electrical or chemical stimulation of the AP might also run over the DMV (Fig. 7).

In conclusion, we confirmed previous observations that chemical stimulation of the AP by local application of apo-

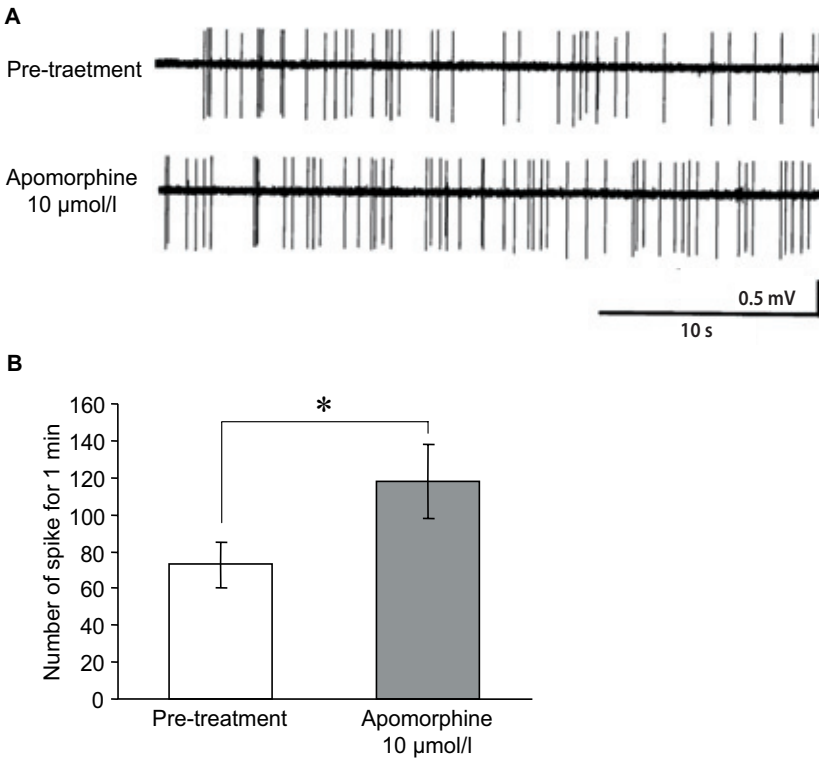

Figure 6. Effect of apomorphine (10 μmol/l) on the spontaneous activity recorded extracellularly from area postrema (AP) neurons in slice preparations. **A.** An actual trace showing the effects of apomorphine (10 μmol/l) on the spontaneous discharge recorded from AP neurons. **B.** The mean value of the effects of apomorphine (10 μmol/l) on the spontaneous activity ($n = 8$). * $p < 0.05$ (paired t-test).

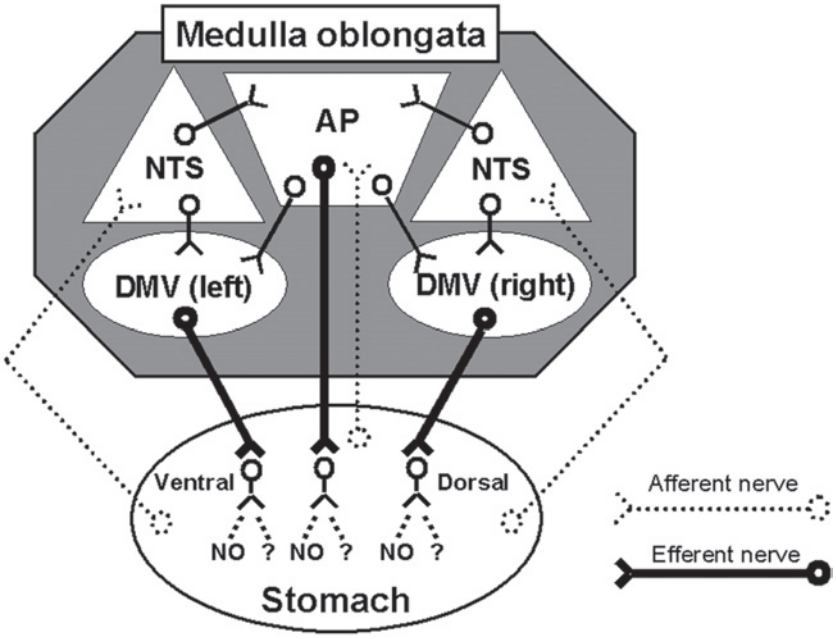

Figure 7. Schematic illustration of the possible efferent neural pathways from area postrema (AP) or dorsal motor nucleus of vagus (DMV) to stomach. Bold and dotted lines indicate efferent and afferent nerves, respectively. NTS, nucleus tractua solitarius.

morphine induces gastric relaxation, and showed that ES of the AP neurons also evokes gastric relaxation, thereby indicating the possible role of AP in the control of the motility of the stomach in physiological or pathophysiological conditions.

References

Abrahamsson H., Jansson G., Martinson J. (1973): Vagal relaxation of the stomach induced by apomorphine in the cat. Acta Physiol. Scand. **88**, 296–302

Andrews P. L., Davis C. J., Bingham S., Davidson H. I., Hawthorn J., Maskell L. (1990): The abdominal visceral innervation and the emetic reflex: pathways, pharmacology, and plasticity. Can. J. Physiol. Pharmacol. **68**, 325–345

Andrews P. L., Kovacs M., Watson J. W. (2001): The anti-emetic action of the neurokinin1 receptor antagonist CP-99, 994 does not require the presence of the area postrema in the dog. Neurosci. Lett. **314**, 102–104

Blessing W. W. (1997): Anatomy of the lower brainstem. In: The Lower Brainstem and Body Homeostasis. pp. 29–100, Oxford University Press, Oxford

Bongianni F., Mutolo D., Srinivasan M., Staderini G., Baccari M. C., Calamai F., Pantaleo T. (1994): Gastric relaxation in response to chemical stimulation of the area postrema in the rabbit. Brain Res. **646**, 307–311

Borison H. L. (1989): Area postrema: chemoreceptor circum ventricular organ of the medulla oblongata. Prog. Neurobiol. **32,** 351–390

Borison H. L., Wang S. C. (1953): Physiology and pharmacology of vomiting. Pharmacol. Rev. **5**, 193–230

Browning K. N., Renehan W. E., Travagli R. A. (1999): Electrophysiological and morphological heterogeneity of rat dorsal vagal neurons which project to specific areas of the gastrointestinal tract. J. Physiol. **517,** 521–532

Buxton I. L., Cheek D. J., Eckman D., Westfall D. P., Sanders K. M., Keef K. D. (1993): N^G-nitro-L-arginine methyl ester and other alkyl esters of arginine are muscarinic receptor antagonists. Circ. Res. **72**, 387–395

Carpenter D. O., Briggs D. B., Knox A. P., Strominger N. (1988): Excitation of area postrema neurons by transmitters, peptides, and cyclic nucleotides. J. Neurophysiol. **59**, 358–369

Ciriello J., Hrycyshyn A. W., Calaresu F. R. (1981): Glossopharyngeal and vagal afferent projections to the brain stem of the cat: a horseradish peroxidase study. J. Auton. Nerv. Syst. **4**, 63–79

Contreras R. J., Kosten T., Bird E. (1984): Area postrema: part of the autonomic circuitry of caloric homeostasis. Fed. Proc. **43**, 2966–2968

Gatti P. J., Souza J. D., Taveira Da Silva A. M., Quest J. A., Gillis R. A. (1985): Chemical stimulation of the area postrema induces cardiorespiratory changes in the cat. Brain Res. **346**, 115–123

Goodchild A. K., Dampney R. A. L., Bandler R. A. (1982): A method for evoking physiological response by stimulation of cell bodies, but not axons of passage within localized regions of the central nervous system. J. Neurosci. Methods **6**, 351–363

Kalia M., Mesulam M. M. (1980): Brain stem projections of sensory and motor-components of the vagus complex in the cat: II. Laryngeal, tracheobronchial, pulmonary, cardiac, and gastrointestinal branches. J. Comp. Neurol. **193**, 467–508

Kalia M., Sullivan J. M. (1982): Brainstem projections of sensory and motor components of the vagus nerve in the rat. J. Comp. Neurol. **211**, 248–265

Koga T., Fukuda H. (1992): Neurons in the nucleus of the solitary tract mediating inputs from emetic vagal afferents and the area postrema to the pattern generator for the emetic act in dogs. Neurosci. Res. **14**, 166–179

Koga T., Kobashi M., Mizutani M., Tsukamoto G., Matsuo R. (2003): Area postrema mediates gastric motor response induced by apomorphine in rats. Brain Res. **960**, 122–131

Lefebvre R. A., Willems J. L., Bogaert M. G. (1981): Gastric relaxation and vomiting by apomorphine, morphine and fentanyl in the conocious dog. Eur. J. Pharmacol. **69**, 139–145

Myers R. D., Adell A., Lankford M. F. (1998): Simultaneous comparison of cerebral dialysis and push-pull perfusion in the brain of rats: a critical review. Neurosci. Biobehav. Rev. **22**, 371–387

Paxinos G., Watson C. (2005): The Rat Brain in Stereotaxic Coordinate. (5th ed.), Elsevier Academic Press, San Diego

Rogers R. C., Herman G. E., Travagli R. A. (1999): Brainstem pathways responsible for oesophageal control of gastric motility and tone in the rat. J. Physiol. **514,** 369–383

Sabbatini M., Molinari C., Grossini E., Mary D. A., Vacca G., Cannas M. (2004): The pattern of c-fos immunoreactivity in the hindbrain of the rat following stomach distension. Exp. Brain Res. **157**, 315–323

Srinivasan M., Bongianni M., Fontana G. A., Pantaleo T. (1993): Respiratory responses to electrical and chemical stimulation of the area postrema in the rabbit. J. Physiol. **463**, 409–420

Sun M. K., Spyer K. M. (1996): GABA-induced inhibition of medullary vasomotor neurons by area postrema stimulation in rats. J. Physiol. **436**, 669–684

Tsukamoto G., Adachi A. (1994): Neural responses of rat area postrema to stimuli producing nausea. J. Auton. Nerv. Syst. **49,** 55–60

van der Kooy D. (1984): Area postrema: site where cholocystokinin acts to decrease food intake. Brain Res. **295,** 345–347

Yuan C. S., Barber W. D. (1993): Area postrema: gastric vagal input from proximal stomach and interactions with nucleus tractus solitarius in cat. Brain Res. Bull. **30,** 119–125

Ziegygansberger W., Puil E. A. (1973): Actions of glutamic acid on spinal neurons. Exp. Brain Res. **17,** 35–49

Received: May 8, 2008
Final version accepted: July 15, 2008