Low-sodium Resistant Non-adrenergic Inhibitory Neurotransmission in the Guinea-pig Duodenum

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Abstract

Ohkawa, H. Low-sodium resistant non-adrenergic inhibitory neurotransmission in the guinea-pig duodenum. Japanese Journal of Smooth Muscle Research, 22(1), 1-10. — The evoked inhibitory potentials (i.p.s) in the longitudinal smooth muscle cells of the guinea-pig duodenum were recorded intracellularly. The i.p.s were not blocked by adrenergic blocking agents, guanethidine (10^-6 g/ml), propranolol (10^-6 g/ml) and phentolamine (10^-6 g/ml), and atropine (10^-6 g/ml). Tetrodotoxin (10^-7-10^-6 g/ml) abolished the non-adrenergic non-cholinergic i.p.s evoked by single or repeated stimulation without changes in the resting membrane potential of the longitudinal smooth muscle. In the presence of atropine (10^-6 g/ml), acetylcholine (5×10^-9-7×10^-7 g/ml) had no effect on the amplitude of the i.p.s d-tubocurarine (2×10^-4 g/ml) reduced the amplitude of the i.p.s and produced a small depolarization in the muscle membrane. The longitudinal muscle membrane was slightly depolarized by Li-solution and the i.p.s could be evoked in the Li-solution for a long period, accompanying with the slight decrease in the amplitude of the i.p.s. In the choline-solution, the depolarization of the muscle membrane and the slight increase in the amplitude of the i.p.s were obtained. The i.p.s were abolished in the Ca^2+-free choline-solution. In the sucrose-solution, the decrease of the resting membrane potential of the longitudinal smooth muscle was observed and the amplitude of the i.p.s was gradually reduced during the perfusion of the sucrose-solution. Chloride deficiency had no considerable effect on the amplitude of i.p.s.

The results obtained suggest that the non-adrenergic non-cholinergic inhibitory neurotransmission is low-sodium resistant and the excitation-secretion coupling in the non-adrenergic non-cholinergic inhibitory nerves is mainly dependent on the external calcium ions but not sodium ions.

Introduction

Although there are many electrophysiological investigations on the non-adrenergic inhibitory neurotransmission, details of the non-adrenergic inhibitory mechanism in the gastrointestinal tract still remain unclear. As to the non-adrenergic inhibitory transmitter, the purinergic nerve hypothesis (Burnstock, 1972) and the peptidergic hypothesis (Fahrenkrug, 1979; Goyal et al., 1980) have been proposed. However, it seems that there is no convincing evidence to
support these hypotheses. On the other hand, there is evidence that ATP, VIP and some opioids are not inhibitory mediators (Bauer et al., 1982; Cocks et al., 1979; Daniel et al., 1983; Mackenzie et al., 1980).

The electrical activity of the non-adrenergic inhibitory nerves has not been successfully recorded. This is a disadvantage in promoting studies on the non-adrenergic inhibitory innervation of the gastrointestinal canal. Daniel et al. (1977) reported no structural distinction between cholinergic and non-adrenergic axons based on the histological study. It is also interesting how to relate the non-adrenergic inhibitory nerves with the enteric neurons and extrinsic nerves (Beari et al., 1971; Goyal et al., 1975).

The release of the non-adrenergic inhibitory transmitter was influenced by external calcium and magnesium ions (Holman et al., 1975; Lang, 1979; Ohkawa, 1984). This property was similar to that in the motor nerve endings (Kharasch et al., 1981; Rahaminoff, 1970; Silinsky, 1978). On the other hand, it is known that tetrodotoxin blocks the evoked inhibitory potentials in the small intestine (Bauer et al., 1982; Bülbbring et al., 1967; Ito et al., 1971). Therefore, it is of interest to study whether or not the external sodium ions relate to the release of the non-adrenergic inhibitory transmitter. In the present experiments, effects of external sodium ions on the generation of the non-adrenergic inhibitory potential in the duodenal smooth muscles were examined. The results obtained may supply guides to understand the properties of the non-adrenergic inhibitory nerves and the release mechanism of the non-adrenergic inhibitory transmitter.

**Methods**

Guinea-pigs of either sex weighing 300-400 g were stunned and bled, and a short segment (3 cm from a pylorus of stomach) of the duodenum was removed. The duodenum was opened along the mesenteric border and full-thickness strips (4 mm x 2 mm) were cut parallel to the longitudinal direction. The strips were mounted serosal surface uppermost in an organ bath with approximately equal lengths of tissue being placed within the stimulating electrodes. Inhibitory potentials in the longitudinal smooth muscle were recorded with intracellular micro-electrodes placed less than 1 mm from one of the stimulating electrodes in response to rectangular pulses (usually 0.3 msec duration at constant strength). The modified Krebs solution contained (mM): NaCl 122, KCl 4.9, NaHCO₃ 15.5, KH₂PO₄ 1.2, CaCl₂ 2.5, MgCl₂ 1.2 and glucose 11.5. Low sodium Krebs solution was made by replacing NaCl by equal molar concentrations of LiCl (Li-solution) or choline chloride (choline-solution) or by twice the molar concentration of sucrose (sucrose-solution). In these solutions 15.5 mM of NaHCO₃ was still present. Low chloride Krebs solution was made by replacing NaCl by equal molar concentrations of NaBr (Br-solution) or Na glutamate (glutamate-solution). In these solutions 2.5 mM of CaCl₂, 1.2 mM of MgCl₂ and 4.9 mM of KCl were still present.

The drugs used are as follows; atropine sulfate, acetylcholine chloride (Nakarai Chemicals), guanethidine sulfate, phentolamine hydrochloride (Regitine, Ciba), propranolol hydrochloride (Inderal, Sumitomo), tetrodotoxin (Sigma) and d-tubocurarine chloride (Tokyo Kasei).

Values of the measured parameters of muscle membrane and inhibitory potentials were expressed as the mean ± S.E. (n = number of penetrations of the micro-electrodes or number
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of observed inhibitory potentials). Statistical significance was assessed using Student’s $t$-test.

Results

General property of the non-adrenergic inhibitory potentials

When a brief pulse was given to a preparation, a transient, small hyperpolarization of the longitudinal smooth muscle cell membrane (inhibitory potential, i.p.) was produced. The parameters on the i.p.s elicited by single stimuli were similar to those reported previously (Ohkawa, 1983). The i.p.s were not blocked by adrenergic blocking agents, guanethidine $10^{-6}$ g/ml, propranolol $10^{-6}$ g/ml and phentolamine $10^{-6}$ g/ml. In these preparations, spontaneous and evoked excitatory junction potentials were not observed. The following experiments were carried out under the absence of atropine and guanethidine except the experiment on the effects of acetylcholine.

Effects of TTX on the inhibitory potentials

Fig. 1 (A-C) shows the effects of TTX ($10^{-7}$-$10^{-6}$ g/ml) on the i.p.s elicited by single and repeated stimuli. The membrane potentials were $-54.0 \pm 0.9$ mV ($n=20$) in control and $-52.8 \pm 0.8$ mV ($n=12$) in the presence of $10^{-6}$ g/ml TTX. The amplitude of the i.p.s evoked by single stimuli was reduced by TTX $10^{-7}$ g/ml and a complete inhibition of the i.p. was observed at the concentration of $10^{-6}$ g/ml TTX (Fig. 1A). The i.p.s evoked by repeated stimulation (1-10 Hz) were abolished by $10^{-7}$ and $10^{-6}$ g/ml of TTX (Fig. 1B and C).

![Fig. 1. Effects of TTX on the inhibitory potentials](image)

A: TTX $10^{-7}$ and $10^{-6}$ g/ml on the i.p.s evoked by single stimuli.
B: TTX $10^{-7}$ and $10^{-6}$ g/ml on the i.p.s evoked by repeated stimulations (1-10 Hz).
C: TTX $10^{-7}$ and $10^{-6}$ g/ml on the i.p.s evoked by single stimuli.
D: Inhibition of the i.p.s evoked by repeated stimulations in the Ca$^{2+}$-free, 1 mM EGTA solution.
It has been reported that the amplitude of the evoked i.p. was reduced in a low-\(\text{Ca}^{2+}\) solution (Holman et al., 1975; Lang, 1979; Ohkawa, 1984). For comparison with the inhibition of the i.p. in the low-\(\text{Ca}^{2+}\) solution, the effect of the \(\text{Ca}^{2+}\)-free solution containing 1 mM of EGTA on the i.p. was examined. This solution slightly depolarized the membrane from \(-50.4 \pm 2.5 \text{ mV} \) (\(n=5\)) to \(-47.8 \pm 1.5 \text{ mV} \) (\(n=12\)). The i.p.s were completely blocked by this solution, as shown in Fig. 1D.

**Effects of acetylcholine on the inhibitory potentials**

In the presence of atropine (10\(^{-6}\) g/ml), acetylcholine (5\(\times\)10\(^{-9}\) and 1.7\(\times\)10\(^{-7}\) g/ml) was added into the solution. Spontaneous action potentials in the longitudinal smooth muscle due to application of a cetylcholine were not observed. The resting membrane potential of the longitudinal smooth muscle was \(-51.3 \pm 1.1 \text{ mV} \) (\(n=20\)) in the atropine (10\(^{-6}\) g/ml) solution. No detectable changes in the membrane potential were observed by addition of acetylcholine at 5\(\times\)10\(^{-9}\) g/ml (51.1 \pm 1.6 mV, \(n=12\), \(p<0.20\)) but the resting membrane potential was slightly decreased at 1.7\(\times\)10\(^{-7}\) g/ml of acetylcholine (\(-49.2 \pm 0.7 \text{ mV} \), \(n=10\), \(p<0.001\)). However, the evoked i.p.s were not affected by acetylcholine (Fig. 2A and B).
Fig. 3. Effects of the Li-solution on the i.p.s evoked by single and repeated stimulations.
A: I.p.s evoked by single stimulus in control and the Li-solution. Times indicate the perfusion period.
B: I.p.s evoked by repeated stimulation (2 Hz) in control and the Li-solution (30 min).
C: I.P.s were completely blocked in the Li-solution including TTX 10^{-6} g/ml. The resting membrane potentials were shown at the left side.

On the other hand, d-tubocurarine (2 \times 10^{-5} g/ml) gradually decreased the amplitude of the i.p.s evoked by single and repeated stimuli (Fig. 2C and D) and the i.p.s were abolished finally. At this concentration of d-tubocurarine, the membrane potential was changed from $-53.0 \pm 1.0$ mV (n=10) to $-47.0 \pm 1.8$ mV (n=4, p<0.005).

**Effects of low-sodium solutions on the inhibitory potentials**

After recording the i.p.s in normal solution, the solution was substituted by a solution in which 89% of the sodium was replaced by lithium (Li-solution), choline (choline-solution) or sucrose (sucrose-solution).

The membrane was slightly depolarized by the Li-solution, i.e.; it was $-55.0 \pm 1.0$ mV (n=25) in normal solution, the mean membrane potentials were $-47.8 \pm 0.6$ mV (n=25, p<0.001) in the Li-solution during the initial 30 min of perfusion, $-49.5 \pm 0.5$ mM (n=6, p<0.10) during the second 30 min, $-46.0 \pm 0.8$ mV (n=18, p<0.001) during the third 30 min and $-46.4 \pm 0.6$ mV (n=18, p<0.001) during the forth 30 min, respectively.

Fig. 3A shows the i.p.s evoked by single stimuli in the Li-solution until 120 min of perfusion. The evoked i.p.s were not blocked by the long perfusion with this solution. The mean amplitude of the i.p.s were as follows; 16.9 \pm 1.4 mV (n=13, 6 cells) in normal solution, 14.5 \pm 0.9 mV (n=20, 9 cells, p<0.001) during the first 30 min in the Li-solution, 21.0 \pm 0.7 mV (n=6, 3 cells, p<0.50) during the second 30 min, 15.4 \pm 0.6 mV (n=17, 10 cells, p<0.005) during the third
Fig. 4. Effects of choline- and sucrose-solutions on the i.p.s evoked by single and repeated stimulations.


B1: Control (3 Hz), B2: I.p.s in the choline-solution.

C: I.p.s in the choline-solution. 1 and 2 also show the i.p evoked by single stimulus during the post-stimulus depolarization.

D1: Control, D2-5: I.p. evoked by single stimulus in the sucrose-solution.

E1: Control i.p.s evoked by repeated stimulation (4 Hz), E2-4, I.p.s in the sucrose-solution.

Times indicate the perfusion period.

30 min and 10.9 ± 0.6 mV (n=25, 17 cells, p<0.001) during the forth 30 min, respectively.

Fig. 3B shows the i.p.s evoked by repeated stimulation (3 Hz) in normal solution and the Li-solution. These i.p.s were also not blocked in the Li-solution. However, these i.p.s in the Li-solution were completely blocked by the addition of TTX 10⁻⁶ g/ml, as shown in Fig. 3C.

The effects of choline-solution on the i.p.s were also examined. In the normal solution, the membrane potential of the duodenal smooth muscle cell was −59.6 ± 1.7 mV (n=14) and the choline-solution depolarized the membrane to −41.0 ± 1.3 mV (n=15, p<0.001) during the initial 30 min of the perfusion. Fig. 4A and B show the i.p.s evoked by single and repeated stimuli (3 Hz) in the choline-solution. The i.p.s were not blocked in this solution. The parameters of the i.p.s evoked by single stimuli in normal solution and the choline-solution were as follows; the latencies were 56.0 ± 2.3 msec (n=16, 6 cells) and 67.3 ± 2.7 msec (n=15, 8 cells, p<0.005), the times to peak of the i.p were 116.8 ± 3.0 msec (n=16, 6 cells) and 166.3 ± 6.5 msec (n=15, 8 cells, p<0.001), the amplitudes of the i.p. were 23.5 ± 0.6 mV (n=25, 10 cells) and 25.6 ± 1.1 mV (n=28, 17 cells, p<0.10) and the rates of the hyperpolarization of the i.p. were 200.7 ± 11.0 mV/sec (n=16, 6 cells) and 150.6 ± 8.0 mV/sec (n=15, 8 cells, p<0.005) in normal and choline-solutions, respectively.

Fig. 4C (1 and 2) shows the effect of single stimuli during the post-stimulus depolarization due to a preceded field stimulation. During the post-stimulus depolarization, several action potentials generated. The amplitude of the i.p. evoked during the post-stimulus depolarization
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Fig. 5. Effects of the low-chloride solutions on the i.p.s evoked by repeated stimulation.

A: Control.
B: I.p.s evoked by repeated stimulation (1 Hz) in the Br-solution.
C: I.p.s evoked by repeated stimulation (1 Hz) in the glutamate-solution. The resting membrane potentials were indicated in each panel.

was larger than that of the preceded i.p. The i.p.s were completely abolished by a removal of external Ca\(^{2+}\) from the choline-solution.

In the sucrose-solution, the membrane potential was increased. The membrane potential was \(-54.1 \pm 0.9\) mV (n=25) in control and the mean membrane potentials in the sucrose-solution were \(-58.5 \pm 1.4\) mV (n=16, p<0.005) during the first 30 min of perfusion, \(-60.8 \pm 2.4\) mV (n=5, p<0.005) during the second 30 min and \(-60.5 \pm 1.4\) mV (n=10, p<0.005) during the third 30 min, respectively. In the forth 30 min, the mean membrane potential was again decreased to \(-55.0 \pm 1.9\) mV (n=5, p<0.50).

The i.p.s evoked by single stimuli were not abolished but those amplitudes were gradually reduced with the perfusion period of the sucrose-solution. Thus, the mean amplitudes were 32.1±0.2 mV (n=15, 10 cells) in control, 15.8±0.4 mV (n=39, 14 cells, p<0.001) during the first 30 min, 11.4±1.1 mV (n=10, 5 cells, p<0.001) during the second 30 min, 12.0±1.7 mV (n=12, 8 cells, p<0.001) during the third 30 min and 8.2±0.9 mV (n=5, 3 cells, p<0.001) during the last 30 min, respectively.

Prolongation of the latency, the time to peak of the i.p. and the rate of hyperpolarization of the i.p. were significantly in the sucrose-solution (Fig. 4D and E). The generation of the successive i.p.s evoked by repeated stimulation also deteriorated in this solution, as shown in Fig. 4E.

Effects of low-chloride solutions on the inhibitory potentials

The membrane potential of the duodenal smooth muscle cells was not altered in the Br-solution, i.e., \(-55.1 \pm 1.5\) mV (n=13) in control and \(-53.3 \pm 2.0\) mV (n=11) in the Br-solution, while in the glutamate-solution the membrane potential was slightly increased \((-60.8 \pm 1.7\) mV,
n = 10, p < 0.005).

Fig. 5 shows the i.p.s. evoked by repeated stimulation (1 Hz) in the low-chloride solutions. Changes in the parameters of the i.p.s. elicited by single stimuli were as follows; the latency was slightly prolonged in the glutamate-solution (53.6 ± 0.5 msec, n = 14, 8 cells in control and 69.1 ± 1.0 msec, n = 9, 4 cells, p < 0.001 in the glutamate-solution). The amplitude of the i.p.s were 19.0 ± 0.6 mV (n = 33, 14 cells) in control, 17.1 ± 0.3 mV (n = 28, 7 cells, p < 0.01) in the Br-solution and 17.1 ± 0.3 mV (n = 28, 7 cells, p < 0.01) in the glutamate-solution. The time to peak of the i.p. and the rate of hyperpolarization of the i.p. in these solutions were nearly the same to these in control.

Discussion

The obtained results and previous reports provide that TTX blocks the evoked i.p.s. in the intestinal smooth muscles (Bauer et al., 1982; Büllbring et al., 1967; Ito et al., 1971). These results suggest that the excitation in the non-adrenergic inhibitory nerves is external Na+-dependent. Therefore a resultant inhibition of the excitation-secretion coupling in the nerves is expected by the low-sodium treatment. However, as shown in the results, the i.p.s. could be evoked in the low-sodium solutions during a long perfusion period. A substitution of the role of sodium ions on the mechanism of the excitation in the nerves by lithium or choline ions is deniable because the i.p. could be elicited in the sucrose-solution. In the used low-sodium solutions, the 16 mM of Na⁺ was still remained. One possibility is that the excitation of the non-adrenergic inhibitory nerves is supported by such small amount of external sodium ions.

It is known that the amplitude of elicited i.p. depends on the external calcium concentrations (Holman et al., 1975; Lang, 1979; Ohkawa, 1983). A decreased amplitude of i.p. in the low-calcium solution was restored by addition of barium or strontium ions (Ohkawa, 1983). These results indicate that the release of the non-adrenergic inhibitory transmitter requires Ca²⁺ influx into the nerve terminals. In the present experiments, the i.p. was abolished in the Ca²⁺-free low-sodium solution but not in the low-sodium solutions. Therefore, an alternative explanation is that the excitation-secretion coupling in the non-adrenergic inhibitory nerves is mainly dependent on the external calcium ions but not sodium ions. Katz and Miledi (1967) showed that sodium ions were not necessary for transmitter liberation in the neuromuscular junction (also c.f., Rahaminoff, 1970). However, a partial inactivation on the release mechanism, as shown in the neuromuscular transmission (Colomo et al., 1968) was not clear. The amplitude of i.p. was not affected by reducing chloride ions. The release of the non-adrenergic inhibitory transmitter may not be affected by the external chloride ions. Muchnik and Gate (1968) concluded that chloride ions do not have any significant role in the transmitter release at the neuromuscular junction.

In vivo, it is considered that the non-adrenergic inhibitory nerves release neurotransmitter when the nerves receive some inputs from other nervous elements including sensory nerves in the intestinal wall. In the present experiments, acetylcholine had no effect on the elicited i.p. in the presence of atropine. This suggests that the non-adrenergic inhibitory nerve has no nicotinic receptors. Hirst and McKirdy (1974) reported that d-tubocurarine decreased the amplitude of the evoked i.p. Similar results were obtained in the present experiments. These
results suggest that a nicotinic mechanism relates to the non-adrenergic inhibitory pathway. The interaction between cholinergic and non-adrenergic inhibitory nerves has been proposed in stomach and lower esophageal sphincter (Beani et al., 1971; Goyal et al., 1975; Bülbbring et al., 1967), whereas no synaptic transmission has been suggested in the caecum (Ito et al., 1973).

References


