ELSEVIER

European Journal of Pharmacology 294 (1995) 75–81

ejp

Dual effects of trimebutine on electrical responses of gastric smooth muscles in the rat

Lin Xue, Hiroyasu Fukuta, Yoshimichi Yamamoto, Hikaru Suzuki *

Department of Physiology, Nagoya City University Medical School, Mizuho-ku, Nagoya 467, Japan

Received 19 April 1995; revised 8 August 1995; accepted 11 August 1995

Abstract

The effects of trimebutine on the electrical properties of smooth muscle membranes were studied in the isolated rat stomach, the objective being to elucidate the dual actions of this drug on gastric motility. Transmural nerve stimulation elicited a cholinergic excitatory junction potential (e.j.p.) and a nonadrenergic noncholinergic inhibitory junction potential (i.j.p.), and trimebutine inhibited the e.j.p. more than the i.j.p., with no significant change in the acetylcholine-induced depolarization. Trimebutine reduced the interval and, at high concentrations, the amplitude of slow waves. In enzymatically dispersed single cells, the Ca^{2+} current elicited by depolarization of the membrane was also inhibited by trimebutine. Thus, trimebutine increases slow wave frequency and inhibits cholinergic transmission and Ca^{2+} influx. The former would enhance while the latter two would depress gastric motility.

Keywords: Stomach; Smooth muscle, gastric; Membrane potential; Junction potential; Slow wave; Trimebutine

1. Introduction

Trimebutine maleate is effective for the treatment of both hypermotile and hypomotile gastrointestinal tracts, such as irritable bowel syndrome and dyspepsia (Abei et al., 1977; Moshal and Herron, 1979; Fukudo et al., 1986). The dual actions of this drug on intestinal motility are also shown in in vivo experiments using conscious dogs, i.e., an i.v. application of trimebutine activates gastric movements during the quiescent period, and depresses the elevated motility induced by feeding (Takenaga et al., 1982a). In isolated smooth muscles of the guinea-pig stomach, this drug again shows dual actions on spontaneous activity, as the regular activity is inhibited whereas the irregular activity is regularized upon exposure to trimebutine (Takenaga et al., 1982b). These actions are considered to be the result of a direct action of trimebutine on smooth muscles, and they are insensitive to sympathetic (phentolamine) or parasympathetic (atropine) inhibitors (Takenaga et al., 1982b). The inhibitory and excitatory actions of trimebutine on gastric muscles are reportedly due to inhibition of Ca^{2+} influx and depolarization of the membrane, respectively (Furukawa and Kimoto, 1984). The inhibitory actions of trimebutine on Ca^{2+} influx (Shimada et al., 1990; Nagasaki et al., 1990) and on the release of intracellular stored Ca^{2+} (Nagasaki et al., 1991) have been confirmed in guinea-pig intestinal smooth muscles. In isolated ileum smooth muscles of the guinea-pig, however, the dual actions of trimebutine on muscle motility are indirect, through modulation of excitatory cholinergic and inhibitory adrenergic neuro-muscular transmission mechanisms (Taniyama et al., 1991).

We investigated the effects of trimebutine on the electrical properties of the membrane of isolated smooth muscles from the rat stomach, using intracellular microelectrode techniques, the objective being to elucidate the cellular mechanisms of the dual actions of this drug on gastric motility. The electrical properties of gastric smooth muscles are documented, and the membranes generate slow waves in the antrum, the amplitude and frequency of which can be used as an indicator of gastric motility (Tomita, 1981; Komori and Suzuki, 1988). Transmural nerve stimulation evokes a cholinergic excitatory junction potential (e.j.p.) and a nonadrenergic noncholinergic (NANC) inhibitory junc-

* Corresponding author. Tel.: 052 853 8129; fax: 052 (842) 1538.

SSDI 0014-2999(95)00513-7

tion potential (i.j.p.) (Hoyle and Burnstock, 1989). The actions of trimebutine on the neuromuscular transmission were estimated from the junction potentials evoked by transmural nerve stimulation.

2. Materials and methods

2.1. Tissue preparation

Albino rats (Wister strain), aged 5–6 weeks, were bled during anesthesia with ether. The stomach was excised and opened by cutting along the small curvature. The contents of the stomach were removed, muscles were transferred to the oxygenated Krebs solution, and the mucosal layers were removed using forceps and scissors, under binocular microscopic control.

2.2. Intracellular recordings

Circular muscle preparations of 1–1.5 mm width and 10–15 mm long were prepared and immobilized on the silicon rubber plate fixed in the recording chamber, by using tiny pins, with the circular muscle layer upwards. The recording chamber was made from Lucite plate with a capacity of about 1 ml, and the tissue preparations were superfused with warmed (35°C) Krebs solution at a constant flow rate of about 3 ml/min.

Conventional microelectrode techniques were applied to record the electrical responses of smooth muscle membrane, using glass capillary microelectrodes filled with 3 M KCl (the tip resistance, 40–60 MΩ). Point stimulating methods allowed us to apply transmural electrical stimulation to intramural nerves in the stomach. Square current pulses with 50–100 μs duration and 10–50 V intensity were supplied from an electric stimulator (SEN-3301, Nihon-Kohden, Japan). The recorded responses were displayed on a cathode-ray oscilloscope (SS-7602, Iwatsu, Japan) and also on a pen-writing recorded (RM-6100, Nihon-Kohden, Japan).

The Krebs solution contained (in mM); Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, Cl^- 134 and glucose 11.5. The solution was aerated with O_2 containing 5% CO_2, to maintain the pH of the solution at 7.2–7.3.

2.3. Cell isolation

Tissues from the stomach fundus were incubated in a physiological salt solution (PSS: composition in mM; NaCl 135, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, Hepes 10, glucose 5) at 37°C for 30 min and then transferred to a low Ca^{2+} PSS (Ca^{2+} 0.1 mM) for 30 min. The tissue was then minced and incubated in low Ca^{2+} PSS containing 0.05% (w/v) collagenase (Amano Pharmaceut. Co., Nagoya, Japan) and 0.05% elastase (Type III, Sigma Chem. Co., St. Louis, MO, USA) with gentle stirring (12 rpm) at 4°C. After 1 h, the temperature of the solution was raised to 37°C and the incubation was continued for an additional 30 min with gentle stirring (30 rpm). The tissue was then rinsed with 'KB medium' (Isenberg and Klögner, 1982). Single cells were harvested by gentle agitation with a wide-bore pipette and stored at 4°C.

2.4. Whole-cell patch clamp recording

The tight-seal patch-clamp method was used in whole-cell configuration (Hamill et al., 1981), and experimental procedures were similar to those reported previously (Yamamoto et al., 1993). In brief, smooth muscle cells in the experimental chamber were continuously superfused with bath solution at 25°C. Electrodes were made from Kimax tubing (1.5 mm o.d.) and the diameter of the tip opening was 2–3 μm. The electrode was positioned using a micro-manipulator (MX-1, Narishige Sci. Inst., Tokyo, Japan). Current signals were acquired using an EPC-7 patch-clamp system (List-Medical, Darmstadt, Germany). The signal was filtered at a cut-off frequency (−3 dB) of 1 K Hz with a 4-pole low-pass Bessel filter (E-3201A, NF Circuit Design Block, Yokohama, Japan), and it was then digitized at a sampling rate of 5 K Hz with a 12-bit resolution A/D converter (Labmaster TM-100, Scientific Solution, Solon, OH, USA) for further analysis performed with a microcomputer (APC-IV, NEC, Tokyo, Japan). The currents were digitally corrected for capacitive and leakage currents using current traces obtained with small hyperpolarizing pulses.

2.5. Drugs

Acetylcholine chloride, atropine sulfate, guanethidine sulfate, and indomethacin were obtained from Sigma (St. Louis, MO, USA), phentolamine maleate from CIBA Geigy (Switzerland), tetrodotoxin from Sankyo Pharm. (Osaka, Japan) and trimebutine maleate from Tanabe Pharm. (Osaka, Japan). Indomethacin (5 mM) was dissolved in an equimolar concentration of Na_2CO_3 solution. Other drugs were dissolved in distilled water at concentrations of 1–100 mM, and they were further dissolved in Krebs solution to obtain final concentrations shown in the Results. Addition of these drugs did not change the pH of the solution.

2.6. Data analysis

Observed values were expressed as means ± standard deviation (S.D.), and their statistical significances were determined using Student's *t*-test. Proba-

bilities of less than 5% ($P < 0.05$) were considered significant.

3. Results

3.1. Properties of smooth muscle membrane and junction potentials

In the rat stomach, the electrophysiological properties of the membranes of the smooth muscle cells recorded using intracellular microelectrodes were similar to those of the guinea-pig (Komori and Suzuki, 1986). Briefly, the membranes of muscles from the fundus were quiescent, while those from the middle and lower parts of the antrum generated slow waves. In the upper part of the antrum, the slow waves were small in amplitude (up to 10 mV). In antrum muscles close to the pylorus, the amplitude of the slow wave was larger than that in the middle part of the stomach (15–25 mV), and a burst of spike discharges was often generated on top of the depolarization. The slow waves were resistant to tetrodotoxin (3×10^{-7} M), atropine (10^{-6} M) or guanethidine (5×10^{-6} M), suggesting that they were not of neurogenic origin.

In muscles from the fundus, transmural application of brief current stimuli evoked a biphasic response, an initial depolarization followed by hyperpolarization. The initial depolarization was transient with a duration of 200–300 ms, and its amplitude varied between cells (up to 10 mV). The subsequent hyperpolarization reached a peak amplitude of 5–15 mV at about 800 ms, and then decayed slowly to the resting level within 2–3 s. In muscles from the antrum and pylorus, application of electrical stimulation generated a hyperpolarizing response. In the presence of tetrodotoxin (3×10^{-7} M), the resting membrane potential remained unaltered (control, -43.0 ± 1.5 mV, $n = 7$; in tetrodotoxin, $-43.4 \pm$ 6 mV, $n = 15$), and application of these current stimuli did not evoke any detectable change in the membrane potential. Thus, we considered that the electrical responses evoked by current stimuli were junction potentials, and that the depolarization and hyperpolarization were the excitatory and inhibitory junction potentials (e.j.p. and i.j.p.), respectively.

In muscles from the fundus, application of 10^{-6} M atropine altered the membrane responses to nerve stimulation to an i.j.p. alone, indicating that the e.j.p. was cholinergic in nature. The i.j.p. generated in the presence of atropine (10^{-6} M) was inhibited by apamin (10^{-7} M) and remained unaltered by phentolamine (10^{-6} M), guanethidine (5×10^{-6} M), or indomethacin (10^{-5} M), suggesting that this potential was nonadrenergic noncholinergic (NANC) in nature. The i.j.p.s generated in muscles from the antrum were not significantly altered by atropine, and showed properties similar to those of i.j.p.s generated in muscles from the atropinized fundus.

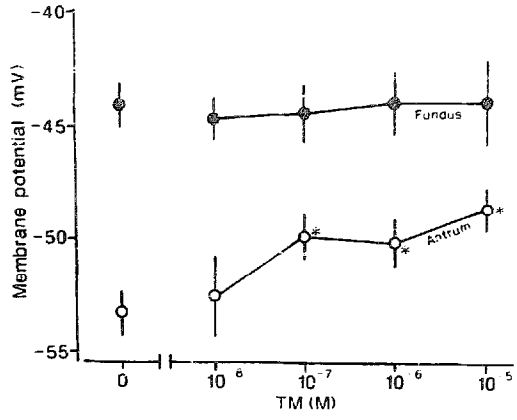

Fig. 1. Effects of trimebutine (TM) on membrane potentials in smooth muscle cells from the fundus (●) and antrum regions (○) of the rat stomach. Means ± S.D. ($n > 10$). The resting membrane potential: fundus, -44.1 ± 1.1 mV, $n = 26$; antrum, -53.3 ± 1.0 mV, $n = 22$. * Significantly different from the resting membrane potential (equal to trimebutine at 0 M).

3.2. Effects of trimebutine on electrical responses of gastric smooth muscles

The resting membrane potential differed between regions of the stomach, and in muscles from the fundus the values were $-40 \sim -45$ mV (mean value of 26 tissues, -44.1 ± 1.1 mV). In muscles from the antrum region, the resting membrane potential was difficult to determine, due to the spontaneous generation of slow waves. When membrane potentials at the most negative state (i.e., at the diastolic phase of the slow wave) were measured, the values varied between -50 and -55 mV in the antrum (mean value of 22 tissues, -53.3 ± 1.0 mV).

Membrane potentials of smooth muscle cells of the rat fundus and antrum measured in the presence of various concentrations of trimebutine are summarized in Fig. 1. The most negative potential between slow waves was measured in muscles from the antrum. Trimebutine ($> 10^{-7}$ M) concentration dependently depolarized the membrane in the antrum but not in the fundus (up to 10^{-5} M).

The effects of trimebutine on junction potentials were observed in muscles from the fundus. Application of 10^{-6} M trimebutine reduced the amplitudes of both the e.j.p. and i.j.p.; the former was reduced more than the latter (Fig. 2B). The inhibitory actions of trimebutine on junction potentials were long-lasting, and full recovery was not observed after removal of this drug from the superfusate, for up to 1 h (Fig. 2C, D).

The effects of different concentrations of trimebutine on the evoked junction potentials are summarized in Fig. 3, in which the i.j.p.s were generated in the

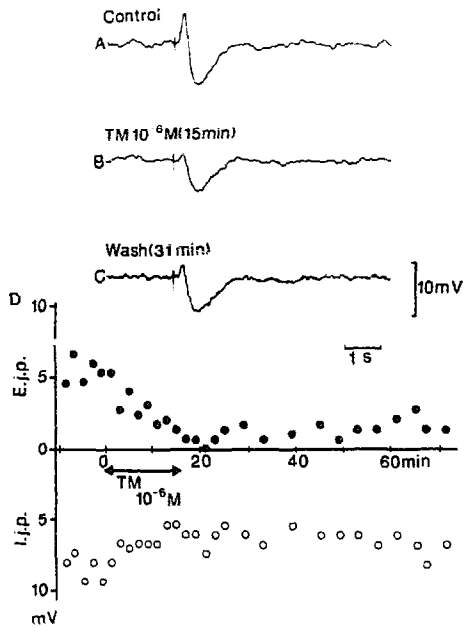

Fig. 2. Effects of trimebutine (TM) on junction potentials recorded from circular smooth muscle cells of the rat fundus. The junction potentials were recorded before (A) and during application of 10^{-6} M trimebutine for 15 min (B) and after removal of trimebutine from the superfusate for 31 min (C). Nerve stimulation: 30 μs duration, 50 V intensity. D, changes in amplitudes of the e.j.p. (●) and i.j.p. (○) produced by application of 10^{-6} M trimebutine for 15 min in the rat fundus circular muscle. Transmural nerve stimulation was applied every 2–6 min, and peak amplitudes of the e.j.p. and i.j.p. were measured. All points were obtained from the same cell (the membrane potential measured at the end of this experiment, −43 mV). These effects of trimebutine on junction potentials were found to be reproducible in eight tissues.

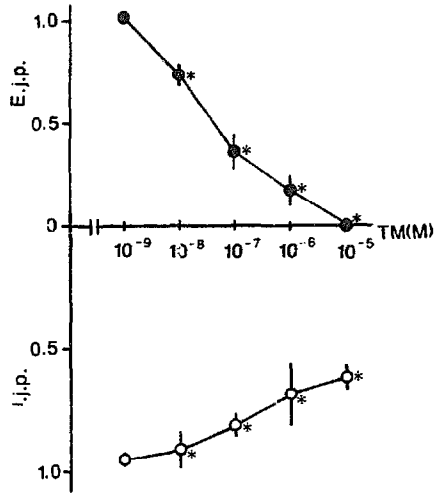

Fig. 3. The concentration-response relationship of the trimebutine-induced inhibition of junction potentials (●, e.j.p.; ○, i.j.p.) in the rat fundus. The amplitude of junction potentials evoked by single stimuli was measured in the presence of trimebutine for over 15 min, and the values relative to the control (= before application of TM) are expressed by the means ± S.D. (n = 5–12 tissues). The i.j.p.s were obtained in the presence of 10^{-6} M atropine. Mean amplitude of junction potentials measured before application of TM: e.j.p., 5.5 ± 2.3 mV; i.j.p., 6.2 ± 2.8 mV. TM = trimebutine. * Significantly different from the control.

presence of atropine. Trimebutine inhibited the e.j.p. at concentrations over 10^{-8} M; complete inhibition was observed at 10^{-5} M. The i.j.p. was rather resistant to trimebutine, and although a significant reduction in amplitude of the i.j.p. was noted at 10^{-8} M, the peak inhibition of about 55% of the control was observed at 10^{-6} M.

Attempts were made to observe the effects of trimebutine on membrane depolarizations produced by acetylcholine in smooth muscles from the fundus, the objective being to determine the possible inhibition of acetylcholine receptors by trimebutine. Application of acetylcholine (10^{-6} M) depolarized the membrane by about 11 mV (from -44.0 ± 2.0 mV, $n = 10$, to -33.0 ± 3.0 mV, $n = 5$). In the presence of 10^{-5} M trimebutine, acetylcholine depolarized the membrane from -43.8 ± 2.2 mV ($n = 8$) to -32.5 ± 2.0 mV ($n = 5$), the depolarized level being unaltered by trimebutine ($P > 0.05$). Thus, we considered that trimebutine does not inhibit muscarinic receptors in smooth muscle of the rat stomach.

In muscles from the antrum, the effects of trimebutine on slow waves were investigated. As shown in Fig. 4B, 10^{-6} M trimebutine reduced the interval between slow waves, and also the amplitude and duration of individual slow waves. Increasing the concentration of trimebutine to 10^{-4} M caused a reduction in the amplitude and duration of the slow waves (Fig. 4C).

The effects of different concentrations of trimebutine on slow waves are summarized in Fig. 5. The figure shows that trimebutine reduced the amplitude of slow waves at concentrations over 10^{-5} M, while the intervals were reduced by trimebutine in concentrations between 10^{-7} and 10^{-5} M. A high concentration (10^{-4} M) of trimebutine inhibited the amplitude and duration of the slow waves, with no significant change in their interval.

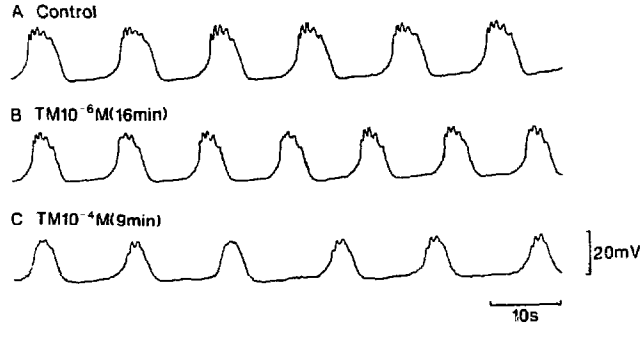

Fig. 4. Effects of trimebutine (TM) on slow waves recorded from the rat antrum circular muscles. Slow waves were recorded from a single cell, before (A) and after application of trimebutine (B, 10^{-6} M for 16 min; C, 10^{-4} M for 9 min). The membrane potential was measured at the end of trace C, −47 mV. These effects of trimebutine were also observed in six tissues for 10^{-6} M and eight tissues for 10^{-4} M.

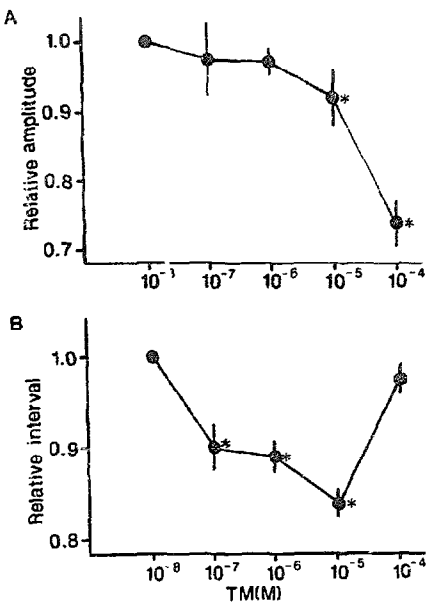

Fig. 5. Effects of trimebutine (TM) on the (A) amplitude and (B) interval of slow waves recorded in circular smooth muscles from the rat antrum. Relative values are expressed by the means ± S.D. (n = 5–20 tissues). In the absence of TM, the mean amplitude of slow waves was 33.3 ± 5.8 mV (n = 20) and the mean interval of slow waves was 10.6 ± 2.8 s (n = 20). * Significantly different from the control.

3.3. Effects of trimebutine on Ca^{2+} current

In enzymatically dispersed single smooth muscle cells from the fundus, the effects of trimebutine (10^{-5} M) on inward currents triggered by a depolarizing pulse were investigated. K^+ currents were blocked with Cs^+ in the pipette and tetraethylammonium (TEA) in the bath. Inward currents with a reproducible amplitude were produced by a depolarizing pulse of 100 ms duration, to +10 mV from the holding potential of −40 mV, which was close to the resting membrane potential (Fig. 1). This current was considered to be carried mainly by Ca^{2+}, since most of the Na^+ channels were inactivated at this holding potential (Yamamoto et al., 1993). Application of trimebutine reduced the amplitude of the inward current (Fig. 6A). The time course of the decrease in peak amplitude of the inward current is shown in Fig. 6B. In the absence of trimebutine, the peak amplitude of the inward current was about 210 pA, and the value was decreased to about half by 10^{-5} M trimebutine. The inhibitory actions of trimebutine on the inward current remained for more than 10 min (n = 3) after removal of this drug from the superfusate.

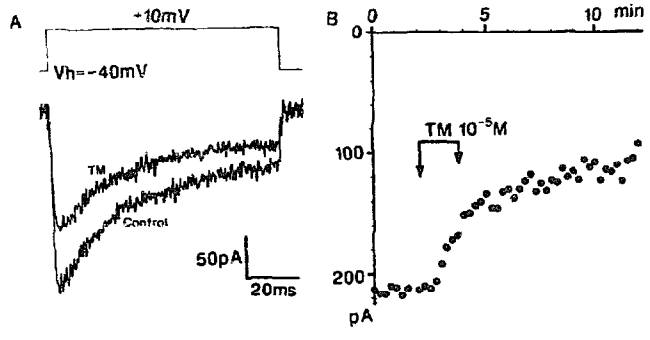

Fig. 6. Effects of trimebutine on Ca^{2+} current recorded in enzymatically dispersed single smooth muscle cell of the rat fundus. (A) The inward current evoked by depolarizing pulse of 100 ms duration, to +10 mV from the holding potential of −40 mV, before (control) and after application of 10^{-5} M trimebutine (TM). (B) Changes in peak amplitude of the inward current produced by application of 10^{-5} M trimebutine for 2 min (between arrows). All the data were obtained from a single cell. The effects of trimebutine on Ca^{2+} currents were confirmed in three cells.

4. Discussion

The present experiments identified the electrical properties of smooth muscle cells of the rat stomach: (1) the resting membrane potential is more negative in muscles from the antrum than in those from the fundus, (2) slow waves are generated in muscles from the antrum, while the membranes are quiescent in muscles from the fundus, (3) transmural nerve stimulation evokes an e.j.p. and following an i.j.p. in the fundus, while an i.j.p. alone is evoked in the antrum, (4) the e.j.p. is atropine-sensitive, i.e., cholinergically mediated, and (5) the apamin-sensitive i.j.p. is mediated by NANC transmitter(s). These electrical properties are similar to those found in the guinea-pig stomach (Furukawa and Kimoto, 1984; Komori and Suzuki, 1986; Ono and Suzuki, 1987). Some differences in the properties of the junction potentials were noted between rat and guinea-pig; in the rat fundus, nerve stimulation evoked an e.j.p. which was followed by an i.j.p., while the e.j.p. was not followed by the i.j.p. in the guinea-pig.

In the rat gastric muscles, trimebutine reduced (1) the amplitude of junction potentials, (2) the amplitude of slow waves, (3) the duration of slow waves, and (4) the inward current produced by depolarization, and increased the interval between slow waves. The e.j.p. was atropine-sensitive, and therefore may be produced by acetylcholine, while the i.j.p. may be mediated by vasoactive intestinal polypeptide (VIP, Ito et al., 1990). The evidence that the acetylcholine-induced depolarization was not inhibited by trimebutine, suggests that the reduction by trimebutine of the e.j.p. amplitude is not due to inhibition of the postjunctional muscarinic receptor, but probably due to the inhibition of the release of acetylcholine. Although the present experiments could not elucidate the cellular mechanisms related to the difference in actions of trimebutine on excitatory and inhibitory transmissions, the inhibition by trimebutine of the e.j.p. was stronger than that of the i.j.p. by more than 1000-fold, suggesting that this

drug predominantly inhibits cholinergic transmission in the rat stomach. Gastric motility is enhanced by parasympathetic nerve excitation, and therefore the vagally mediated excitation of the stomach would be suppressed by trimebutine. However, the reduced motility of the stomach produced by activation of NANC inhibitory nerves, if any, may not be fully restored by trimebutine.

In the rat stomach, acetylcholine increases intracellular Ca^{2+} concentrations, by increasing Ca^{2+} influx through the voltage- and receptor-gated channels and also the release of stored Ca^{2+} (Ohta et al., 1992). Trimebutine inhibits voltage-activated Ca^{2+} currents in intestinal smooth muscles (Shimada et al., 1990). The present experiments showed that in smooth muscles of the rat stomach, trimebutine inhibited the inward current produced by depolarization. In the rat stomach, the inward currents produced by membrane depolarizations are carried by Na^+ and Ca^{2+}, and these currents can be separated by changing the holding potential, i.e., Na^+ channel currents, but not Ca^{2+} channel currents, are inactivated by depolarization of the membrane to above −50 mV (Muraki et al., 1991; Yamamoto et al., 1993). At the membrane potential held in the present experiments (equal to −40 mV), the inward currents produced by depolarizing pulses are probably carried mainly by Ca^{2+}. Thus, the results indicated that trimebutine inhibits the Ca^{2+} current in smooth muscle cells of the rat stomach. Similar actions of trimebutine have been suggested from binding experiments with tritium-labeled dihydropyridine agonists (Nagasaki et al., 1990).

The slow wave is generated by an unidentified ionic mechanisms, and an increased influx of Ca^{2+} during the depolarization triggers gastric contractions (Tomita, 1980). The pace-making site of the slow wave is the interstitial cells, which possesses Ca^{2+} channels with a threshold lower than that of smooth muscle cells (Lee and Sanders, 1993). The reduced duration of individual slow waves produced by trimebutine may be due to inhibition of Ca^{2+} channels, and this can also contribute to the inhibition of gastric motility. Trimebutine also increased the frequency of slow waves, with associated depolarization of the smooth muscle membrane. The frequency and amplitude of the slow wave generated in the guinea-pig stomach are sensitive to the membrane potential, and depolarization accelerates the frequency and reduces the amplitude of slow waves (Ohba et al., 1975; Tomita, 1980). The reduction of the interval between slow waves (equal to the increase in frequency) by trimebutine may be, therefore, partly due to depolarization of the membrane. The depolarizing actions of trimebutine are due to inhibition of K^+ current (Nagasaki et al., 1993), and as such would result in accelerated peristaltic movements of the stomach.

In summary, the smooth muscles of the rat stomach are spontaneously active, and they are neurally controlled mainly by cholinergic excitatory and NANC inhibitory nerves. The actions of trimebutine on gastric muscles are (1) inhibition of the e.j.p., (2) reduction of the duration of slow waves, (3) inhibition of Ca^{2+} channels, (4) inhibition of the i.j.p., (5) depolarization of antrum smooth muscle membrane, and (6) increase in the frequency of slow wave generation. The former three actions may contribute to the depression of gastric motility, while the latter three actions could enhance peristaltic movements. Clinically, ingestion of a cerekinon tablet (which contains 500 mg trimebutine maleate) causes the plasma concentration of trimebutine to rise to over 40 ng/ml (equal to about 10^{-7} M, personal communication from Tanabe Pharm. C.), and these concentrations of trimebutine are effective for modulation of junctional transmissions and slow waves. Thus, the above-mentioned actions may be involved in the dual effects of trimebutine on gastric muscles.

Acknowledgements

Trimebutine maleate was a gift from Tanabe Pharm. C., Ltd. (Osaka, Japan). L.X. received support from Iwaki Scholarship in 1992-1993.

References

Abei, T., S. Isogai, T. Onuki, T. Okada, M. Osada, A. Kawamura, Y. Kiryu, H. Koizumi, K. Suzuki, S. Takagi, H. Takahashi, M. Tsuchiya, K. Nakamura, T. Namuba, M. Muraoka, Y. Yokoi and A. Yokoyama, 1977, A multi-institutional double blind control study to compare the effectiveness of TM-906 and metochlopramide in controlling functional gastrointestinal symptoms, Clin. Eval. 5, 189 (Japanese with English abstract).

Fukudo, S., J. Suzuki and Y. Tanaka, 1986, The effect of trimebutine maleate on colonic motility and controlled comparative trials in irritable bowel syndrome, Yakuri to Chiryo 14, 5841 (Japanese with English summary).

Furukawa, K. and Y. Kimoto, 1984, Effects of trimebutine maleate (TM-906) on electrical and mechanical activities of smooth muscles of the guinea-pig stomach, Jpn. J. Pharmacol. 35, 307.

Hamill, O.P., A. Marty, E. Neher, B. Sakmann and F.J. Sigworth, 1981, Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches, Pflügers Arch. 391, 85.

Hoyle, C.H.V. and G. Burnstock, 1989, Neuromuscular transmission in the gastrointestinal tract, in: Handbook of Physiology, Section 6, The Gastrointestinal System, Vol. I, Motility and Circulation, Part 1, eds. S.G. Schultz, J.D. Wood and B.B. Rauner (Am. Physiol. Soc., Bethesda, MD) p. 435.

Isenberg, G. and U. Klögner, 1982, Calcium tolerant ventricular myocyts prepared by preincubation in a 'KB medium', Pflügers Arch. 395, 6.

Ito, S., A. Kurokawa, A. Ohga, T. Ohta and K. Sawabe, 1990, Mechanical, electrical and cyclic nucleotide responses to peptide VIP and inhibitory nerve stimulation in rat stomach, J. Physiol. 430, 337.

Komori, K. and H. Suzuki, 1986, Distribution and properties of excitatory and inhibitory junction potentials in circular muscles of the guinea-pig stomach, J. Physiol. 370, 339.

Komori, K. and H. Suzuki, 1988, Modulation of smooth muscle activity by excitatory and inhibitory nerves in the guinea-pig stomach, Comp. Biochem. Physiol. 91C, 311.

Lee, H. and K.M. Sanders, 1993, Comparison of ionic currents from interstitial cells and smooth muscle cells of canine colon, J. Physiol. 460, 135.

Moshal, M.G. and M. Herron, 1979, A clinical trial of trimebutine (Mebutine) in spastic colon, J. Int. Med. Res. 7, 231.

Muraki, K., Y. Imaizumi and M. Watanabe, 1991, Sodium currents in smooth muscle cells freshly isolated from stomach fundus of the rat and ureter of the guinea-pig, J. Physiol. 442, 351.

Nagasaki, M., H. Kurosawa, K. Naito and H. Tamaki, 1990, Allosteric interaction of trimebutine maleate with dihydropiridine binding sits, Eur. J. Pharmacol. 189, 71.

Nagasaki, M., T. Kobayashi and H. Tamaki, 1991, Effects of trimebutine on cytosolic Ca^{2+} and force transitions in intestinal smooth muscle. Eur. J. Pharmacol. 195, 317.

Nagasaki, M., S. Komori, H. Tamaki and H. Ohashi, 1993, Effect of trimebutine on K^+-current in rabbit ileal smooth muscle cells. Eur. J. Pharmacol. 235, 197.

Ohba, M., Y. Sukamoto and T. Tomita, 1975, The slow wave in the circular muscle of the guinea-pig stomach, J. Physiol. 253, 505.

Ohta, T., S. Ito, T. Noto, R. Tachibana, Y. Nakazato and A. Ohga, 1992, The inhibitory action of cyclic AMP on responses to carbachol dependent on calcium stores in rat gastric smooth muscle, J. Physiol. 453, 367.

Ono, H. and H. Suzuki, 1987, Effects of histamine on the guinea-pig stomach: excitation of smooth muscle and inhibition of transmitter release, Br. J. Pharmacol. 91, 839.

Shimada, T., Y. Kurachi, A. Terano, E. Hamada and T. Sugimoto, 1990, Trimebutine maleate has inhibitory effects on the voltage-dependent Ca^{2+} inward current and other membrane currents in intestinal smooth muscle cells, Jpn. J. Gastroenterol. Soc. 25, 175 (in Japanese).

Takenaga, H., T. Magaribuchi, K. Nosaka and H. Tamaki, 1982a, Effects of trimebutine maleate on gastrointestinal motility in conscious dogs, Folia Pharmacol. Japon. 80, 271 (in Japanese with English summary).

Takenaga, H., T. Magaribuchi and H. Tamaki, 1982b, Effects of trimebutine maleate (TM-906) on the spontaneous contraction of the isolated guinea-pig stomach, Folia Pharmacol. Japon. 80, 163 (in Japanese with English summary).

Taniyama, K., I. Sano, S. Nakayama, S. Matsuyama, K. Takeda, C. Yoshihara and C. Tanaka, 1991, Dual effect of trimebutine on contractility of the guinea-pig ileum via the opioid receptors, Gastroenterology 101, 1579.

Tomita, T., 1980, Electrical activity (spikes and slow waves) in gastrointestinal smooth muscles, in: Smooth Muscle, eds. E. Bulbring, A.F. Brading, A.W. Jones and T. Tomita (E. Arnold, London) p. 127.

Yamamoto, Y., H. Fukuta and H. Suzuki, 1993, Blockade of sodium channels by divalent cations in rat gastric smooth muscle, Jpn. J. Physiol. 43, 785.