Responses of the Smooth Muscle Membrane of Guinea Pig Jejunum Elicited by Field Stimulation

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ABSTRACT Field stimulation of the jejunum elicited successively an action potential of spike form, a slow excitatory depolarization, a slow inhibitory hyperpolarization, and a postinhibitory depolarization as a rebound excitation. The slow depolarization often triggered the spike. The inhibitory potential showed lower threshold than did the excitatory potential. Both the excitatory potentials were abolished by atropine and tetrodotoxin. Effective membrane resistance measured by the intracellular polarizing method was reduced during the peak of the excitatory potential, but the degree of reduction was smaller than that evoked by iontophoretic application of acetylcholine. Conditioning hyperpolarization of the muscle membrane modified the amplitude of the excitatory potential. The estimated reversal potential level for the excitatory potenialt was about 0 mv. No changes could be observed in the amplitude of the inhibitory potential when hyperpolarization was induced with intracellularly applied current. Low $[K]_o$ and $[Ca]_o$ blocked the generation of the excitatory potential but the amplitude of the inhibitory potential was enhanced in low $[K]_o$. Low $[Ca]_o$ and high $[Mg]_o$ had no effect on the inhibitory potential.

INTRODUCTION

Gillespie (1962 *a*, *b*) studied the effects of autonomic nerve stimulation on the activity of intestinal smooth muscle and found that repetitive stimulation of the parasympathetic nerve caused depolarization of the membrane and increased the spike frequency. On the other hand, sympathetic nerve stimulation had the reverse effect on membrane activity.

Burnstock, Campbell, Bennett, and Holman (1963, 1964), Burnstock, Campbell, and Rand (1966), and Bennett, Burnstock, and Holman (1966 *a*) described inhibition of the taenia coli in response to repetitive stimulation of the perivascular nerves, and showed that these nerves had properties typical of sympathetic postganglionic adrenergic fibers elsewhere. In contrast to this adrenergic inhibition, the inhibition of intestinal movement and membrane

activity elicited by transmural stimulation with less than 5 pulse/sec was not affected by the treatment with sympathetic blocking agents. Both transmural and field stimulation of the guinea pig taenia coli, in the presence of atropine, produce a transient hyperpolarization of the membrane and block spike generation (Bennett, Burnstock, and Holman, 1966 *b*; Bülbring and Tomita, 1967). The hyperpolarization of the membrane lasted several hundred msec with a latency of about 100 msec. The inhibitory potential elicited by the transmural stimulation is thought to arise from the stimulation of inhibitory nerves whose cell bodies are in Auerbach's plexus. Tetrodotoxin blocked the generation of the inhibitory potential without any change in the membrane activity and the chronaxie for the inhibitory potential was less than 1 msec whereas that for the muscle spike was 20–30 msec (Bülbring and Tomita, 1967). Kuriyama, Osa, and Toida (1967 *b*) have reported that storage of the tissue at 4°C for more than 100 hr blocked the inhibitory potential but not the spontaneous membrane activity of the muscle. The present experiments were intended to investigate further the properties of the excitatory and inhibitory potential in response to field stimulation of the guinea pig jejunum.

METHODS

The smooth muscle of the isolated guinea pig jejunum was used. Guinea pigs weighing 250–300 g were stunned and bled. The jejunum was dissected from the abdomen, and the mesentery was removed carefully in Kreb's solution at room temperature. Pieces, 1.0–1.2 cm in length, were used and each piece was placed in an organ bath through which solution flowed continuously at a temperature of 35–36°C.

A modified Krebs solution of the following composition was used (mM): Na^+, 137.4; K^+, 5.9; Ca^{2+}, 2.5; Mg^+, 1.2; Cl^-, 134.0; HCO_3^-, 15.5; $H_2PO_4^-$, 1.2; glucose, 11.5, equilibrated with 97% O_2 + 3% CO_2.

Two Ag-AgCl electrodes were used for extracellular stimulation of the tissue. One electrode (diameter 2 mm) was placed at a distance of 3–4 cm from the tissue, with the other (diameter 0.5 mm) placed on the tissue. For intracellular stimulation a single electrode was used for electrical recording as well as for stimulation by the Wheatstone bridge method as described previously by Kuriyama and Tomita (1965). The resistance of a microelectrode filled with 3 M KCl varied between 40 and 80 MΩ. A stimulating electrode was placed on the tissue and another electrode was placed at a distance of 3 cm from the tissue. The recording microelectrode was placed at a distance of 0.5 mm from the stimulating electrode.

Iontophoretic application of acetylcholine was carried out according to the method described by Eccles (1964) with the glass capillary filled with 2 M acetylcholine. The drug concentrations used in the experiments will be described below.

RESULTS

Responses of Single Cells to Field Stimulation

The membrane potential of the guinea pig jejunum ranged from −48 to −65 mv and the mean value was −56 mv (SD = ±4.1, n = 120).

Field stimulation (0.1–0.3 msec pulse) of the jejunum elicited a transient depolarization (excitatory junction potential) which was followed by a transient hyperpolarization (inhibitory junction potential). The former was blocked by atropine and both potential changes were blocked by tetrodotoxin. The inhibitory potential appeared with a latency of 60–150 msec and the duration of the recovery process from the peak of the inhibitory potential to 40 % in amplitude was 220–480 msec. These values confirm previous observations on the guinea pig taenia coli by Bennett et al. (1966 *a*, *b*), Bülbring and Tomita (1967), and Kuriyama et al. (1967 *a*, *b*). However, the minimum latency of the excitatory potential was 20–65 msec (45 ± 6.5 SD, n = 20), a time much shorter than that observed by Bennett (1967).

Fig. 1 shows the responses of the jejunum to field stimulation (0.2 msec

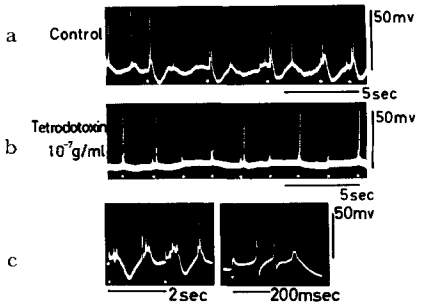

FIGURE 1. Responses of the jejunum smooth muscle to field stimulation (0.2 msec pulse duration) and the effect of tetrodotoxin (10^{-7} g/ml). (a) Responses of the muscle membrane to field stimulation. (b) Responses of the muscle membrane in the presence of tetrodotoxin. (c) Rapid sweep of photograph (explanation is the same as for (a)).

pulse) and the effect of tetrodotoxin (10^{-7} g/ml) on these responses. Field stimulation of the jejunum evoked a spike, an excitatory potential, and an inhibitory potential successively (Fig. 1 a and c) and these responses were blocked by tetrodotoxin except for the spike in response to the stimulation of the muscle. Although at these particular stimulus parameters treatment with tetrodotoxin did not consistently elicit the spikes, the spike could always be evoked by increasing current intensity or duration, or by reduction of stimulus frequency.

Responses of the Membrane to Field Stimulations of Different Polarity and Different Parameters

The response of the jejunum smooth muscle to field stimulation varied with the parameters of the stimulus.

Fig. 2 shows the effects of stimulus polarity on the activity of a strip of jejunum membrane during a period of electrical and mechanical quiescence (silent period). When an anodal pulse of 0.2 msec duration was applied to the

tissue, an inhibitory potential was elicited (Fig. 2 a), whereas a cathodal pulse of the same duration and intensity triggered a spike, excitatory potential, and inhibitory potential successively (Fig. 2 b). In Fig. 2 c, the responses of the membrane to two different intensities of anodal pulse are superimposed. The pulse of weaker intensity is the same as that used in Fig. 2 a. The pulse of strong intensity elicited a spike, excitatory junction potential, and inhibitory potential successively as observed in Fig. 2 b. These observations indicate that the threshold of the inhibitory potential was lower than that of the

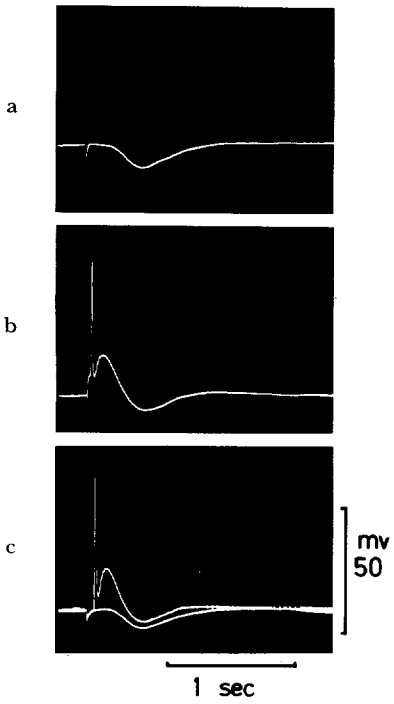

FIGURE 2. Effects of stimulus polarity in eliciting the membrane activity of the jejunum during the silent period. (a) 0.2 msec anodal pulse; (b) 0.2 msec cathodal pulse; (c) two different intensities of anodal pulse were applied successively.

excitatory potential, and further, that cathodal pulses were more effective than anodal pulses in eliciting an excitatory potential.

Fig. 3 shows the effects of changing the duration (a and b) and the intensity (c and d) of the stimulus in eliciting inhibitory potentials (a and c) and also excitatory with inhibitory potentials (b and d). In Fig. 3 a and c, only the inhibitory potentials were recorded. Increased duration and intensity of the stimulus enhanced the amplitudes of the inhibitory potential but the peak times of the inhibitory potential were nearly the same. In Fig. 3 b and d, the excitatory potential together with the inhibitory potential could be elicited using a stronger stimulus intensity than that applied in Fig. 3 a and c, This increased intensity and duration enhanced the amplitude of the excitatory potential but no enhancement in inhibitory potential amplitude was observed, because it had already reached a steady level.

Excitatory Potentials

Excitatory slow depolarizations could be recorded in response to field stimulation. It was difficult, however, to record the excitatory potential alone without generation of the inhibitory potential as described previously; thus causing difficulty in measuring the decay time of the excitatory potentials. The shape of the excitatory potential was modified by the generation of the spike and the inhibitory potential. When the spike was triggered by the excitatory potential the falling phase of the spike wiped out the course of the excitatory potential (see Fig. 4 b). The inhibitory potential generated with a latency

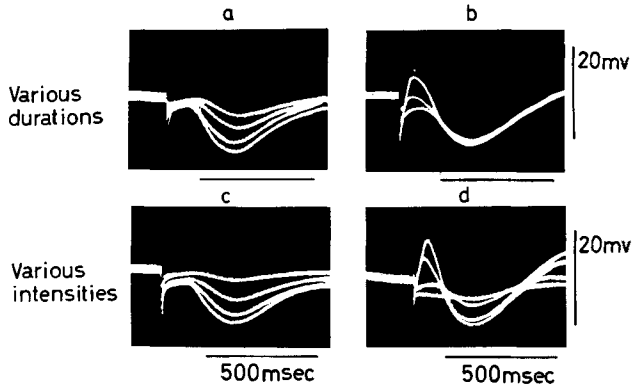

FIGURE 3. Effects of various durations (a and b) and intensities (c and d) of the anodal pulse stimulus in eliciting the inhibitory potentials (a and c) and excitatory potentials with the inhibitory potentials (b and d). (b) and (d) were recorded using a stimulus of stronger intensity than that recorded in (a) and (c). Stimulus duration used in (a) was 0.2, 0.3, 0.4, and 0.5 msec and in (b), 0.3, 0.5, and 0.7 msec. Stimulus intensity used in (c) was 5, 7.5, 10, and 12.5 v applied extracellularly and in (d) it was 5, 7.5, 15, and 20 v, respectively.

longer than that of the excitatory potential reduced the amplitude of the falling phase of the excitatory potential further, as shown in Fig. 2 c. The excitatory potential usually appeared as the tissue response to the field stimulation. However, on some occasions, the excitatory potential followed the spike which was generated as a response of the muscle membrane to the field stimulation and also followed the spontaneously generated spike. In the latter case, the latency depended on the time for the muscle spike generation. Fig. 4 shows two different types of membrane excitatory potentials elicited by the field stimulation. In Fig. 4 a, the excitatory potential followed the spontaneously generated spike and elicited a second spike. In Fig. 4 b, the excitatory potential in response to the field stimulation elicited the spike. When the spike was elicited by the excitatory potential, the spike partly wiped out the falling phase of the excitatory potential (Fig. 4 a and b). In Fig. 4 c, two sweeps of

membrane activities, i.e. the spike, excitatory potential, and inhibitory potential elicited by the field stimulation, were superimposed. One of them, the spike with the following excitatory potential appeared with long latency, but the peak times of the inhibitory potential remained the same in both records.

An excitatory potential appearing after the cessation of the inhibitory potential, the postinhibitory rebound, has been described by Bennett (1966 *a*) and Kuriyama et al. (1967 *b*). This inhibitory potential sometimes gave rise to a depolarization of the membrane and generated the spikes. The large depolarization of the membrane appearing after the cessation of the inhibitory

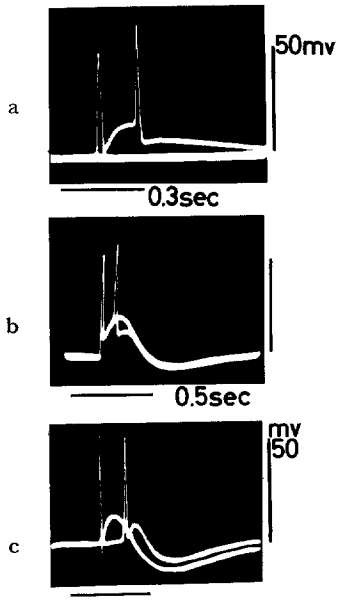

FIGURE 4. Two different types of excitatory potentials of the jejunum smooth muscle cells elicited by field stimulation. (a) The excitatory potential was elicited by the spontaneously generated spike. The excitatory potential also generated the second spike. (b) The excitatory potential in response to field stimulation. When the excitatory potential elicited the spike, the falling phase of the excitatory potential was partly wiped out. (c) The spike, excitatory potential, and inhibitory potential in response to field stimulation. The latency of the spike and the excitatory potential differed in the two superimposed records but the crest time of the inhibitory potential remained the same.

potential is probably not due to the reappearance of the excitatory potential which was previously lost during the generation of the inhibitory potential.

In some cells, it was difficult to decide whether the spike discharge which appeared after the inhibitory potential was due to postinhibitory rebound or to the inherent spontaneous activity of the smooth muscle.

Fig. 5 shows spontaneous membrane activities recorded from the jejunum (a) and membrane activities elicited by field stimulation (b). Both records were taken from the same cell. When field stimulation (0.2 msec pulse) was applied to the tissue, spikes, excitatory potentials, and inhibitory potentials could be recorded. The inhibitory potential transiently blocked the spike generation although the membrane potential level remained at a more depolarized level than the resting membrane potential. During such spontaneous bursts of spikes the sustained depolarization continued for more than 5 sec. Casteels and Kuriyama (1965, 1966) reported that the excitability of

the membrane varied periodically in the rat uterus and guinea pig taenia coli and that when a single stimulus was applied during the low threshold period of the excitability cycle, a train of discharges could be elicited. Presumably the phenomena observed in Fig. 5 b might correspond to those described by Casteels and Kuriyama.

All the excitatory potentials produced by field stimulation were blocked by treatment with atropine [5×10^{-6} g/ml) and tetrodotoxin (10^{-7} g/ml), and were enhanced by treatment with $BaCl_2$ (0.5 mM) and prostigmine (5×10^{-6} g/ml). These effects of drugs on the excitatory potentials confirmed the observations made by Kuriyama et al. (1967 *b*).

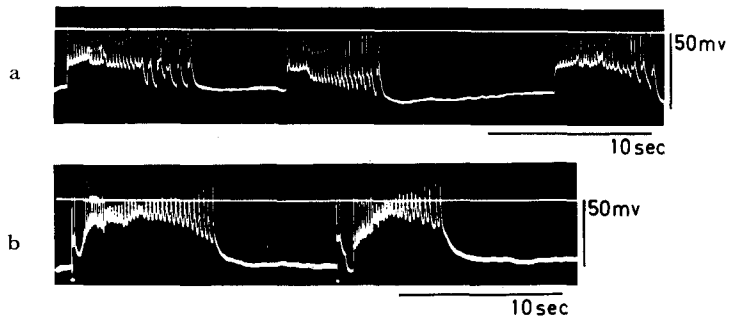

FIGURE 5. Membrane activities recorded from the jejunum. (a) Spontaneous discharges. (b) Membrane activities elicited by field stimulation. The tissue was the same as the one whose activities are recorded in (a).

Effect of Changes in Membrane Potential Produced by Intracellular Polarization

Fig. 6 shows changes in the amplitude of the excitatory and inhibitory potentials resulting from changes in membrane potential. When the membrane was hyperpolarized by inward currents, the amplitude of the excitatory potential increased proportionally. However, no change in the amplitude of the inhibitory potential was observed. The relationships between the amplitude of the excitatory potential (three experiments) and the inhibitory potential (one experiment) and the membrane potential level are illustrated in Fig. 7. The membrane was hyperpolarized to -90 mv. The reversal potential levels for the excitatory potential were estimated by extrapolation from the measured range. Reversal potential levels measured from the three specimens mentioned above ranged from $+5$ mv to -5 mv and the mean value was -3 mv (SD = ± 0.6, $n = 8$). These values agree nicely with those measured from the various excitatory junction potentials recorded from other junctions and synapses where it is likely that the excitatory potential is due to release of acetylcholine from the nerve terminals (cf. Eccles, 1964). However, the above values obtained from the excitatory potential of the jejunum might not indicate the

absolute value of the equilibrium potential for the excitatory junction potential, because smooth muscle cells are innervated diffusely by the excitatory nerves (Burke and Ginsborg, 1956). The amplitude of the inhibitory potential had no relation to the membrane potential level until the membrane was hyperpolarized to −90 mv.

Input Resistances of the Membrane during the Excitatory and Inhibitory Potential and Acetylcholine Potential

The mean input resistance and time constant of the longitudinal muscle membrane calculated from the electrotonic potential in response to intracellular

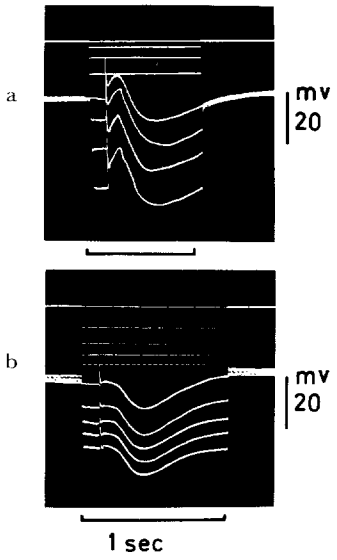

FIGURE 6. Effects of intracellular polarizations on the amplitudes of the excitatory and inhibitory potentials of the jejunum elicited by field stimulation (0.2 msec pulse). Duration of conditioning pulse was 1 sec. (a) Excitatory and inhibitory potentials elicited by field stimulation. (b) Inhibitory potential.

stimulation were 45 MΩ (n = 11) and 3.5 msec (n = 11), respectively. On the other hand, the space constant of the muscle bundles calculated from the electrotonic potentials in response to extracellular stimulation at three different distances from the stimulating electrode was 1.3 mm (n = 3) and the time constant of the membrane measured at 0.5 mm distance from the stimulating electrode was 120 msec at 64 % decay of the electrotonic potential. The current-voltage relation of the membrane observed by the intracellular polarizing method showed a linear relation in the range of −90 mv to −20 mv. However, the current-voltage relation observed by extracellular stimulation showed rectification of the membrane when it was depolarized beyond −42 mv and −48 mv (n = 3). The difference in the current-voltage relation observed with the two different stimulating methods suggests that the input resistance of the membrane measured by the intracellular polarizing method

might not show the true input resistance of the membrane. Nevertheless, the amplitudes of the electrotonic potentials evoked by intracellular polarizing currents during the depolarization phase were reduced by 10–15 % around

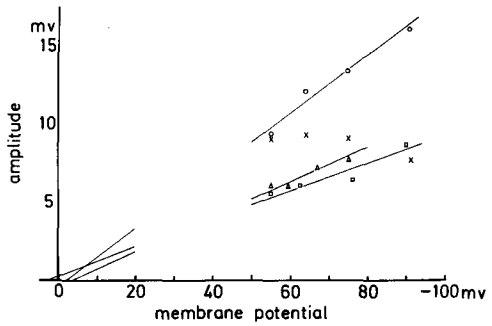

FIGURE 7. Effects of intracellular polarization on the amplitudes of the excitatory (three specimens) and inhibitory (one specimen) potentials elicited by field stimulation (0.2 msec pulse). Unfilled symbols indicate the amplitudes of the excitatory potential under various membrane potential levels. × indicates the amplitudes of the inhibitory potential.

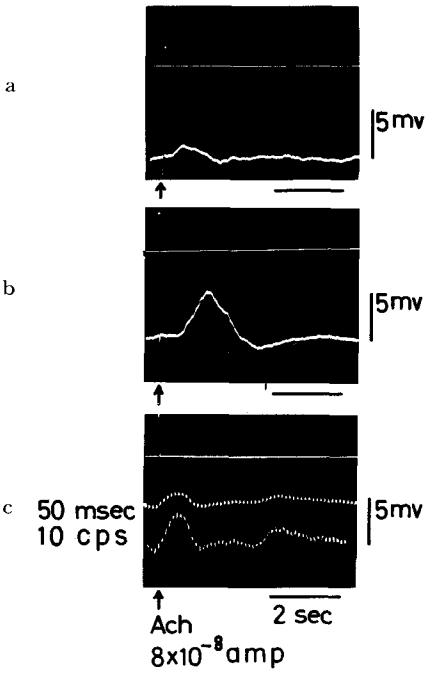

FIGURE 8. Acetylcholine potentials evoked by iontophoretic application of acetylcholine (pulse duration; 10 msec; current intensity, 8 × 10^{-8} amp). (a) Application of cathodal pulse through Ach-filled electrode. (b) Application of anodal pulse. (c) Changes in the electrotonic potentials during the generation of Ach potential were measured by the application of anodal pulses through the recording electrode (2 × 10^{-10} amp, 50 msec pulse 10 cps).

the peak of the excitatory potential. On the other hand, in more than 30 fibers no change could be detected in the input resistance during the hyperpolarization of the inhibitory potential.

Iontrophoretic application of acetylcholine to the jejunum evoked slow de-

polarization of the membrane. Fig. 8 shows this effect. The acetylcholine electrode and the recording electrode were placed within 50 μ. When a current of 8×10^{-8} amp and 10 msec duration was applied to the stimulating electrode, the membrane was depolarized and the input resistance of the membrane measured by intracellularly applied currents during such depolarization was reduced by 30–50 % in comparison to the resistance of the resting membrane.

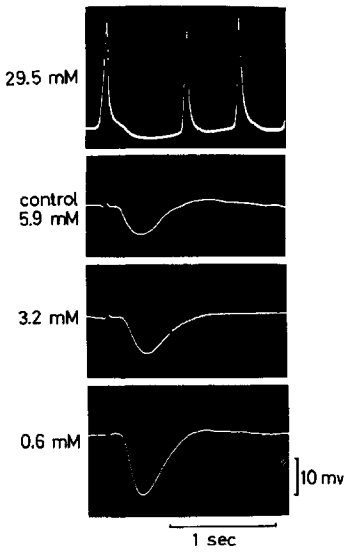

FIGURE 9. Effects of various external potassium concentrations (0.6–29.5 mM) on the inhibitory potentials elicited by field stimulation (0.3 msec pulse).

Effects of Various Ions and Drugs on the Excitatory and Inhibitory Potentials Elicited by Field Stimulation

EFFECTS OF IONS

The effects of variation in potassium concentrations on the inhibitory potential were examined. When the external potassium concentration was reduced, the membrane potential was hyperpolarized and the amplitude of the inhibitory potential was enhanced. Increased external potassium concentration, $[K]_o$, decreased the amplitude of the inhibitory potential. Fig. 9 shows the effects of variation in $[K]_o$ from 0.6 mM to 29.5 mM on the inhibitory potentials elicited by the field stimulation. Lowered $[K]_o$ not only enhanced the amplitude but also increased the velocity of the upstroke of the inhibitory potential. When $[K]_o$ was increased to 29.5 mM (five times the normal solution) reducing NaCl accordingly, the inhibitory potential was almost abolished.

Fig. 10 shows changes in the membrane potential and amplitudes of the inhibitory potential in different external potassium and chloride concentrations.

The test solutions were kept isotonic. In normal $[K]_o$, the membrane potential was −53.1 mv (SD = ±2.1, n = 20) and the inhibitory potential reached −61.7 mv (SD = ±1.7, n = 25). When $[K]_o$ was reduced to a tenth of the normal concentration, the membrane was hyperpolarized to −65.4 mv (SD = ±3.2, n = 20) and the inhibitory potential reached −79.6 mv (SD = ±2.1, n = 45).

When the external chloride was reduced to a tenth of the normal concen-

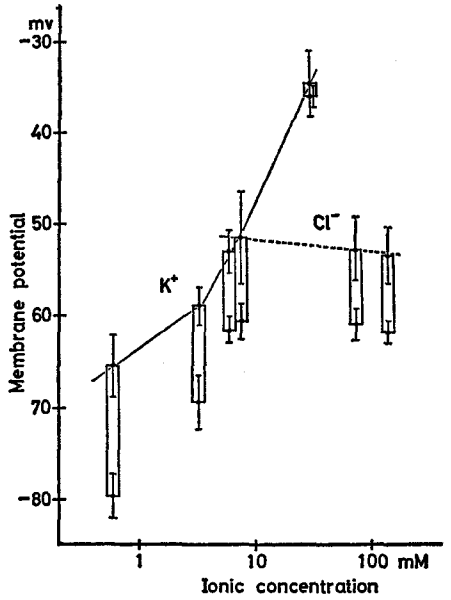

FIGURE 10. Changes in the membrane potentials and amplitudes of the inhibitory potential with various external potassium concentrations (0.6–29.5 mM) and with various external chloride concentration (7.4 mM Cl^- and 70.7 mM Cl^-, glutamate was substituted for chloride). Vertical bars, twice the standard deviation. Continuous line shows changes in the membrane potential caused by the various $[K]_o$, dotted line shows changes in the membrane potential caused by the various $[Cl]_o$. Rectangles show the grades of hyperpolarization of the membrane produced by field stimulation.

tration by substitution with glutamate, the membrane was transiently depolarized and then gradually returned to the normal membrane potential level (control −52.2 mv, SD = ±3.0, Cl-deficient solution −51.6 mv, SD =1 ±4.9). However, no change in the amplitude of the inhibitory potential was observed.

Effects of Ca^{++} and Mg^{++} ions on the inhibitory potential was observed. When the $[Ca]_o$ was reduced to a tenth of the normal concentration (2.5 mM), the membrane was depolarized from −54.6 mv (SD = ±3.8, n = 20) to −43.1 mv (SD = ±4.1, n = 20), but no change in the amplitude of the in-

hibitory potential was observed (control, 8.7 mv, SD = ±2.2, n = 16; in 0.25 mM $[Ca]_o$, 8.7 mv, SD = ±1.5, n = 16). The excitatory potential was completely abolished in Ca-deficient solution. Excess Mg solution (18 mM) slightly hyperpolarized the membrane but no change in the amplitude of the inhibitory potential was observed (control, 8.6 mv, SD = ±1.2, n = 48, 5 times the normal; $(Mg)_o$, 8.6 mv, SD = ± 1.0, n = 43, 10 times the normal; $(Mg)_o$, 8.0 mv, SD = ±1.1, n = 50).

In excess Mg^{++} with low Ca^{++} solution (18 mM Mg^{++} and 0.25 mM Ca^{++}), the inhibitory potential was slightly reduced from 8.7 mv (SD = ±2.2, n = 16) to 6.7 mv (SD = ±1.3, n = 15).

These experiments suggest that (*a*) the inhibitory potential had a close relation with $[K]_o$ but not with $[Cl]_o$; (*b*) excess Mg^{++} and low Ca^{++} had almost no effect on the amplitude of the inhibitory potential. These findings are of interest since it is known that Ca^{++} and Mg^{++} ions exert competitive effects on the release of the chemical transmitter, e.g. excess Ca^{++} increases the release of chemical transmitter, and low Ca^{++} and high Mg^{++} have the opposite effect; (*c*) the membrane potential level, modified by the ionic composition, did not have a close relation with the amplitude of the inhibitory potential; i.e., hyperpolarization of the membrane induced by low potassium enhanced the amplitude of the inhibitory potential and depolarization of the membrane induced by low calcium did not change the amplitude of the inhibitory potential.

The Action of Drugs on the Inhibitory Potential

Effects of various inhibitors of chemical transmission namely, picrotoxin, strychnine, morphine, and lysergic acid (LSD-25), on the inhibitory potential were investigated. 10^{-4} g/ml of picrotoxin and strychnine, and 5×10^{-5} g/ml of morphine and LSD-25 had no specific effect on the inhibitory potential.

10^{-4} g/ml of picrotoxin and strychnine did not change the membrane potential but 5×10^{-5} g/ml of morphine and LSD-25 depolarized the membrane by 5–8 mv (n = 3) and, not only the inhibitory potential, but also the excitatory potential was reduced. Metabolic inhibitors, ouabain (5×10^{-5} g/m), *p*-chloromecuribenzoic acid (PCMB, 5×10^{-6} g/ml), and 2,4-dinitrophenol (5×10^{-5} g/ml), were also studied. These drugs generally depolarized the membrane and reduced the amplitude of both the excitatory and the inhibitory potential was reduced. No specific action on the inhibitory potential was observed.

DISCUSSION

Excitatory Potential

Responses of the guinea pig jejunum to field stimulation differ in several ways from those described previously for the guinea pig taenia coli.

Bennett (1966 *b*) studied the excitatory junction potentials of the taenia coli elicited by field stimulation. He found that in some cells stimulation of the intramural nerves with single pulses of maximum strength gave excitatory junction potentials of about 20 mv amplitude after a latency of 100–200 msec and further, that in the presence of neostigmine there was no detectable increase in the number of cells showing excitatory junction potentials. These excitatory junction potentials were blocked by treatment with atropine.

In the jejunum, the latency of the excitatory potential was only 20–65 msec. However, when the excitatory potential followed the spike, its latency depended on the time when the muscle spike was generated.

For the following reasons excitatory potentials elicited by field stimulation are probably caused by release of acetylcholine from nerve terminals distributed in the jejunum muscle layer: (*a*) atropine and tetrodotoxin blocked the generation of the excitatory potential while prostigmine enhanced its amplitude and prolonged its duration. (*b*) Conditioning hyperpolarization of the membrane enhanced the amplitude of the excitatory potential. These observations might indicate an increased ionic conductance during the depolarization, which was also suggested by the existence of an equilibrium potential around 0 mv (-5 to $+5$ mv) for the excitatory potential.

In normal solution, acetylcholine depolarized the membrane. However, when the membrane was depolarized more than 40 mv (normal resting membrane potential was -53 mv) by excess potassium, acetylcholine hyperpolarized the membrane (Burnstock, 1958; Bülbring and Kuriyama, 1963). The equilibrium potential for acetylcholine added in Krebs solution was roughly estimated to be from -15 to -5 mv. In the jejunum, the reversal potential level for the excitatory potential was estimated to be within the range of -5 to $+5$ mv. Presumably, the generation of the excitatory potential is due to an increase in the sodium and potassium permeabilities of the membrane caused by release of acetylcholine. The slight reduction in the input resistance during the depolarization might be due to a three dimensional spread of the electrotonic current as a consequence of the morphology of the smooth muscle tissue (Kuriyama and Tomita, 1965).

The excitatory junction potentials generated either by field stimulation of the tissue or by an evoked or spontaneous spike might be of the same nature. However, the excitatory junction potential elicited by the spike might be due to the activation of the peripheral nerve fibers by the current arising from the activated muscle fibers.

Inhibitory Potential

The hypothesis that generation of the inhibitory potential is due to release of a chemical transmitter from the nerve terminals is supported by the following experiments.

Bennett et al. (1966 *a*, *b*) showed that the membrane potential changes in the guinea pig taenia coli in response to stimulation of intramural inhibitory nerves differed from those produced by peripheral perivascular inhibitory nerve stimulation in several ways. They therefore postulated that inhibitory nerves which were not of sympathetic origin might be distributed in the taenia coli. Bülbring and Tomita (1967) confirmed the presence of inhibitory nerves in the taenia and carried out further detailed investigations of the properties of the inhibitory junction potential produced by field stimulation. Bülbring and Tomita (1967) deduced that the length of the nerve fibers involved in the inhibitory response is probably only a few millimeters and the space constant of the order of 0.1 mm.

The effect of differences between the inhibitory potential and the excitatory junction potential can be summarized as follows: (*a*) The intensity of stimulus required to elicit the inhibitory potential was lower than that needed to elicit the excitatory junction potential. (*b*) The latency was much shorter for the excitatory junction potential than for the inhibitory potential. (*c*) The effects of membrane polarization on the amplitudes of both potentials differed, as described previously for the taenia coli (Bennett and Roger, 1967). (*d*) In a low $[Ca]_o$ solution, the excitatory junction potential was blocked but almost no change was observed in the inhibitory potential. (*e*) Hyperosmotic solution (2.5 times the normal osmolarity) prepared by excess $[Na]_o$ blocked the excitatory junction potential but not the inhibitory potential (unpublished observation). (*f*) Repetitive stimulation facilitated the amplitude of the excitatory junction potential but not that of the inhibitory potential (unpublished observations).

Similarities in the changes in both potentials could be observed by treatment with tetrodotoxin which blocked the generation of both potential changes completely.

Recently, Ogura, Mori, and Watanabe (1966) reported that treatment with tetrodotoxin inhibited the release of acetylcholine from the isolated guinea pig ileum. Therefore, tetrodotoxin may block the nervous activity without affecting the intestinal smooth muscle (Nonomura, Hotta, and Ohashi, 1966; Kuriyama, Osa, and Toida, 1966).

If the inhibitory potential elicited in the jejunum by field stimulation is due to release of a chemical transmitter from the nerve terminals, we must solve many problems; i.e., the inhibitory nerves may have a larger diameter than the excitatory nerves, because the former have a lower threshold. Furthermore, the inhibitory nerves innervate the smooth muscle more diffusely than the excitatory nerves. Presumably, due to local innervation, excitatory nerves activate the smooth muscle fibers in particular areas, reducing the membrane resistance; hence the amplitude of the excitatory junction potential is modified by conditioning polarization. On the other hand, the inhibitory nerves

may generate the inhibitory potential in a larger area of the tissue due to more diffuse innervation and the relatively long space constant of the membrane (1.2 mm). Therefore, there is probably a difference in current spread in the tissue during the excitatory and the inhibitory potentials. This geometrical factor may account for the difference between the effects of intracellular polarization on the inhibitory potential and on the excitatory potential.

Effects of divalent ions, namely Ca^{++} and Mg^{++}, on the mechanism of release of the inhibitory chemical transmitter must be different from those observed in many excitatory chemical transmissions. Low Ca^{++} (0.25 mM) and high Mg^{++} (18 mM) had no effect on the amplitude of the inhibitory potential.

In low potassium solutions the amplitude of the inhibitory potential was enhanced. This observation confirmed the results obtained by Bennett et al. (1963) who postulated that the inhibitory potential was due to increased potassium permeability caused by chemical transmitter action.

The unknown inhibitory chemical transmitter is probably not 5-hydroxytryptamine or γ-aminobutyric acid, because drugs known to be the competitors of both the drugs had no specific action on the inhibitory potential.

As an alternative hypothesis, the metabolic inhibitors, including the uncoupler of the active transport of ions, were used to investigate the possibility that an electrogenic pump might be responsible for the generation of the inhibitory potential. However, these drugs had no specific action on the inhibitory potential.

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