

NERVOUS CONTROL OF THE MOTILITY OF THE ALIMENTARY CANAL OF THE SILVER CARP

BY Y. ITO AND H. KURIYAMA

*Department of Physiology, Faculty of Dentistry,
Kyushu University, Fukuoka, 812, Japan*

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INTRODUCTION

The classification of the autonomic nervous supply to the alimentary canal made by Langley (1921) was derived from the point of outflow of the preganglionic fibres. However, physiological investigations by many workers suggest that such rigid division has been too far generalized. The vagus nerve, which innervates the alimentary canal, was classified as a parasympathetic excitatory nerve but has since been shown to contain not only excitatory fibres but also inhibitory fibres (Campbell, 1970). Recently, Burnstock (1969) has challenged the classification of the autonomic nervous system from the viewpoint of the comparative evolution of the system in various animals, namely fish, reptiles, birds and mammals. The motility of the alimentary canal of mammals is controlled by the nervous systems, i.e. cholinergic and non-cholinergic excitatory systems and the adrenergic and non-adrenergic inhibitory systems. The cholinergic and adrenergic systems are extrinsic systems and the other two are intrinsic systems which are thought to be situated within Auerbach's plexus (Campbell & Burnstock, 1968; Campbell, 1970; Burnstock, 1969; Ambach, Verney & Zar, 1970; Paton & Vane, 1963).

At the present time, however, only a little information is available concerning the nervous control of the alimentary canal in fish. Mahn (1898) worked on the gut of the tench and showed that vagal stimulation caused an immediate contraction of the striated muscle followed by a slow contraction of the smooth muscle. Burnstock (1958*a, b*) investigated the innervation of the gut of a teleost, the brown trout, and concluded that vagal stimulation caused contraction of the stomach. There was rapid contraction of oesophageal striated muscle during the period of stimulation. When stimulation at low frequency was stopped, there was usually a slight relaxation followed by a large prolonged contraction.

Campbell & Burnstock (1968) re-interpreted the results obtained by Mahn and Burnstock and postulated that contraction of the smooth muscle is a rebound excitation, caused by cessation of stimulation of inhibitory nerve fibres in the vagi, and that the main response to vagal nerve stimulation was relaxation of the foregut. They did not agree with the conclusions of Müller & Liljestrand (1918) and of Young (1936), who thought that there was an excitatory vagal innervation to the gastro-intestinal smooth muscle. The chemical substance released from the vagal nerve was unlikely to be acetylcholine or adrenaline (Burnstock, 1958*a, b*).

Splanchnic nerve stimulation in the brown trout caused excitation of the stomach

and intestine, and this response was mediated primarily by cholinergic nerves. Burnstock (1958*a*) reported that when low-frequency stimulation or brief stimulating pulses were used the most prominent part of the response was excitation, whereas high-frequency stimulation with pulses of long duration tended to cause a distinct inhibition of the spontaneous activity. Campbell & Burnstock (1968) re-interpreted the work done by Burnstock and stated that the splanchnic nerve fibres are mostly adrenergic inhibitory nerves, but that excitatory nerve fibres are probably also present.

The present experiments were carried out to investigate the neural control of the electrical and mechanical activities of the alimentary canal of the silver carp with the double sucrose-gap method and strain-gauge tension-recording method. The results led to the conclusion that the mechanical responses of the muscle to field stimulation could be classified into four different components in the stomach and three components in the intestine: (i) the initial phasic contraction evoked by the excitation of the cholinergic (nicotinic) receptors of the striated muscle; (ii) the relaxation (inhibitory response) induced by the excitation of the non-adrenergic receptors of the smooth muscle; (iii) the slow phasic contraction evoked by the excitation of the cholinergic (muscarinic) receptors of the smooth muscle; and (iv) the delayed contraction of the smooth muscle which might be due to rebound excitation and also due to after discharges of the enteric plexus. The spontaneous slow depolarization and the contraction recorded from the tissue were due to neurogenic and myogenic responses of the membrane.

METHODS

Tissues. The alimentary canal was dissected from the silver carp, *Carassius auratus* (Linné). The connective tissue and parenchymal tissue (gall bladder and pancreas) covering the surface of the tract were carefully dissected away. To prepare the specimens for tension recording a 20–25 mm length of the whole tract was excised. For the double sucrose-gap method the mucosal layer was ablated gently from the muscle layer and a strip of the tissue 30–35 mm in length and 1.0–1.5 mm in diameter was dissected out.

Solution. The solution contained: NaCl, 129.6 mM; KCl, 2.7 mM; CaCl₂, 1.8 mM; NaHCO₃, 2.5 mM. The concentrations of drugs used in the experiments will be described in Results. The experiments were all carried out at room temperature (17–22 °C).

Apparatus. To record tension development, a mechano-transducer manufactured by Nihon Kohden Ltd. (SB-1 T) was used. One end of the tissue was fixed to a metal hook and the other end was connected with a thread to the hook of the tension recorder. Electrical stimulation was applied through an electrode placed parallel to the long axis of the tract with the multi-grid method. The stimulating current therefore passed transversely across the tissue with uniform intensity. To record the electrical and mechanical activities the double sucrose-gap method was used. Fig. 1 shows the schematic arrangement of the double sucrose-gap apparatus for the experiments. Of the total muscle length (30–35 mm) a part less than 1 mm in the middle was exposed to the test solution, while both ends were bathed in sucrose solution. Current pulses were applied across the right sucrose gap through a series resistor (50 MΩ) from the isolation unit of the stimulator. The current intensity was measured across a

100 M Ω resistor inserted between ground and the current source. The voltage change, produced across the cell membrane of the tissue in the centre pool, was measured across the left sucrose gap. Ag-AgCl electrodes were used for stimulating and also for recording. The mechanical activity of the tissue was measured from one end of the tissue (potential recording site) by a mechano-transducer.

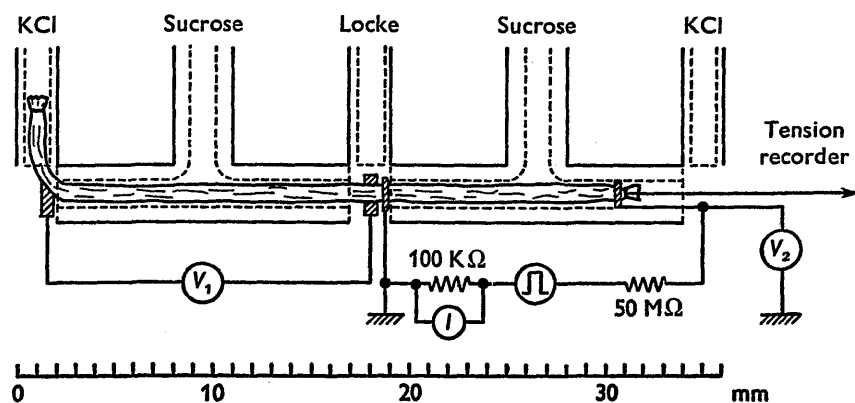


Fig. 1. Schematic diagram of the double sucrose-gap method. Current pulses were applied across the right gap through a 50 M Ω resistor and monitored by a 100 k Ω resistor (I). The voltage produced across the left gap (V_1) and that across the right gap (V_2) were measured by cathode-follower amplifiers. The dimensions of the gap are shown at the bottom.

RESULTS

Effects of field stimulation on the response of the alimentary canal

Spontaneous contractions of the alimentary canal could be recorded, and the frequency of the contractions was very low (2–10/min). The responses of the stomach muscle to field stimulation could be classified into four different responses, i.e. initial rapid phasic contraction, relaxation, slow phasic contraction and delayed contraction. These responses appeared successively after the stimulation.

Fig. 2 shows typical responses of the stomach muscle to field stimulation under various stimulus conditions. The duration of the pulse and intensity of the stimulus were fixed at 0.5 msec and 20 V/cm respectively. When a stimulus of frequency 5 c/s was applied to the tissue, marked relaxation of the tissue appeared. Increase of stimulus duration in steps increased the amplitude of the relaxation proportionally, and the slow contraction and the delayed contraction gradually also developed. At a stimulus frequency of 10 c/s the initial phasic contraction was not observed, but the amplitudes of the relaxation, slow and delayed contractions were consistently increased at any given duration of stimulation compared with those observed at 5 c/s. When the stimulus frequency was increased to 30 c/s, the initial rapid contractions were infrequently observed but the relaxation, the slow phasic and delayed contractions appeared distinctly (see Fig. 2 at 30 c/s with 3 sec and 5 sec stimulation). The initial rapid contraction could be observed clearly when a stimulus frequency of 50 c/s was applied to the tissue. The well-marked initial phasic contraction rapidly developed into relaxation with a large amplitude (except for a stimulus duration of 1 sec at 50 c/s). When field stimulation at a frequency of 50 c/s and more than 3 sec duration was applied to the tissue, the duration of the delayed contraction often exceeded 5 min.

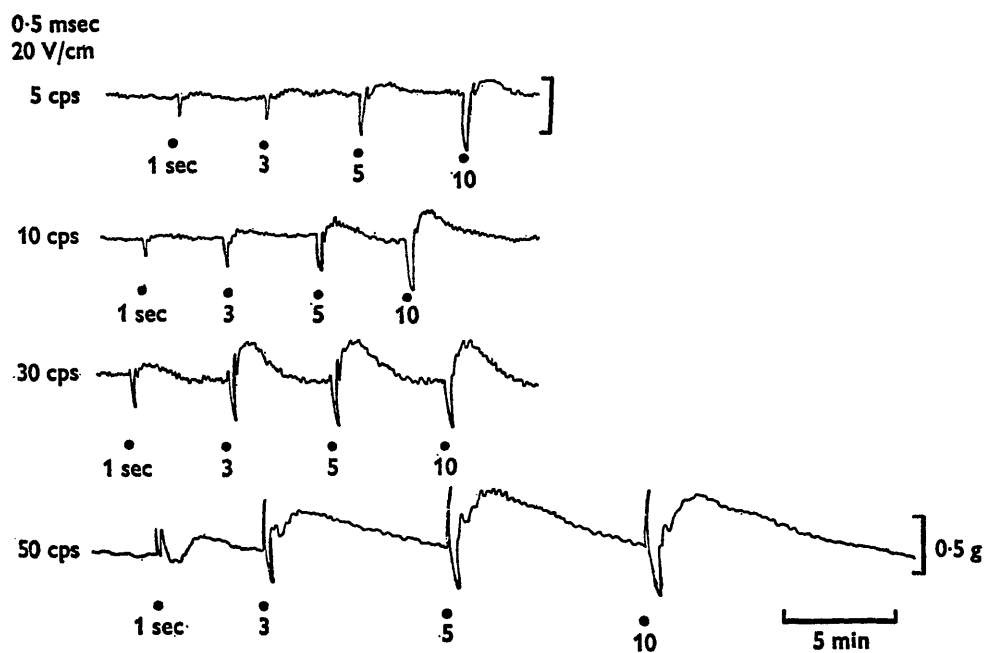


Fig. 2. Typical responses of the stomach muscle to field stimulation under various stimulus conditions. Pulse duration, 0.5 msec; current intensity, 20 V/cm. Stimulus frequencies were varied from 5 to 50 c/s and the stimulus durations were varied from 1 to 10 sec.

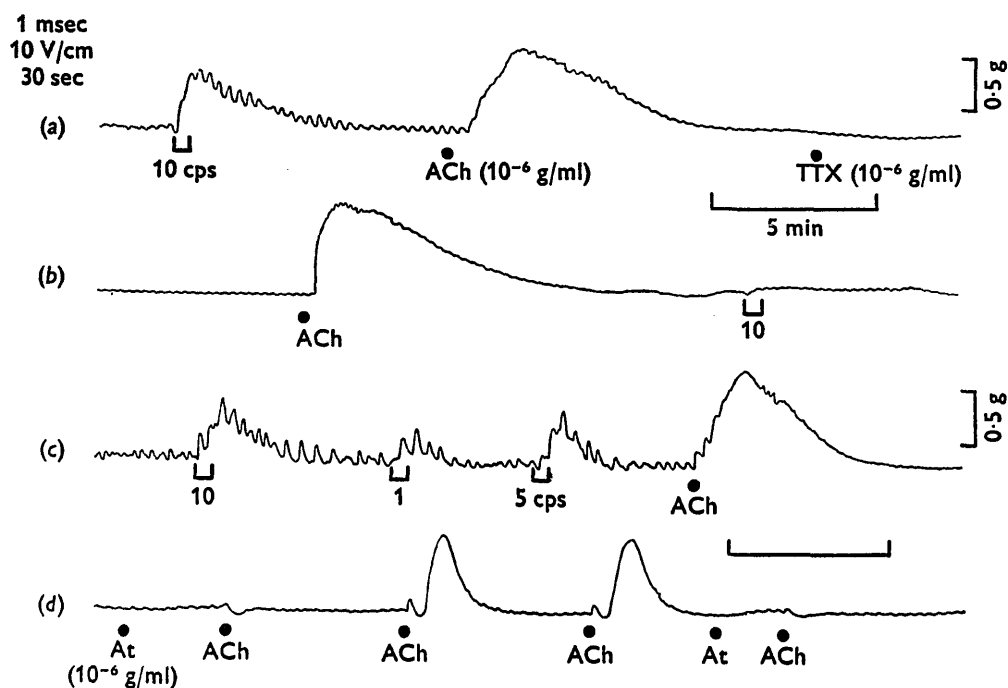


Fig. 3. Effects of tetrodotoxin (10^{-6} g/ml) and atropine (10^{-6} g/ml) on the responses of the stomach muscle evoked by field stimulation and by acetylcholine. The stimulus conditions are illustrated in the figure.

Approximate values for the latencies of onset of the individual components after the onset of the stimulus were 0.5 sec (0.4–0.8 sec, $n = 8$) for the initial rapid contraction, 2 sec (1.2–3.8 sec, $n = 8$) for the relaxation, and 8.2 sec (6–15 sec, $n = 8$) for the delayed contraction; all at 22–24 °C.

To find out whether the responses of the muscle evoked by field stimulation are due to direct stimulation of the muscle or to indirect excitation via nerves, the effects of tetrodotoxin were observed on the muscle responses evoked by treatment with acetylcholine and also by field stimulation. Fig. 3 shows the effects of tetrodotoxin (10^{-6} g/ml) and atropine (10^{-6} g/ml) on the responses of the muscle evoked by field stimulation and by acetylcholine (10^{-6} g/ml). In the control a stimulus of frequency 10 c/s evoked

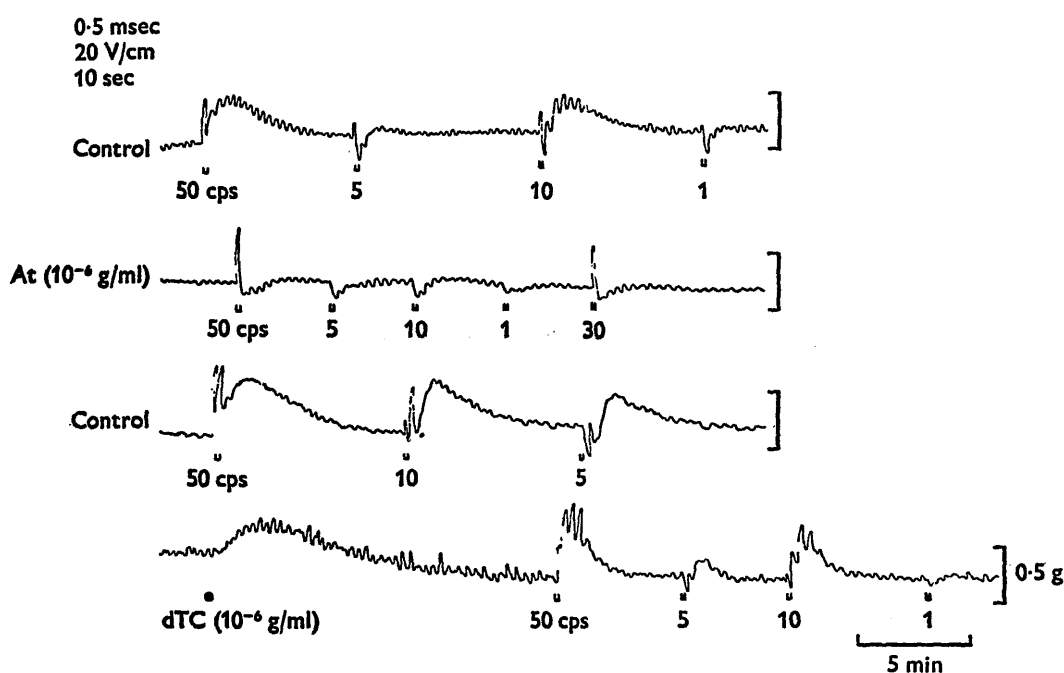


Fig. 4. Effects of atropine (10^{-6} g/ml) and D-tubocurarine (10^{-6} g/ml) on the responses of the stomach muscle evoked by field stimulation (0.5 msec pulse length, 20 V/cm intensity and 10 sec stimulus duration). Atropine and D-tubocurarine were applied to different preparations.

relaxation and a delayed contraction. Acetylcholine also evoked a contraction with a duration of about 5 min. After treatment with tetrodotoxin acetylcholine still evoked the contraction but field stimulation of the tissue failed to evoke the contraction (b). When atropine was applied to the tissue (d), contraction evoked by the acetylcholine was completely abolished and only relaxation was observed (d). When the tissue was rinsed with physiological solution after treatment with atropine, successive applications of acetylcholine to the tissue evoked the initial rapid contraction, relaxation and delayed contraction but failed to evoke the slow phasic contraction (d). These results indicate that the responses of the muscle evoked by field stimulation were mainly due to nervous elements. Furthermore, tetrodotoxin blocked only the nervous activities but not the activity of the muscle membrane.

The effects of atropine and D-tubocurarine on the responses of the muscle evoked by field stimulation were observed in order to discover whether or not the responses of

the muscle were due to cholinergic nerves. Fig. 4 shows the effects of atropine (10^{-6} g/ml) and D-tubocurarine (10^{-6} g/ml) on the responses of the muscle evoked by field stimulation (0.5 msec, 20 V/cm and 10 sec) at various stimulus frequencies. In the control, stimulation at 50 c/s evoked the initial rapid phasic contraction, the slow and the delayed contractions, and a stimulus of frequency 10 c/s evoked the four responses clearly. Treatment with atropine evoked only two responses from the muscle, i.e. a stimulus of frequency more than 30 c/s evoked the initial phasic contraction followed by the relaxation, and stimulation below 30 c/s evoked only the relaxation. On the other hand, when D-tubocurarine was applied (10^{-6} g/ml) the tone of the muscle was transiently increased; then after 5–10 min the tone was gradually reduced to a level just below that before the treatment. Field stimulation markedly suppressed

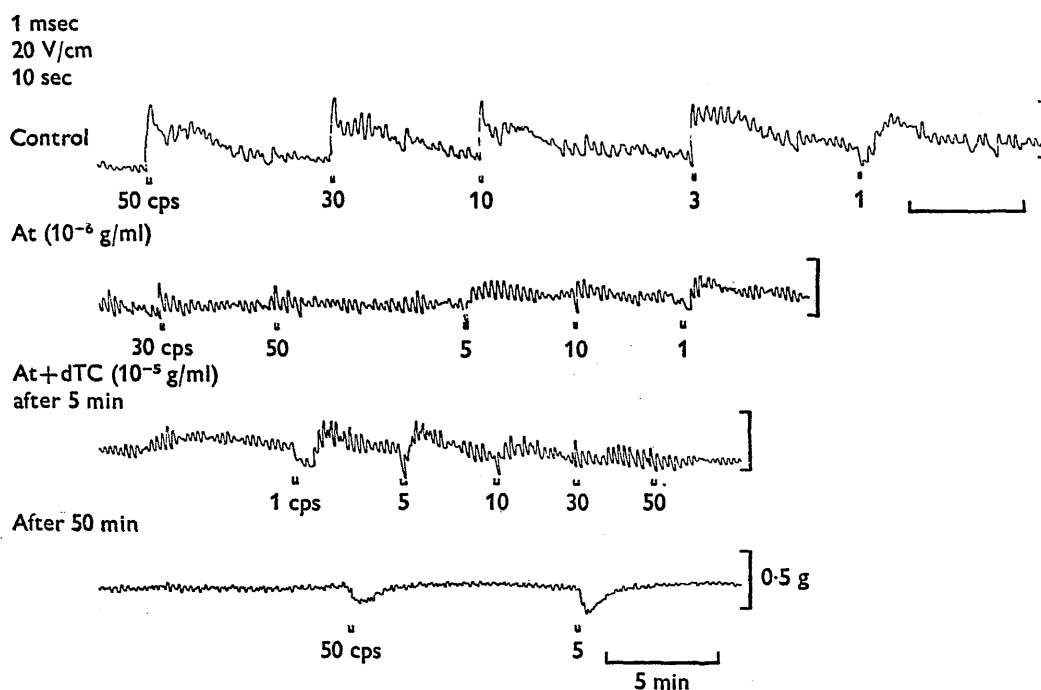


Fig. 5. Effects of atropine (10^{-6} g/ml), and of simultaneous treatments with atropine (10^{-6} g/ml) and D-tubocurarine (10^{-6} g/ml), on the responses of the stomach muscle evoked by field stimulation (1 msec pulse length, 20 V/cm intensity and 10 sec stimulus duration). The effects of the drugs were observed in the same preparation.

the initial phasic contraction compared with the control. However, there was no marked effect on the relaxation, the slow phasic contraction or the delayed contraction.

Fig. 5 shows the effects of atropine (10^{-6} g/ml), and simultaneous treatment with atropine (10^{-6} g/ml) and D-tubocurarine (10^{-6} g/ml), on the responses of the intestine. A typical feature of the responses in the control that should be pointed out is the lack of the initial rapid phasic contraction even when a stimulus frequency of 50 c/s was used. Treatment with atropine suppressed the slow phasic contraction and the delayed contraction. However, the spontaneous contraction could still be recorded. After 50 min of the simultaneous treatment with atropine and D-tubocurarine, electrical stimulation to the tissue at frequencies of both 50 c/s and 5 c/s evoked only the relaxation.

It is likely that the relaxation of the tissue evoked by field stimulation is due to release of chemical transmitter from the nerve terminals, since tetrodotoxin suppressed the relaxation. To investigate further the nature of the relaxation of the muscle, α - and β -adrenergic blocking agents were used.

Fig. 6 shows the effects of phentolamine (10^{-5} g/ml) and propranolol (10^{-5} g/ml) on the responses of the intestinal muscle evoked by field stimulation. In the control, stimulation at 3 c/s evoked more dominant relaxation of the tissue compared with increased frequencies of stimulation. Simultaneous treatment with α -blocker (phentolamine) and β -blocker (propranolol) did not reduce the amplitude of the relaxation after 15 min of the perfusion, but after 40 min the relaxation of the muscle was slightly reduced in amplitude; but this reduction was not specific for the relaxation, because all

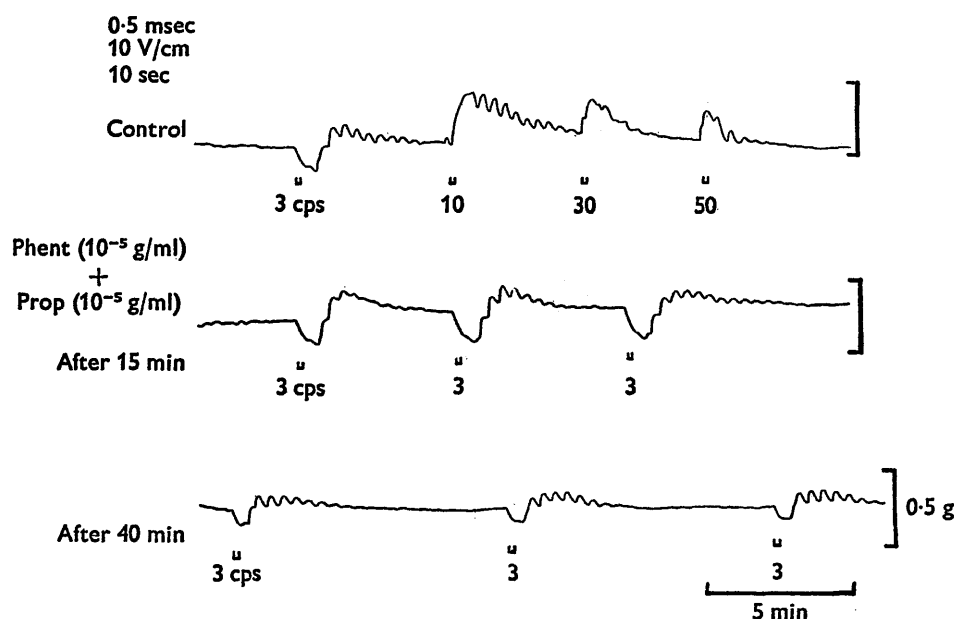


Fig. 6. Effects of adrenergic blocking agents on the responses of the intestinal muscle evoked by field stimulation (0.5 msec pulse length, 10 V/cm intensity and 10 sec stimulus duration). Phentolamine (10^{-5} g/ml) was used as an α -adrenergic blocking agent, on propranolol (10^{-5} g/ml) as a β -adrenergic blocking agent.

the responses evoked by field stimulation were reduced. These effects of adrenergic blocking agents on the relaxation of the tissue caused by field stimulation might indicate that the relaxation of the tissue was not due to release of catecholamines from the nerve terminals but to an unknown inhibitory chemical transmitter as suggested for the mammalian alimentary canal (Burnstock, 1969; Holman, 1970).

It has already been shown in the mammalian alimentary canal that tetra-ethylammonium (TEA) enhanced the amplitude of the neurogenic responses evoked by field stimulation, and inhibited the potassium conductance of the muscle membrane, thus enlarging the amplitude of the evoked spike (Ito, Kuriyama & Sakamoto, 1970). Effects of TEA on the spontaneous contraction and on the responses of the muscle evoked by field stimulation were observed. Fig. 7 shows the effects of TEA (10^{-3} M) alone and of TEA (10^{-3} M) after treatment with tetrodotoxin (10^{-6} g/ml) on the spontaneous contractions and also on evoked responses of the muscle. Treatment with

TEA enhanced the level of the general tone of the muscle (resting tension) and enhanced the frequency and amplitude of the spontaneous contractions. While the resting tension level of the muscle was high, a stimulus of frequency 5 c/s produced marked relaxation of the muscle in spite of the small relaxation in the control. On the other hand, the large initial phasic contraction evoked by field stimulation in the control was suppressed. When the tissue was treated with tetrodotoxin after TEA had been washed out for more than 30 min the amplitude of the resting tension was reduced. However, the spontaneous contraction could be recorded. On re-addition of TEA in the presence of tetrodotoxin the resting tension was again raised close to the level in the absence of tetrodotoxin, although field stimulation did not evoke any response in the muscle.

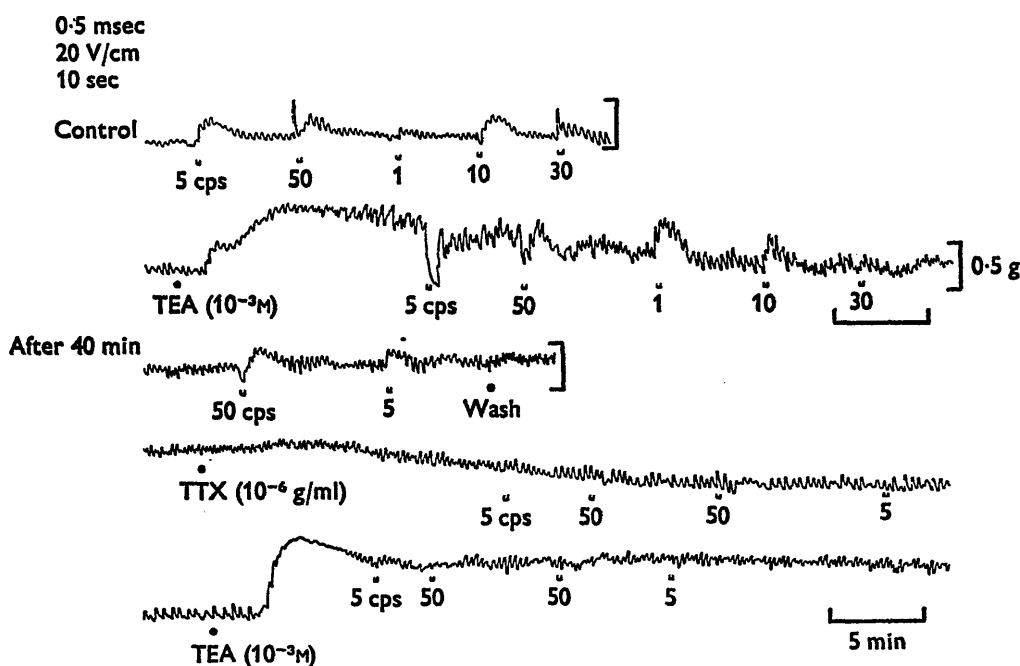


Fig. 7. Effects of tetra-ethylammonium (TEA) (10^{-3} M) and effects of TEA (10^{-3} M) after treatment with tetrodotoxin (TTX) (10^{-6} g/ml) on the spontaneous contraction and evoked response of the stomach muscle (0.5 msec pulse length, 20 V/cm intensity and 10 sec stimulus duration).

*Electrical and mechanical activities of the tissue recorded
with the double sucrose-gap method*

Fig. 8 shows the spontaneous electrical and mechanical activities recorded with the sucrose-gap method. Six different patterns of membrane activity recorded from different specimens are illustrated. Action potentials could not be recorded from all the cells, but the slow depolarization (duration of 3–8 sec and amplitude of 4–18 mV) could be recorded. The duration of the action potential generated in the mammalian stomach was about 30–50 msec (Kuriyama, Osa & Tasaki, 1970) and much shorter than that recorded from the longitudinal and circular muscles of the silver carp stomach (1–3 sec). However, the duration of the depolarization resembled the slow wave recorded from the mammalian stomach. Furthermore, it was very difficult to distinguish the spike and the slow potential from their durations and shapes.

The electrotonic potential of the membrane could be recorded with the sucrose-gap method. Therefore, the existence of electrical connexions between the cells is postulated. Fig. 9 shows the effects of application of inward and outward currents to the tissue. Various intensities of electrical current with pulse duration of 7 sec were applied. No rectifying property of the membrane was observed. However, electrical stimulation could not elicit an active response in the membrane.

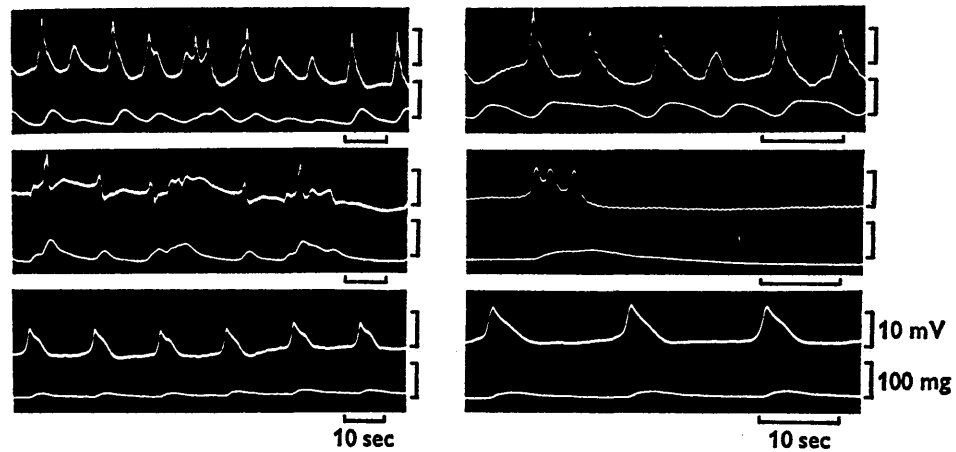


Fig. 8. Spontaneous electrical and mechanical activities recorded from the longitudinal muscle layer of the stomach by the double sucrose-gap method. The six different records were taken from different preparations.

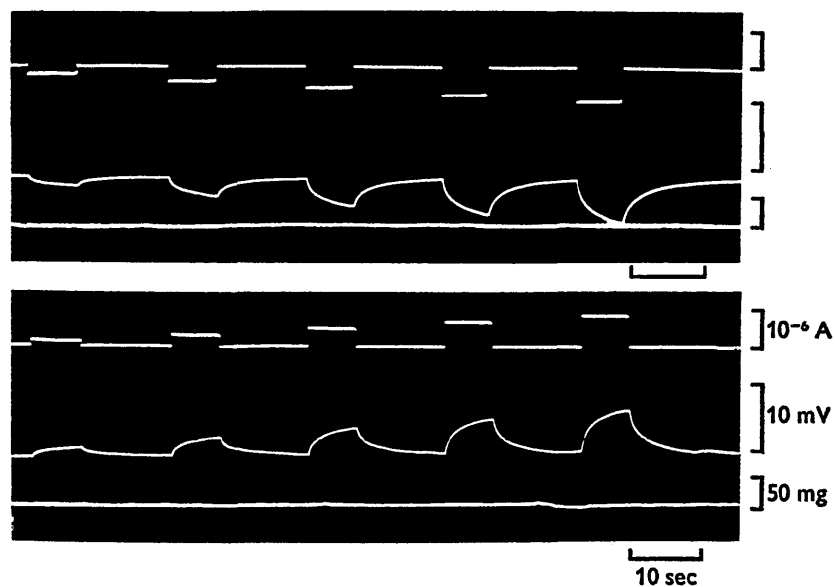


Fig. 9. Effects of application of inward and outward current pulses (7 sec) to the longitudinal muscle of the stomach. The intensity of the current pulse was varied from 2×10^{-6} to 10×10^{-6} A. Neither rectification nor spike generation was observed.

*Effect of various drugs on the electrical and mechanical activities
recorded by the double sucrose-gap method*

Fig. 10 shows the effects of acetylcholine (10^{-5} g/ml) on the spontaneously generated membrane activities and on the contractions. Acetylcholine markedly depolarized the membrane and increased the frequency of the slow depolarization. The depolarization of the membrane often exceeded 20 mV. These depolarizations of the membrane enhanced the amplitude of the contraction and often caused contracture. Similar effects on the membrane activity and on the tension were observed after treatment

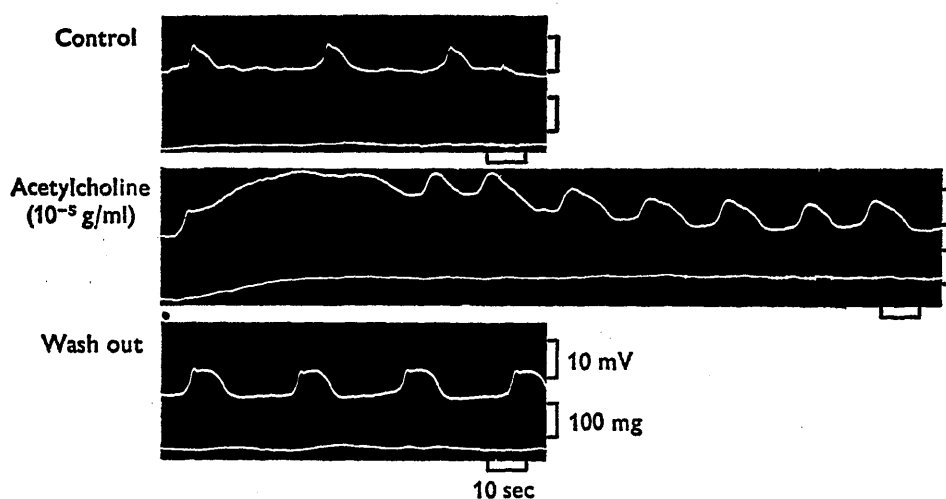


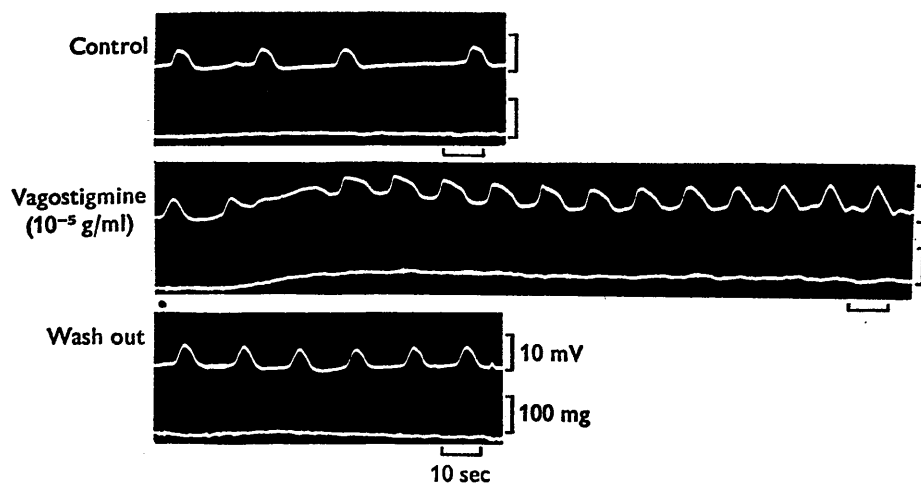
Fig. 10. Effects of acetylcholine (10^{-5} g/ml) on the spontaneously generated electrical and mechanical activities of the longitudinal muscle of the stomach.

with vagostigmine. Fig. 1 (*a*) and (*b*) shows the effects of vagostigmine (10^{-5} g/ml) on the electrical and mechanical activities of the longitudinal muscle (*a*) and circular muscle (*b*) of the stomach. The membrane was depolarized and the frequency of the slow depolarization increased (Fig. 11 *a*). The increased amplitude and duration of the contraction appeared to be in accordance of the increase in membrane activity.

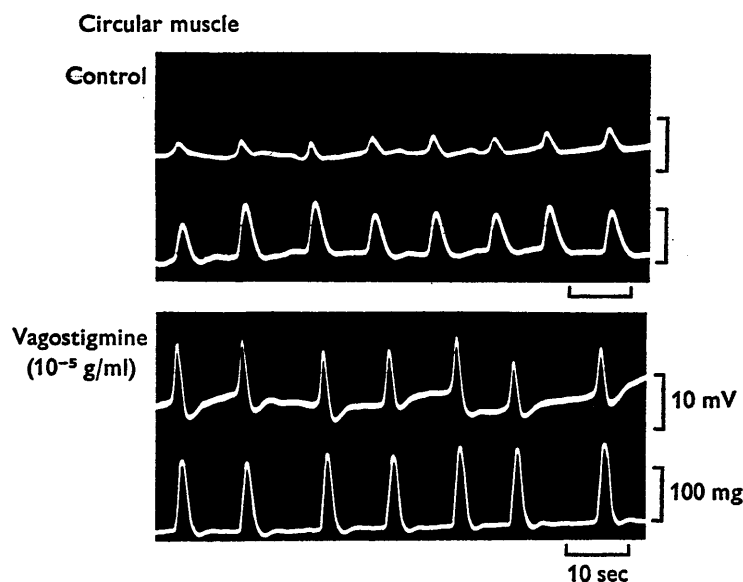
These activities suggested neurogenic responses of the membrane, since acetylcholine and vagostigmine both enhanced the frequency of the slow depolarization. The effects of atropine on the electrical and mechanical activities of the longitudinal muscle were therefore observed in order to elucidate the involvement of the muscarinic responses of the muscle. Fig. 12 shows the effects of atropine (10^{-5} g/ml) on the electrical and mechanical activity of the longitudinal muscle of stomach. The generation of the spontaneous slow depolarizations ceased completely and the tone of the muscle was also reduced. These responses might indicate a neurogenic origin of the slow potential changes. However, in some preparations the frequency and amplitude of the slow depolarizations were not influenced by treatment with tetrodotoxin and atropine. As described previously, the spontaneous contractions recorded from the experiments with the strain gauge were generated neurogenically as well as myogenically, since the spontaneous contractions were sometimes blocked by treatments with atropine and tetrodotoxin. However, in some specimens the spontaneous

contractions were not affected. A similar phenomenon could be observed on the membrane activities of the longitudinal muscle.

Fig. 13 shows an example of the effects of tetrodotoxin (10^{-6} g/ml) and atropine (10^{-5} g/ml) on the electrical and mechanical activities of the longitudinal muscle of the



(a)



(b)

Fig. 11. (a) Effects of vagostigmine (10^{-5} g/ml) on the electrical and mechanical activities of the longitudinal muscle of the stomach. (b) Effects of vagostigmine (10^{-5} g/ml) on the electrical and mechanical activities of the circular muscle of the stomach.

stomach. Tetrodotoxin and atropine had no effect on the generation of the slow depolarization and contraction. However, atropine-resistant and tetrodotoxin-resistant slow depolarizations were completely inhibited by treatment with Mn^{2+} (4 mM).

It is well known that Mn^{2+} blocks the generation of the spike from the mammalian

alimentary canal. The suppression of membrane activity is due to competitive action between Mn^{2+} and Ca^{2+} on the flux of Ca^{2+} generating the spike.

Fig. 14 shows an example of the effects of Mn^{2+} (4 mM) on the electrical and mechanical activities of the circular muscle. The spontaneous slow depolarization of

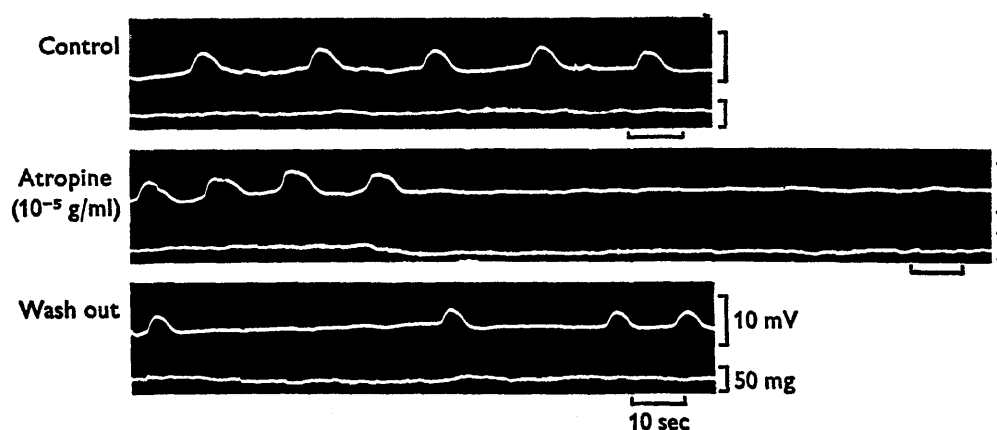


Fig. 12. Effects of atropine (10^{-5} g/ml) on the electrical and mechanical activities of the longitudinal muscle of the stomach.

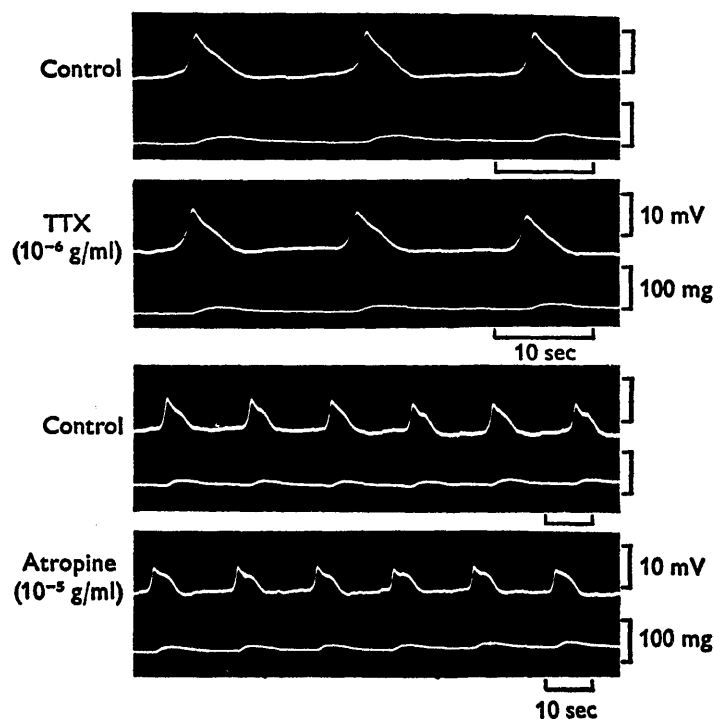


Fig. 13. Effects of tetrodotoxin (TTX) (10^{-6} g/ml) and atropine (10^{-5} g/ml) on the electrical and mechanical activities of the longitudinal muscle of the stomach. The effects of the two drugs were observed on different preparations.

the membrane was blocked even after only 3 min of perfusion. From the above results it might be possible to conclude that there are two types of spontaneous membrane activity classified in accordance with the drug actions as well as in accordance with the

mechanical activities. The neurogenic component of the slow depolarization was blocked by tetrodotoxin and atropine and the myogenic component was blocked by Mn^{2+} .

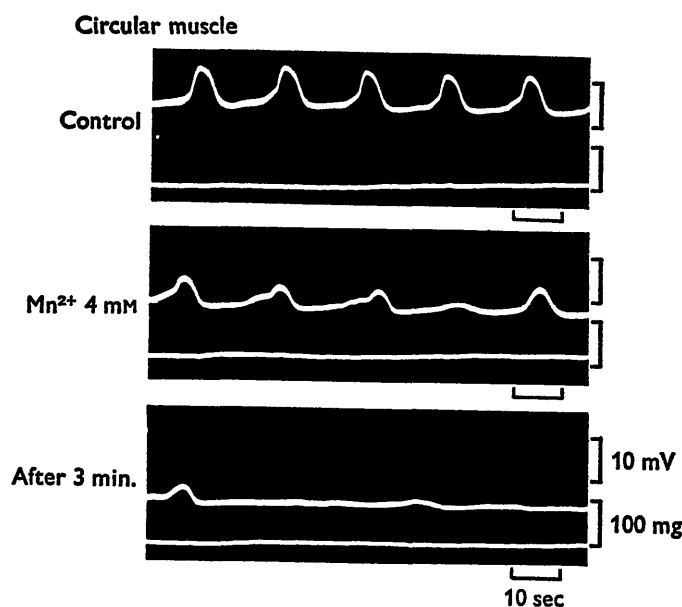


Fig. 14. Effects of Mn^{2+} (4 mM) on the electrical activity of the circular muscle of the stomach.

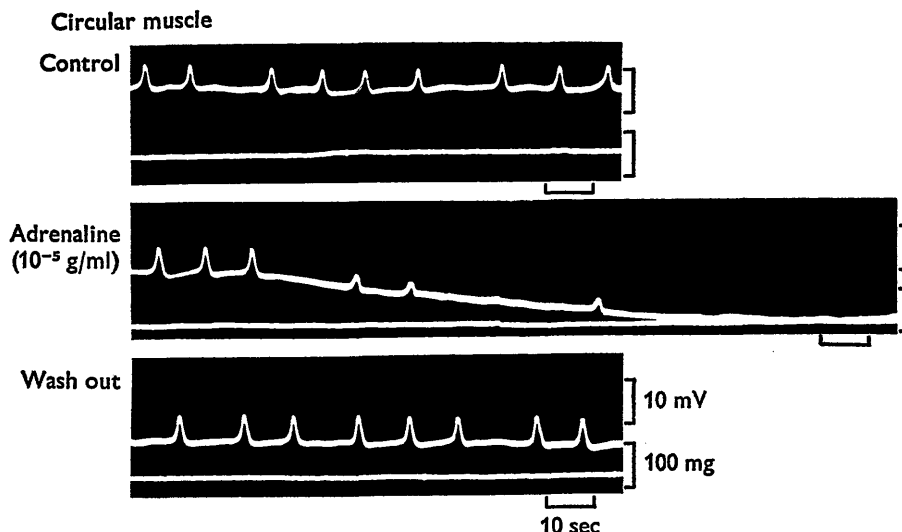


Fig. 15. Effects of adrenaline (10^{-5} g/ml) on the electrical activity of the circular muscle of the stomach.

Effects of catecholamines (adrenaline and noradrenaline) on the electrical and mechanical activities of the circular muscle were investigated, since catecholamines were described as excitatory substances to the alimentary canal of the teleost fish (Young, 1936; Burnstock, 1958*a, b*). Fig. 15 shows the effects of adrenaline (10^{-5} g/ml) on the electrical and mechanical activities of the circular muscle. Adrenaline inhibited the generation of the spontaneous depolarization and hyperpolarized the membrane. As a consequence the tissue was completely relaxed.

On rare occasions spontaneous hyperpolarization of the membrane of the longitudinal muscle could be recorded. During the hyperpolarization of the membrane the tissue was relaxed. Fig. 16 shows spontaneously generated hyperpolarizations of the membrane recorded from the longitudinal muscle. It is unlikely that the hyperpolarizations were due to the generation of miniature inhibitory junction potentials, since the hyperpolarizations were larger in amplitude (more than 8 mV) and longer in duration (3–6 sec) than the miniature inhibitory junction potentials recorded from the neuromuscular junction of crustacean muscle and of earthworm muscle (Fatt & Ginsborg, 1958; Dudel & Kuffler, 1961; Ito, Kuriyama & Tashiro, 1970). These

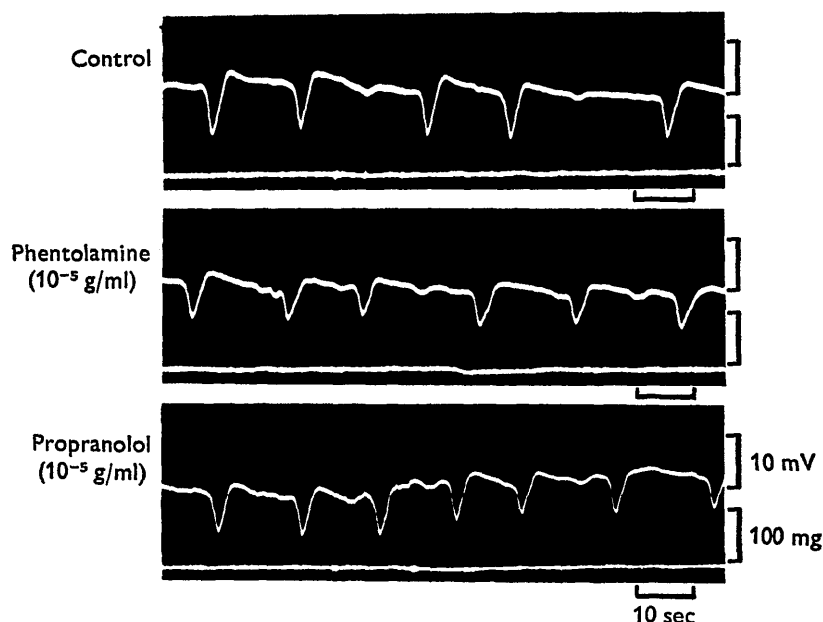


Fig. 16. Effects of phentolamine (10^{-5} g/ml) and propranolol (10^{-5} g/ml) on the spontaneously generated hyperpolarizations of the membrane recorded from the longitudinal muscle of the stomach.

spontaneous hyperpolarizations of the membrane were not blocked by treatment with phentolamine (10^{-5} g/ml) or propranolol (10^{-5} g/ml), although tetrodotoxin (10^{-6} g/ml) blocked the generation of the hyperpolarization. The hyperpolarization of the membrane was therefore not due to the release of catecholamines. Hence it is postulated that they are due to release of an unknown inhibitory chemical substance from spontaneously discharging nerve terminals distributed within the enteric plexus.

DISCUSSION

Young (1936) studied the innervation of the alimentary canal of teleost fish and concluded that the vagi innervate the striated muscles of the oesophagus and the smooth muscle of the stomach, but that probably they do not extend to the intestine. Burnstock (1958*a, b*) confirmed the above conclusion in experiments made on the actions of the alimentary canal to drugs.

Yamamoto (1966) recently studied the fine muscle structure of the alimentary canal in teleost fish (rainbow trout) and found that the inner circular muscle layer of the

intestine is innervated by sympathetic and parasympathetic axons. He concluded that transmission occurs not only through the regions of intimate contacts between swollen portions of axon and smooth muscle but also through those portions of axons that do not make intimate contact. The circular muscle layer of the intestinal bulb in goldfish is composed of smooth muscle and striated muscle, which are mixed in an irregular way. The basic structure of the striated muscle is the same as that of the skeletal muscle, although the triad structure and myoplasmic reticulum are rather poorly developed. Furthermore, the plasma membrane of the striated muscle apposed to the motor ending does not exhibit infoldings. Presumably, the striated muscle in this tissue has the structural property of a tonic muscle rather than of a twitch muscle. The striated muscle and smooth muscle occasionally make close contacts with each other but no nexus was observed. The same motor ending makes junctional contact with both types of muscle. Yamamoto postulated that the axons were sympathetic, and that their vesiculated portions made neuro-muscular contact with both types of muscle. He stated that the general structure of the nerve terminals and of the muscle fibres of the silver carp were the same as those of the goldfish (personal communication). The present experiments showed four different types of response to field stimulation.

(i) Initial rapid phasic contraction evoked from the stomach by high-frequency stimulation. This component was blocked by treatment with either D-tubocurarine or tetrodotoxin. The initial rapid phasic component was not recorded from the lower alimentary canal. It is therefore likely to be due to responses of the striated muscles distributed within the stomach tissue. The show muscle (tonic muscle, red muscle) of the striated muscle in vertebrates is known to contract without generation of a spike, but follows depolarizations of the membrane caused by the release of acetyl-choline from the multiple innervating nerve fibres onto the muscle membrane (Kuffler & Vaughan Williams, 1953). A similar distribution of nerves and properties of the muscle fibres might be postulated in the stomach of the fish producing the initial rapid phasic contraction.

(ii) Relaxation of the tissue evoked by low-frequency stimulation. This component was blocked only by tetrodotoxin and was not observed when the tone of the tissue was low. α - and β -adrenergic blocking agents did not produce any marked change in the relaxation. Furthermore, atropine and D-tubocurarine did not block the relaxation evoked by field stimulation; but hexamethonium decreased the amplitude of the relaxation. The generation mechanism of this relaxation might be the same as that observed in the mammalian alimentary canal (see review of Campbell & Burnstock, 1968; Holman, 1970). In the mammalian alimentary canal an inhibitory junction potential was recorded in response to field or transmural stimulation (Burnstock & Holman, 1966; Bennett, Burnstock & Holman, 1966; Holman, 1970). The spontaneous hyperpolarizations of the membrane might have a causal or close relation with the relaxation of the tissue, since these likewise were not influenced by the α - and β -adrenergic blocking agents but were blocked by tetrodotoxin. The vagus nerve innervating the stomach is known to contain inhibitory nerves, and vagal nerve stimulation evoked diphasic responses of the muscle, i.e. contraction and relaxation. The possibility of a mixed distribution of excitatory and inhibitory nerves within the vagal nerve to the stomach cannot be ruled out from the present experiments.

(iii) The slow phasic contraction of the muscle evoked by field stimulation had a

long latency after the onset of the stimulation. This component ceased after treatment with atropine and tetrodotoxin. In the teleost fish (brown trout) the contraction occurred after a long latency in low-tone preparations. For this reason Campbell & Burnstock (1968) suggested that the contraction was due to rebound contraction after stimulation of inhibitory nerves. In the present experiments the slow phasic response appeared after the generation of the relaxation. However, the response is unlikely to be rebound contraction, since the generation of the slow phasic response interrupted the relaxation, and even in the absence of the relaxation the slow phasic contraction could be recorded. In some experiments relaxation of large amplitude was not followed by the slow phasic contraction. The cholinergic nerves and muscarinic receptors on the muscle membrane might therefore be involved in the generation of the slow phasic contraction.

(iv) The delayed contraction appeared with a latency of 11 sec and had a very long time course of several minutes. This response was more sensitive to atropine and tetrodotoxin than to D-tubocurarine. The latency was too long for one to postulate that it was the time required for neuromuscular transmission of excitation. It was presumably due to release of chemical transmitter from the enteric plexus as an after discharge of the ganglion cells, or to diffusion to the muscle layer of chemical transmitter accumulated during the excitation of the enteric plexus. Treatment with hexamethonium potentiated the amplitude and duration of the membrane depolarization and of the contraction, and these responses were abolished by treatment with atropine and tetrodotoxin. Presumably acetylcholine generates the delayed contraction.

The above four different components could be elicited by field stimulation of the tissue. These responses were produced by the striated and smooth muscles, and the chemical transmitters were acetylcholine and an unknown inhibitory chemical transmitter. The receptors for the acetylcholine were nicotinic (striated muscle) and muscarinic (smooth muscle) receptors. Catecholamines might also be involved in the responses of the muscle to field stimulation. Treatments with noradrenaline and adrenaline hyperpolarized the membrane and blocked the generation of the slow potential changes. These facts contradict the observation made by Burnstock (1958*b*), since in the brown-trout stomach catecholamines produced contraction. In the presence of the α - and β -blocking agents, the above four responses of the muscle could be elicited by field stimulation. The responses of the adrenergic nerve evoked by field stimulation in the present experiment appeared therefore to be only minor responses.

It was difficult to insert a micro-electrode into the muscle cells and the electrical records were therefore made by the double sucrose-gap method. The spontaneous contractions and slow electrical potential changes in the stomach and the intestine originated from two different sources, i.e. myogenic and neurogenic responses. The neurogenic responses were due to release of acetylcholine on to muscarinic receptors on the muscle, since atropine abolished the generation of the slow depolarization and contraction. On the other hand, the myogenic response is closely related with Ca^{2+} , because it was not affected by atropine and tetrodotoxin but was blocked by Mn^{2+} . It is likely to be generated by the smooth muscle of the stomach rather than by the striated muscle, since it is well known that the spike is not due to the influx of Na^+ in mammalian visceral muscle but of Ca^{2+} , and the spike generation is blocked by Mn^{2+} (see reviews of Kuriyama, 1968, 1970). In the present experiments it was difficult to

explain how two different components generate spontaneous depolarization and contraction from the same muscle. For example, after treatment with atropine the slow depolarization and contraction reappeared with nearly the same frequency as before the treatment.

In the presence or absence of tetrodotoxin TEA still increased the magnitude of the tonus of the muscle, and increased the frequency and amplitude of the spontaneously generated contraction. TEA is known to enhance the membrane activity in crustacean muscle and smooth muscle (Fatt & Ginsborg, 1958; Ito, Kuriyama & Sakamoto, 1970). These effects of TEA are thought to be due to suppression of the potassium conductance. On the other hand, in the neuromuscular junction of the frog skeletal muscle TEA increased the amount of acetylcholine released in the presence of tetrodotoxin (Katz & Miledi, 1968). TEA might therefore accelerate the muscle membrane activity and also the release of chemical transmitter from the nerve terminals in the presence of tetrodotoxin.

SUMMARY

1. The electrical and mechanical activities of the alimentary canal of the silver carp, *Carassius auratus*, were investigated using the strain-gauge tension-recording method and also the double sucrose-gap method.

2. In responses to field stimulation of the alimentary canal four different responses from the stomach and three different responses from the intestine could be evoked.

(i) An initial rapid contraction was produced by stimulation of high-frequency or long pulses. The onset of the contraction appeared 0.5 sec after the stimulation. The initial rapid contraction was blocked by tetrodotoxin and D-tubocurarine. This response is thought to be from the striated muscles distributed in the stomach muscle layers.

(ii) With or without the initial rapid contraction, field stimulation of low frequency (1–5 c/s) evoked relaxation of the tissue. The latency for the onset of the relaxation was 2 sec. This response was blocked by tetrodotoxin, but α - and β -adrenergic blocking agents had no effect on it. This response is thought to be from the smooth muscle and to be due to the release of an unknown inhibitory chemical transmitter from the nerve fibres.

(iii) The slow phasic contraction appeared with a latency of 8.2 sec, and the amplitude of the contraction was reduced by treatment with atropine and tetrodotoxin but not D-tubocurarine. This response is thought to result from release from the nerve terminals of acetylcholine, which acts on the muscarinic receptors of the smooth muscle.

(iv) The delayed contraction appeared with a latency of 11 sec after the onset of field stimulation. The contractions often continued for several minutes. The amplitude and duration of the contraction were reduced or abolished by atropine and tetrodotoxin and were slightly reduced by D-tubocurarine. This response is thought to result from release from the enteric plexus of acetylcholine, which diffuses on to the muscarinic receptors of the muscle.

3. It was difficult to demonstrate a response of the muscle to the excitation of the adrenergic nerve by field stimulation, since α - and β -adrenergic blocking agents had no marked effects on the responses evoked by field stimulation. However, treatment

with adrenaline and noradrenaline hyperpolarized the circular muscle membrane and blocked the spike generation.

4. The spontaneous slow potential changes and contractions originated from the myogenic and the neurogenic components. The former was blocked by Mn^{2+} but not by tetrodotoxin and atropine. The latter was blocked by atropine and tetrodotoxin.

5. Spontaneous hyperpolarizations of the membrane could be recorded. The adrenergic blocking agents had no effect on them.

6. Tetraethyl-ammonium enhanced the tone of the muscle and the amplitude and frequency of the slow depolarizations and contractions recorded from the longitudinal as well as from the circular muscle, in the presence or absence of tetrodotoxin.

7. From the above results it was concluded that there are two kinds of slow depolarization of the muscle, i.e. neurogenic and myogenic. There are three kinds of nerve distributed in the muscle, i.e. excitatory vagal nerve, inhibitory vagal nerve and inhibitory sympathetic nerve. Excitatory nerves innervated two different receptors, i.e. nicotinic striated muscle receptors and muscarinic smooth-muscle receptors.

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