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Antroduodenal coordinated contractions as studied by chemical ablation of myenteric neurons in the gastroduodenal junctional zone.

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Abstract

Antroduodenal contractions were studied in rat preparations. Augmented duodenal contractions occurred spontaneously in coordination with antral contractions in normal and saline-pretreated preparations. The coordination did not occur when muscle layers and the myenteric plexus were transversely cut at the duodenum just anal to the gastroduodenal junction. In silent preparations, the coordinated contractions were produced by neostigmine or domperidone. When the antroduodenal junctional zone was pretreated with benzalkonium chloride, the augmented duodenal contractions did not occur spontaneously, and even after administration of neostigmine and domperidone although antral contractions occurred spontaneously. In these preparations, there were notably few myenteric neurons in the junctional zone, but the neurons were distributed normally in the areas where motility was recorded. The results suggest that myenteric neurons mediate antroduodenal coordinated contractions and that the coordination is modified by myenteric cholinergic excitatory and dopaminergic inhibitory pathways.

KEYWORDS: gastroduodenal motility, coordinated contraction, myenteric plexus, rat

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Antroduodenal contractions were studied in rat preparations. Augmented duodenal contractions occurred spontaneously in coordination with antral contractions in normal and saline-pretreated preparations. The coordination did not occur when muscle layers and the myenteric plexus were transversely cut at the duodenum just anal to the gastroduodenal junction. In silent preparations, the coordinated contractions were produced by neostigmine or domperidone. When the antroduodenal junctional zone was pretreated with benzalkonium chloride, the augmented duodenal contractions did not occur spontaneously, and even after administration of neostigmine and domperidone although antral contractions occurred spontaneously. In these preparations, there were notably few myenteric neurons in the junctional zone, but the neurons were distributed normally in the areas where motility was recorded. The results suggest that myenteric neurons mediate antroduodenal coordinated contractions and that the coordination is modified by myenteric cholinergic excitatory and dopaminergic inhibitory pathways.

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It has been postulated that an antral contraction causes an augmented contraction following a relaxation in the uppermost duodenum (gastroduodenal coordination), and that this coordinated contraction or electrical activity (pace-setter potentials, slow waves or basic electrical rhythm) is controlled through intramural myogenic or neurogenic pathways.

Bortoff and Weg (1) and Bortoff and Davis (2) stated that the coordinated contraction is due to transmission of the antral pace-setter potential via the longitudinal muscle fibers which cross the pylorus from the stomach into the duodenum.

Atanassova (3, 4) reported that the coordination of the contraction is a function of the intramural nervous system. Allen *et al.* (5) explained that the coordination is accomplished through intrinsic nerve connections which transverse the antroduodenal junction, or by the chyme as it passes from the stomach to the duodenum. Bedi and Code (6) stated that coordination of electric activities of the antrum and duodenum is accomplished by messages transmitted via the neural or muscular elements in the wall of the antroduodenal junction.

It has recently been demonstrated in the rat that surfactants applied to the serosal surface selectively ablate the myenteric neurons of the

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colon, anorectum (7) and jejunum (8, 9). Based on this demonstration, we studied whether the antroduodenal coordinated contractions occur through the neural connections in isolated rat antroduodenal preparations.

Materials and Methods

Twenty-five Wister rats of either sex, weighing 170–370 g were used. They were fasted for 24 h before the experiments, but tap water was given *ad libidum*. Animals were anesthetized with ether and exsanguinated. The antroduodenal preparation consisted of 1 cm of the antrum and 1 cm of the uppermost duodenum. The residual contents of the preparation were washed thoroughly with warm Tyrode solution (145 mM NaCl, 2.7 mM KCl, 1.5 mM CaCl₂, 0.7 mM MgCl₂, 4.8 mM NaHCO₃, 0.3 mM NaH₂PO₄ and 11.1 mM glucose). Then, the preparation was suspended in a bath filled with 15 ml Tyrode solution bubbled with 95 % O_2 and 5 % CO_2 at 37 ± 1°C. The circular muscle contractions of the antrum (3 mm oral) and the duodenum (3 mm anal to the antroduodenal junction) were separately recorded under a 1-g load with isotonic transducers (Fig. 1). In some cases, neostigmine methylsulfate (1 μM) (Shionogi-Seiyaku Co., Ltd., Osaka, Japan) or domperidone (3 μM) (Kyowa-Hakko Co., Ltd., Tokyo, Japan) was administered during the recording.

To ascertain if the antral contraction reached the duodenum across the antroduodenal junction, the longitudinal and circular muscle layers and the myenteric plexus located between these muscle layers were sectioned circumferentially at a level just anal to the junction under a dissecting microscope. The myoelectrical activity of the duodenum was also recorded by monopolar suction electrode. The mechanical activities of the antrum and duodenum were recorded simultaneously.

The following procedures were used to ablate the myenteric neurons of the antroduodenal junctional zone. The animals were anesthetized with pentobarbital sodium (50 mg/kg, i.p., Abbott Lab., North Chicago, USA). A midline incision of the abdomen was made, and the antroduodenal junctional zone was floated from the neighboring tissue. Modifying the methods of Fox *et al.* (9), a 1 mm thick cotton string soaked with a mixture containing 0.062 % benzalkonium chloride (Takeda-Yakuhin Co. Ltd., Osaka, Japan) and 0.9 % NaCl was placed on the serosa around the junctional zone every 5 min for 30 min

(total of six applications). Afterwards, the serosa of the treated area and the neighboring tissue was thoroughly rinsed with 0.9 % saline solution. In the control group, a cotton string soaked with 0.9 % saline solution was applied in the same as that in the benzalkonium-pretreated group. Twenty-eight days after the treatment, the same antroduodenal area that taken from normal rats was prepared.

To histologically examine the effect of benzalkonium chloride on the myenteric neurons, the preparation was fixed with 10 % formalin after the experiment. Serial and longitudinal sections (20 μm thick) of paraffine-embedded preparations were prepared, and every twentieth section was stained by the thionine or van Gieson's methods. The ganglion cells in the myenteric plexus were counted in more than 15 sections from each animal under a light microscope. The results were expressed as the number of ganglion cells per section per millimeter length in the longitudinal direction from the mid-point of the antroduodenal junctional zone.

In all cases, statistical comparisons were made using Student's *t-test*. All the data are expressed as the mean ± SEM, and n represents the number of preparations from which the data were obtained.
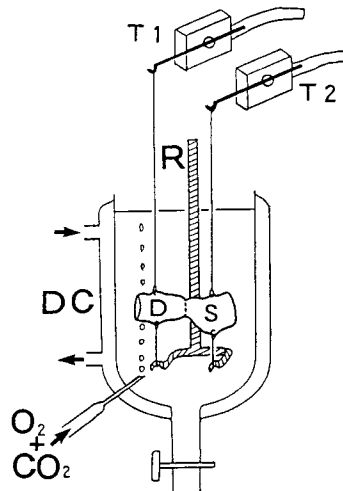
Fig. 1   Experimental arrangement. S and D: Antral part and duodenal part of the isolated gastroduodenal preparation. R: Rod for supporting the antrum and the duodenum separately. T_1 and T_2: Isotonic transducers to record circular muscle motility of the antrum and the duodenum. DC: Double walled chamber, $O_2 + CO_2$: Gas mixture of 95 % O_2 and 5 % CO_2.

Results

Normal preparations. In 9 of 17 preparations, augmented duodenal contractions following antral contractions occurred spontaneously with a constant time lag (Table 1). These contractions were characterized by a marked increase in tone on which 2 to 3 small contractions were superimposed (Fig. 2A). As shown in Fig. 3, spike bursts of the duodenal muscles synchronous to the augmented upward deflections on the mechanical activity recording of duodenum were recorded in the duodenal muscles (n = 3), indicating that

Table 1   Time lag between antral and augmented duodenal contractions

| Contractions	Time lag (sec)	n	Probability
Spontaneous	2.3 ± 0.1	4	$p < 0.01^a$, $p < 0.001^b$
Domperidone-induced	3.2 ± 0.2	3	$p < 0.01^a$, $p < 0.001^c$
Neostigmine-induced	1.3 ± 0.1	3	$p < 0.001^{b,c}$

The data are expressed as mean $\pm$ SEM. a: spontaneous *vs* domperidone, b: spontaneous *vs* neostigmine, c: domperidone *vs* neostigmine.
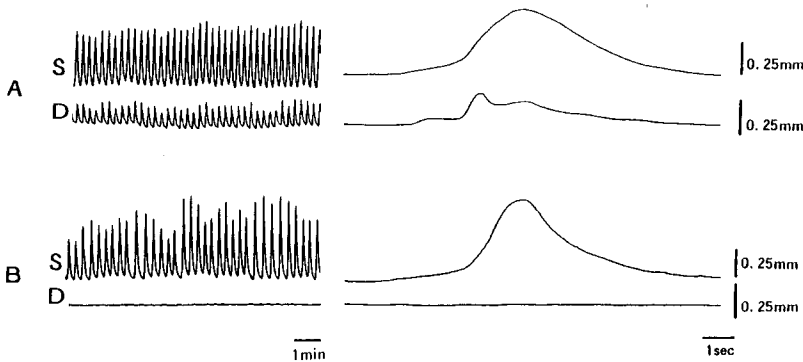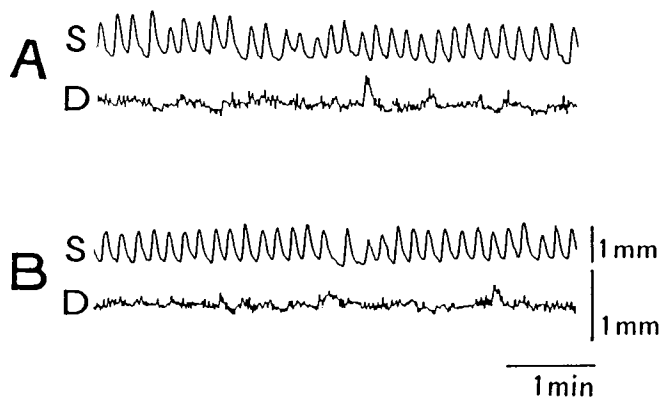

Fig. 2   Motility of the antrum (S) and duodenum (D). The augmented duodenal contraction with apparently the same frequency and duration as those of antral contraction were seen in normal preparation (A). However, in a preparation with completely cut muscle layers, including the myenteric plexus on the anal side of the pylorus, no augmented contractions occurred (B). Each preparation was prepared from two different rats.
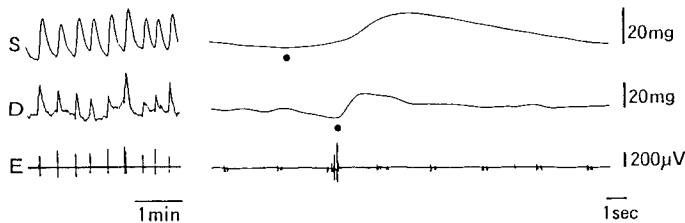
Fig. 3   Motility of the antrum (S) and duodenum (D) and action potentials (E) recorded in the duodenum just oral to the motility recording site. Large spike bursts synchronous to augmented duodenal contractions were recorded. Closed circles show the initiation points of the antral and augmented duodenal contractions.
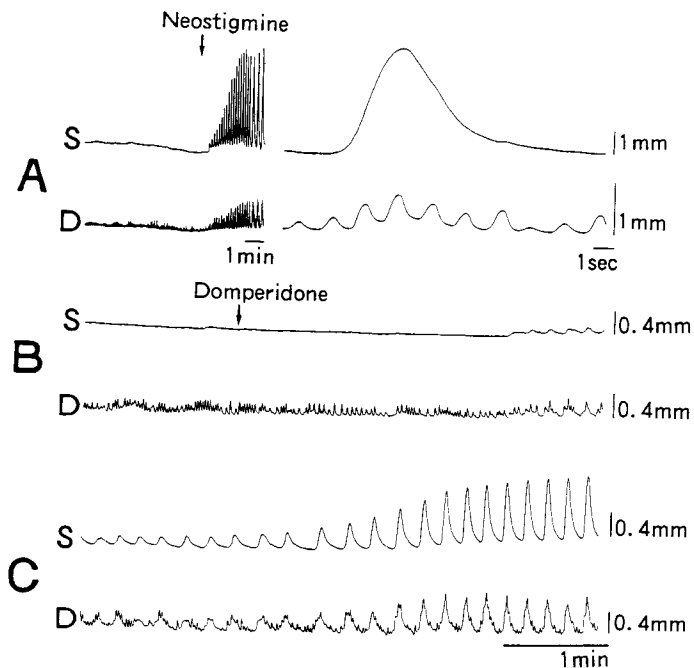
Fig. 4   Antral (S) and augmented duodenal (D) contractions were induced in normal but silent preparations by administration of neostigmine (1 μM, A) and domperidone (3 μM, B and C). B and C are continuous recordings. Each preparation was made from a different rat.

Fig. 5   Motility of the antrum (S) and duodenum (D) in a preparation pretreated with benzalkonium chloride (0.062 %) 28 days before the experiment. A and B are continuous recordings. Histological examination revealed that myenteric neurons near the junctional zone including the antrum and duodenum were ablated but distributed normally at motility-recording sites as shown in Fig. 6.

these upward deflections are due to active contractions of the duodenum. Intervals of the augmented duodenal contractions were closely related to those of the antral contractions (Table 2). The augmented duodenal contraction was abolished by circumferential transection of the muscle layers and myenteric plexus at the gastroduodenal junction (Fig. 2B, n = 4).

In 8 preparations, however, the antral and augmented duodenal contractions did not occur spontaneously. In these preparations, administration of neostigmine or domperidone induced antral contractions and augmented duodenal contractions (Table 2, Fig. 4 A, B, C), which were similar to the spontaneous ones observed in the non-treated preparations (see above).

Time lags between the antral and the augmented duodenal contractions evoked by neostigmine and domperidone were respectively shorter and longer than that of the spontaneous contractions (Table 1).

Preparations pretreated with benzalkonium and saline solution. The augmented duodenal contractions did not occur in any of the benzalkonium-pretreated preparations (n = 4), although the spontaneous antral contractions with an interval equivalent to that in the non-treated or saline-pretreated preparations were recorded (Table 2, Fig. 5). Neither neostigmine nor domperidone induced augmented duodenal contractions in any of the six preparations tested in this study (neostigmine, n = 3; domperidone, n = 3).

On the contrary, the spontaneous antral and the augmented duodenal contractions were observed in all the saline-pretreated preparations (n = 4). In three preparations, however, these contractions ceased during the recording. In such preparations, administration of neostigmine or domperidone evoked the antral and augmented duodenal contractions. The differences in intervals between the evoked contractions were not significant (Table 2).

Distribution of myenteric neurons. In the antral and duodenal zones from the mid-point of the gastroduodenal junction (pylorus) to points to 2 mm oral and 2 mm anal to the junction, there were considerably fewer myenteric neurons when the preparation was pretreated with benzalkonium. However, no significant change was observed in the antral zone from points 2 to 3 mm oral to the junction and in the duodenal zone from points 2 to 3 mm anal to the junction, where motilities of the circular muscles were recorded (Fig. 6).
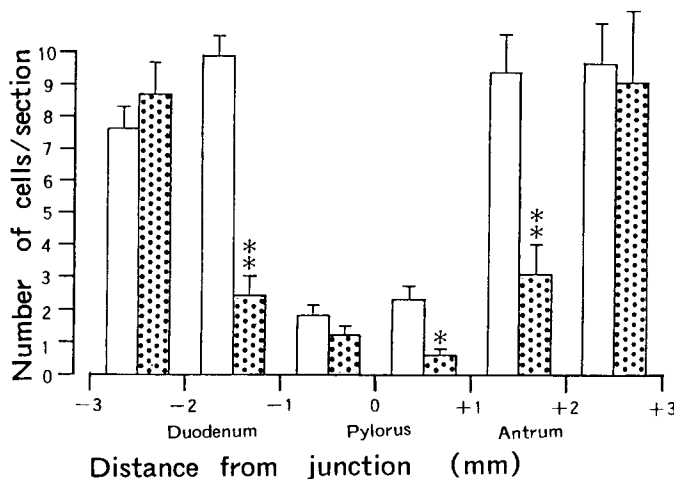

Fig. 6   Histograms showing distribution of myenteric neurons in preparations pretreated with 0.9 % saline solution (open column) and in preparations pretreated with benzalkonium (dotted column). The results were obtained from 6 rats. * p < 0.01, ‡ p < 0.001.

Matsuo *et al.*

Table 2 Intervals of antral and augmented duodenal contractions induced spontaneously, and by neostigmine and domperidone in preparations isolated from normal rats, and 0.062 % benzalkonium- and 0.9 % saline-pretreated rats

Treatment	Spontaneous		Neostigmine		Domperidone	
	Antrum	Duodenum	Antrum	Duodenum	Antrum	Duodenum
Non	18.2 ± 0.5	18.0 ± 0.5	14.4 ± 0.2	14.3 ± 0.2	16.4 ± 0.7	16.5 ± 0.7
Benzalkonium	16.6 ± 1.1	— —	13.6 ± 0.2	— —	14.9 ± 0.3	— —
Saline	18.7 ± 1.0	18.7 ± 1.0	12.1 ± 1.3	12.2 ± 1.2	21.2 ± 0.5	21.4 ± 0.5

Each datum was obtained from 3–4 preparations and is expressed as mean ± SEM. There were no significant differences between antral and augmented duodenal contractions in non- and saline-pretreated preparations. The augmented duodenal contractions did not produce in benzalkonium-pretreated preparations.

Discussion

The present study showed that, in normal and saline-pretreated antroduodenal preparations, antral contractions precede augmented duodenal contractions. The intervals of antral contractions which occurred spontaneously and were induced by neostigmine or domperidone were found to be closely related to those of augmented duodenal contractions. No augmented contractions were recorded after circumferential section of the myenteric plexus together with the muscle layers. Thus, the present study indicates that antral contractions spread to the duodenum across the gastroduodenal junction and produce coordinated (augmented) duodenal contractions. The coordination of these contractions may be mediated by the nervous system.

By application of benzalkonium, myenteric neurons were largely ablated in the area between the anal end of the antrum and the oral end of the duodenum, but they were distributed normally in areas where motility was recorded. In this preparation, it was remarkable that no augmented duodenal contraction (antroduodenal coordinated contraction) occurred even after the administration of neostigmine and domperidone although the antral contraction occurred. This finding strongly suggests that the myenteric neurons in the gastroduodenal junction mediate the coordination of augmented duodenal contractions with antral contractions.

In the normal and saline-pretreated prepara-

tions, neostigmine, a cholinesterase inhibitor, and domperidone, a specific dopamine inhibitor (10) evoked augmented duodenal contractions following antral contractions. In preparations in which myenteric neurons of the gastroduodenal junctional zone were ablated, augmented duodenal contractions were not produced by neostigmine and domperidone. These results suggest that activation of a cholinergic excitatory pathway and/or inhibition of a dopaminergic inhibitory pathway are involved in the initiation of the coordinated antroduodenal contractions.

Domperidone has been demonstrated to induce gastroduodenal coordinated contractions by blocking the dopaminergic inhibitory mechanism in the guinea-pig isolated preparation (11). Dopamine has been shown to be present in significant amounts in the myenteric plexus of the proximal gut in the rat (12), and to have a potent inhibitory effect on the gastrointestinal motility in the dog (13) and the guinea pig (14).

In conclusion, myenteric neurons in the gastroduodenal junctional zone may mediate antroduodenal coordinated contractions. The coordination may be controlled through myenteric cholinergic excitatory and dopaminergic inhibitory pathways.

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