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Hindbrain GABA Receptors Influence Parasympathetic Outflow to the Stomach

Abstract. *Blockade of γ-aminobutyric acid receptor function by direct microinjection of bicuculline into the nucleus ambiguus in cats produced a marked increase in gastric motility which was mediated by the vagus nerve. This effect was reversed by muscimol. These data indicate that the nucleus ambiguus may be an important brain site influencing gastric function and that the neurotransmitter controlling parasympathetic outflow from this nucleus to the stomach is γ-aminobutyric acid.*

There are large gaps in our knowledge of the central nervous system (CNS) pathways and neurotransmitters that control parasympathetic outflow to the gastrointestinal tract. Anatomical and physiological evidence indicates that the nucleus tractus solitarius, dorsal motor nucleus of the vagus, parabrachial nucleus, hypothalamus, and central nucleus of the amygdaloid complex comprise part of the pathways for gastrointestinal control (*1–3*). The neurotransmitters associated with these pathways are unknown. However, recent cardiovascular studies suggest that γ-aminobutyric acid (GABA) may function as a CNS neurotransmitter controlling parasympathetic outflow (*4*). In addition, these studies have revealed that the nucleus ambiguus is also important for control of parasympathetic outflow and that activation of GABA receptors on this nucleus exerts a profound effect on parasympathetic activity to the heart (*4*). To obtain new information on CNS control of gastrointestinal function, we studied the effect of augmentation and reduction of GABA receptor activity at the nucleus ambiguus on contractile activity of the stomach.

Cats were anesthetized with α-chloralose (70 to 80 mg/kg, intravenously) and artificially ventilated with room air through a tracheal cannula. The animals were then paralyzed with decamethonium bromide (0.25 mg/kg, intravenously), given every 45 minutes or as needed. The femoral artery was catheterized for recording blood pressure and limb leads (lead 2) were placed on the extremities for recording the electrocardiogram. Arterial blood pressure and the electrocardiogram were monitored on a Gould brush recorder. Rectal temperature was monitored and maintained between 36° and 38°C with an infrared lamp. A midline abdominal incision was made and extraluminal strain gage force transducers (*5*) were sutured to the antrum and pylorus in the transverse axis to record circular muscle activity. The force transducers were calibrated prior to use and had equal sensitivities. Smooth muscle activity was registered on a Grass polygraph and motility indices (*6*) were calculated for antral and pyloric responses. The splanchnic nerves were sectioned and the adrenal glands were ligated in each animal because adrenergic innervation of the stomach and circulating catecholamines from the adrenal glands oppose parasympathetic effects on gastric motility (*7*).

The cats were then mounted in a David Kopf stereotaxic instrument and the dorsal surface of the lower brainstem was exposed by limited occipital craniotomy. Coordinates from Berman (*8*) were used to locate the nucleus ambiguus. In a few experiments we studied the effect on gastric motility of reducing GABA receptor activity at the dorsal motor nucleus of the vagus. This nucleus was also located with Berman's coordinates. The shaft of a 26-gauge Quincke (Babcock) spinal needle that had been cut from its base and filed smooth was guided stereotaxically at a 36° caudal angle into the nuclear area. The needle was attached to a 10-μl Unimetrics syringe with PE-20 tubing. Pharmacologic agents that augment (muscimol) and reduce (bicuculline methiodide) GABAergic tone (*9*) were dissolved in artificial cerebrospinal fluid (*10*) and administered by infusion with a Sage infusion pump at the rate of 0.1 μl/min for 10 minutes. For control infusions artificial cerebrospinal fluid alone was used. At the termination of each experiment the animal was killed and the brain was removed and placed in 10 percent Formalin. Frozen 50-μm sections were cut and mounted on slides. From these sections the cannula track and the injection site were identified and verified.

Microinjections of the GABA receptor antagonist bicuculline (10 ng/min for 10 minutes) into the left nucleus ambiguus resulted in pronounced increases in antral and pyloric contractile activity (Fig. 1B). The GABA receptor agonist muscimol was then microinjected (10 ng/min for 10 minutes) into the same site, and a striking decrease in gastric motility was observed (Fig. 1C). The same procedure was carried out for the right nucleus ambiguus (Fig. 1, D and E).

From the gastric motility tracings, the

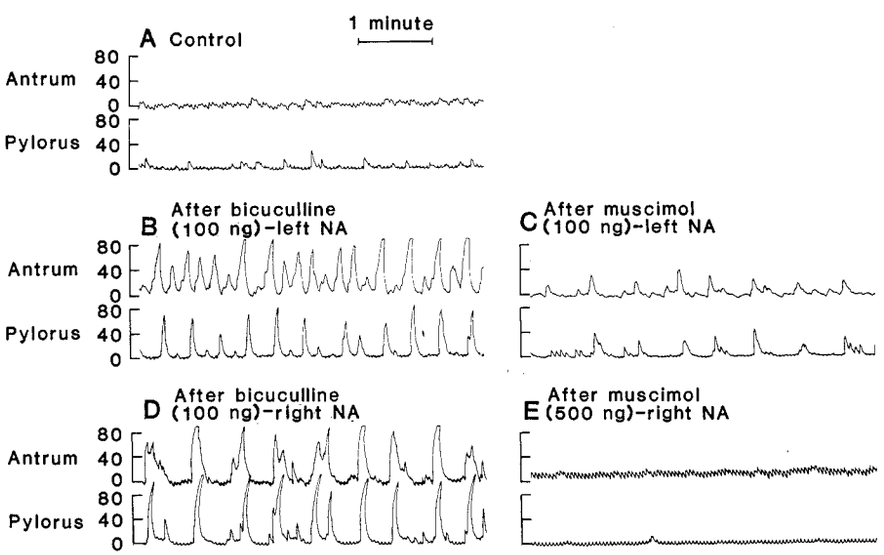

Fig. 1. (A to E) Effects of GABA receptor blockade (bicuculline) and stimulation (muscimol) at the nucleus ambiguus (*NA*) on antral and pyloric smooth muscle activity. Values on ordinate represent force in grams.

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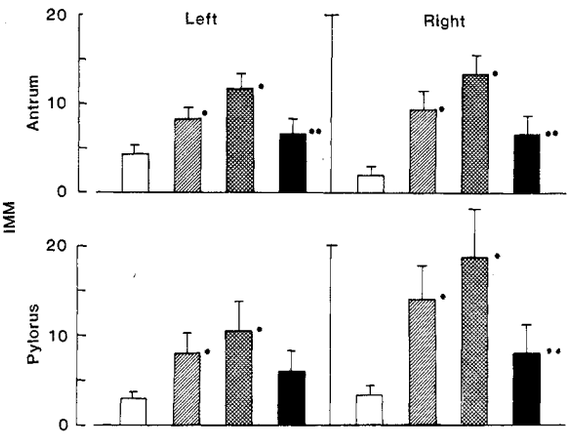

Fig. 2. Effects of microinjection of bicuculline into the left or right nucleus ambiguus on gastric motility, and the effect of a subsequent microinjection of muscimol into nucleus ambiguus after a maximal stimulatory effect of bicuculline was noted. Open histograms represent baseline IMM. Histograms with slanted shading or cross-hatching represent the IMM during the first and second 10-minute period after bicuculline microinjection, respectively. The solid histograms indicate the effect of muscimol microinjection given at the time of the peak of the bicuculline-induced response (that is, at the end of the second 10-minute period). Histograms with one asterisk indicate that the IMM was significantly greater than the corresponding control value ($P < .05$, paired t-test). Histograms with two asterisks indicate that the IMM was significantly less than the corresponding value obtained during the periods when bicuculline was administered ($P < .05$). Values are means ± standard errors for seven animals.

index of motility per minute (IMM) was calculated at 10-minute intervals before, during, and after the microinjection of GABAergic drugs into the left and right nucleus ambiguus of seven animals (Fig. 2). Microinjection of bicuculline into the left or right nucleus ambiguus produced a significant increase in the IMM. Subsequent microinjection of muscimol into this nucleus at the peak of the bicuculline-induced response produced a significant decrease in the IMM. Microinjection of cerebrospinal fluid, however, produced no statistically significant change. Baseline values for heart rate and arterial pressure were 201 ± 11 beats per minute and 111 ± 4 mmHg. Bicuculline microinjected into the left nucleus ambiguus decreased heart rate to 146 ± 13 beats per minute and arterial pressure to 74 ± 7 mmHg. With muscimol administration, heart rate and blood pressure increased toward the control values. Subsequent microinjection of bicuculline into the right nucleus ambiguus decreased heart rate to 130 ± 18 beats per minute and arterial pressure to 86 ± 8 mmHg.

In two additional experiments, bicuculline (100 ng) was microinjected into the left nucleus ambiguus of one cat and the right nucleus ambiguus of a second cat. At the time of a peak increase in gastric motility, the ipsilateral vagus nerve in the cervical region was sectioned. This abolished the bicuculline-induced increase in motility, indicating that the effect of bicuculline is indeed mediated by the parasympathetic nerves. Also, three experiments were performed where the dose of bicuculline found to be effective when microinjected into the nucleus ambiguus was administered into the dorsal motor nucleus of the vagus. No effect on gastric motility was observed.

In summary, microinjection of a drug that antagonizes GABA receptors in the nucleus ambiguus increases parasympathetic outflow to the gastrointestinal tract. To our knowledge this is the first evidence implicating a specific CNS neurotransmitter in the control of gastric motility. Earlier data indicate the presence of a significant amount of GABA and high-affinity binding sites for GABA at the nucleus ambiguus (*11*). Furthermore, the enzyme responsible for synthesizing GABA, glutamic acid decarboxylase—which is also a marker for GABA neurons—is present in significant quantities at the nucleus ambiguus (*12*).

The dorsal motor nucleus of the vagus traditionally has been accepted as being responsible for regulating parasympathetic control of the gastrointestinal tract. This has been shown by recording gastrointestinal function and electrically stimulating the dorsal motor nucleus (*2*), injecting horseradish peroxidase into the stomach wall and noting reaction product in the nucleus (*3*), and using a metabolic mapping technique (the autoradiographic 2-deoxy-D-[^{14}C]glucose method) to determine brain areas affected during insulin-dependent hypoglycemia (*3*). While our study was in progress, Kalia and Mesulum (*13*) showed that some of the parasympathetic innervation of the stomach arises from the nucleus ambiguus: horseradish peroxidase was injected into the stomach wall of the cat and labeled cell bodies were noted in the nucleus ambiguus. Our findings are consistent with those of Kalia and Mesulum and, in addition, indicate that a GABAergic mechanism at the nucleus ambiguus strongly influences parasympathetic outflow to the gastric smooth muscle.

DANIEL J. WILLIFORD
Department of Pharmacology, Georgetown University, Washington, D.C. 20007
HERBERT S. ORMSBEE III
Department of Surgery, University of Maryland School of Medicine, Baltimore 21201
WESLEY NORMAN
Department of Anatomy, Georgetown University
JOHN W. HARMON
Department of Surgery, Walter Reed Army Institute of Research, Washington, D.C. 20012
THOMAS Q. GARVEY III
National Institute of Arthritis, Metabolism, and Digestive Diseases, Bethesda, Maryland 20205
JOSEPH A. DIMICCO
RICHARD A. GILLIS
Department of Pharmacology, Georgetown University

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