RHYTHMIC CONTRACTIONS INDUCED BY VAGOTOMY
IN THE FUNDUS OF RAT STOMACH

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Abstract—Continuous rhythmic contractions were observed in longitudinal muscle strips of fundus obtained from the chronically vagotomized rat stomach. These vagotomy-induced rhythmic contractions (VRC) were not blocked either by tetrodotoxin \((3 \times 10^{-6} \text{ M})\) or atropine \((3 \times 10^{-7} \text{ M})\), indicating that the VRC was myogenic in origin. It was also found that the VRC had the following characteristics. 1) The VRC was blocked either by \(10^{-5} \text{ M D600}\) or by \(\text{Ca}^{2+}\) deprivation from the medium. 2) On replacement of Na with sucrose or of K with Na, the tone of fundus strips was concomitantly elevated with the cessation of the VRC. 3) \(10^{-5} \text{ M ouabain}\) blocked the VRC. 4) The VRC was blocked by cooling. 5) The VRC disappeared under anoxic conditions. Based on these results possible mechanisms of the VRC are discussed in relation to an energy dependent activity of the cell membrane.

Among the smooth muscle from different tissues which exhibit rhythmic spontaneous contractions \textit{in vitro} are those from the taenia coli, portal vein, etc. These spontaneous activities seem to be myogenic in origin, since various drugs which interfere with the nervous activity or transmission do not abolish the activities (1–3).

On the other hand, rat stomach fundus strips free of mucosa show no spontaneous mechanical activity under usual conditions \textit{in vitro} (4). In a previous paper, however, we reported that when fundus strips were prepared from chronically vagotomized rat stomach they exhibited spontaneous rhythmic contractions and that their maximum contractile responses to cholinergic agonists and to serotonin decreased to an appreciable extent. These findings suggest that vagotomy exerts a profound influence on smooth muscle activities (5).

This study was undertaken to obtain insight into the mechanisms underlying the spontaneous rhythmic contractions. For this purpose the effects of some agents, of deprivation of ions from the bathing fluid, and also of chronic sympathectomy were investigated.

MATERIALS AND METHODS

Male rats of Donryu strain weighing from 240 to 300 g were used. Chronic sympathectomy was performed by excision of celiac ganglion and some length of postganglionic nerve bundle as reported previously (5). Chronic vagotomy was performed by bilateral

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excision of vagus nerves at the gastric end of the esophagus. The animals were fed for 7 to 10 days until excision of the stomach.

Animals were sacrificed by a blow on the head. The stomach was excised, the mucous layer was removed with the aid of an excision microscope, and the fundus strip preparation approximately 30 mm long and 3 mm wide was made essentially according to the method of Vane (6). The strip was cut out along the longitudinal muscle. The circular muscle was not removed from the strip.

The fundus strips were suspended in a 30 ml-organ bath containing modified Krebs-Ringer bicarbonate buffer solution at 37 °C and aerated with 95% O₂ and 5% CO₂. The tension loaded on the strip was 0.5 g. Contractions (tension developments) were recorded isometrically with a force displacement transducer (Nihon-Kohden, FD-5) connected to a polygraph (Nihon-Kohden, RM-25).

The composition of the modified Krebs-Ringer bicarbonate buffer solution was as follows (mM): NaCl 120, KCl 4.7, CaCl₂ 2.2, MgCl₂ 1.2, NaHCO₃ 25, KH₂PO₄ 1.2 and glucose 14. K⁺-free Krebs' solution was prepared by replacing KCl and KH₂PO₄ with equimolar NaCl and NaH₂PO₄ respectively. Na⁺-free Krebs' solution was prepared by replacing NaCl and NaHCO₃ with sucrose, and pH was adjusted to 7.4 with 5 mM Tris buffer. Ca²⁺-free Krebs' solution was prepared by replacing CaCl₂ with NaCl.

RESULTS

Vagotomy-induced rhythmic contractions

Fig. 1 shows typical records of the spontaneous mechanical activity of the normal, the chronically vagotomized and the sympathectomized fundus strips. The normal and the chronically sympathectomized tissues were usually quiescent, while all of chronically vagotomized fundus strips (n=30) exhibited developments of spontaneous rhythmic tension (vagotomy-induced rhythmic contractions, VRC) 1 to 2 g in amplitude and 2 to 3/min in frequency. The effects of morphine (10⁻³ M), hexamethonium (10⁻⁴ M), tetrodotoxin (3 x 10⁻⁶ M), eserine (3 x 10⁻⁶ M) were also examined on vagotomized strips. These agents failed to affect the VRC (Fig. 2). When indomethacin was applied to the medium cumula-

![NORMAL](image1)

![VAGOTOMY](image2)

![SYMPATHETOMY](image3)

FIG. 1. Spontaneous isometric tension development of rat stomach fundus strips.

![NORMAL](image4)

![VAGOTOMY](image5)

![SYMPATHETOMY](image6)

FIG. 2. Effects of agents on the chronically vagotomized strips.
tively, the VRC was inhibited at a relatively high concentration ($3 \times 10^{-5}$ to $10^{-4}$ M).

**Influence of sympathetic nerves on the VRC**

Each of five animals was simultaneously subjected to both sympathectomy and vagotony, and another five animals were given a dose of 5 mg/kg of reserpine intraperitoneally 24 hr before the experiment. In the fundus strips obtained from these animals, the VRC was not observed as depicted in Fig. 3.

**Influence of external ions on the VRC**

When Ca ions were removed from Krebs' solution, the VRC disappeared and the resting tone was also decreased. Similarly, treatment with $10^{-5}$ M D600 resulted in a decrease in resting tone and a disappearance of the VRC (Fig. 4).

When the tissues were exposed to Na⁺-free or K⁺-free Krebs' solution, a large transient
contraction occurred and was followed by a gradual relaxation at the same time the VRC ceased. When the external Na\(^+\) or K\(^+\) concentration in the bath was returned to normal, the VRC gradually recovered.

**Effects of metabolic depletion on the VRC**

Influence of treatment which would lead to metabolic depletion of the strips on the VRC was examined. As shown in Fig. 5, when 10\(^{-5}\) M DNP was added, the VRC gradually decreased and was completely suppressed within 3 to 5 min. When the bath temperature was gradually lowered to below 20°C, frequency of the VRC was reduced and eventually the VRC was nearly suppressed. When the strips were exposed to anoxia, the VRC disappeared within 5 min.

**Effects of ouabain on the VRC**

If the Na-K pump is makes an important contribution to the generation of the VRC, treatment such as addition of ouabain or removal of K\(^+\) which will abolish the pump activity should reduce or inhibit the VRC. The effects of K\(^+\)-free Krebs' solution and ouabain (5 x 10\(^{-5}\) M) on the VRC were compared. As shown in Fig. 6, K\(^+\)-free Krebs' solution and ouabain inhibited the VRC in a similar manner and simultaneously increased the resting tone. Although ouabain reportedly promotes the release of norepinephrine from adrenergic nerve in various tissues (7), it is unlikely that the inhibition of the VRC by ouabain is due to this effect, since the tone was elevated. This assumption was also supported by the results that in the presence of both 10\(^{-5}\) M phentolamine and 10\(^{-5}\) M propranolol ouabain still inhibited the VRC.

**DISCUSSION**

Muren (8), Jansson and Martinson (9) and Jansson and Lisander (10) reported that the chronically vagotomized canine stomach exhibited in vitro an increased mechanical activity which was blocked by either or both of atropine and hexamethonium. Their observations suggest that an increased activity of parasympathetic intramural plexuses and postganglionic neurons was responsible for the increased motility of the stomach following vagotomy. In the present study, in contrast to their results in the dog, it was found that the chronically vagotomized stomach of the rat was fully dilated and mechanically quiescent in situ. When the longitudinal muscle strip of the vagotomized stomach fundus was placed in an organ bath, however, spontaneous rhythmic contractions were observed. Such an activity was never seen in the normal or chronically sympathectomized preparations.

The vagotomy-induced rhythmic contractions (VRC) were neither blocked by TTX, atropine, morphine, hexamethonium nor enhanced by eserine. These facts indicate that the VRC is myogenic in origin.

Indomethacin is known to produce its action on various tissues via an inhibition of the biosynthesis of prostaglandins at concentrations of 2.8 x 10\(^{-5}\) to 1.4 x 10\(^{-5}\) M (11), while at higher concentrations a nonspecific inhibitory action has been reported (12-13). In the present studies, indomethacin did not inhibit the VRC until concentrations as high as 3 x 10\(^{-5}\)}
M were applied. The contribution of prostaglandins to the VRC is therefore rather unlikely.

The VRC was prevented by either sympathectomy or reserpinization. These findings suggest that sympathetic nerves exert a profound influence on the gastric smooth muscle which gives an automaticity in vitro to the fundus. Sympathetic nerves may exert these influences on the muscle by a direct influence on the muscle, influences via intramural plexus or via possible chronic hypoxia as a result of constriction of blood vessels.

The VRC was sensitive to Ca²⁺-free medium and Ca²⁺-antagonist (D600) indicating that such is dependent on extracellular Ca ions. A rapid contraction was observed on exposure to Na⁺-free solutions. Similar phenomena have been reported in the mouse myometrium and have been explained by an increase in Ca²⁺ permeability, as the contracture and depolarization are prevented by Mn²⁺ ions (14). Such a mechanism may be involved in the contracture of the rat stomach fundus strip. Removal of K⁺ ions from the medium, an application of ouabain and cooling produced an increase in resting tone accompanied by a suppression of rhythmic activity. In the spontaneously active smooth muscle it is known that slow waves precede action potentials. It has been demonstrated in the cat that the jejunal slow waves may be generated by the oscillatory action of an electrogenic Na pump, as they are blocked by ouabain or by the removal of external K⁺ (15-17). According to these authors, oscillation of slow wave is determined by an initial passive Na⁺ influx causing a depolarization followed by an electrogenic extrusion of Na⁺ and K⁺ influx which induces the repolarizing phase. The results obtained from the present experiments, together with these cited above, suggest that the electrogenic Na-pump may be involved in generating the VRC.

In summary, we found that chronic vagotomy of the rat stomach gave an automaticity to the fundus strip preparation in vitro; the normally quiescent fundus strip exhibited spontaneous rhythmic tension developments. We suggest that these tension developments may be myogenic and energy dependent, and that sympathetic nerves may play a role in vivo in inducing such a change in the characteristics of the muscle.

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