The Inhibitory Effect of Tiamulin on High K^+ -Induced Contraction in Guinea Pig Intestinal Smooth Muscle

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ABSTRACT—Tiamulin with an IC_{50} of 1.7×10^{-6} M inhibited both the rapid and sustained contractions induced by hyperosmotically added 60 mM K^+ (Hyper 60 K^+) without changing the membrane potential in the intestinal muscle. Tiamulin inhibition (2×10^{-6} – 2×10^{-5} M) of the Ca^{2+} -induced contraction in depolarized muscle was competitively antagonized by raising external Ca^{2+} . Tiamulin (2×10^{-5} M) slightly affected the Hyper 60 K^+ -induced phasic contraction under hypoxia and the carbachol-induced phasic contraction. Moreover, tiamulin (2×10^{-5} M) inhibited the Hyper 60 K^+ -induced contraction with decreasing $[Ca^{2+}]_{\text{cyt}}$ level. Although the inhibitory effect of 10^{-7} – 10^{-5} M monensin, an inhibitor of mitochondrial respiration, on the Hyper 60 K^+ -induced contraction was reduced under hypoxia, the effect of tiamulin (2×10^{-7} – 2×10^{-4} M) was not modified. Tiamulin changed neither the intracellular Na^+ and K^+ content of the depolarized muscle nor the Ca^{2+} -induced contraction in the chemically skinned preparations. These results suggest that the inhibitory action of tiamulin on the Hyper 60 K^+ -induced tonic contraction is possibly due to the competitive inhibition of Ca^{2+} entry through the voltage-dependent Ca^{2+} channel of the intestinal smooth muscle cell.

Tiamulin is an antibiotic produced in cultures of the basidiomycete, *Pleurotus mutilis*, and it is a pleuromutilin derivative, 14-deoxy-14[(2-diethylaminoethyl)mercaptoacetoxy]-mutilin (1, 2). Tiamulin is frequently used to treat infections caused by avian mycoplasma and gram-negative and gram-positive bacteria. In pharmacological studies, tiamulin at a high dose induced a slight increase in the QT interval of the electrocardiograph in dogs (3). Tiamulin inhibits the isotonic contractions induced by acetylcholine, norepinephrine, histamine and high potassium concentration in smooth muscles. However, the effect of tiamulin on the smooth muscle function is not yet fully understood. In the present study, we

have examined the effects of tiamulin on the phasic and tonic component of high K^+ -induced contraction of guinea pig intestinal smooth muscle, and we compared the effect of tiamulin with that of verapamil, an organic Ca^{2+} antagonist. Our data indicate that tiamulin inhibits the high K^+ -induced contraction, possibly by inhibiting the Ca^{2+} influx through a voltage-dependent Ca^{2+} channel of intestinal smooth muscle.

MATERIALS AND METHODS

Preparations

Male guinea pigs (300–400 g) were bled after stunning. The ileum and taenia coli were

quickly removed. The muscle strip was suspended in an organ bath containing 15 ml of physiological salt solution (PSS); one end of the muscle was connected to a holder and the other connected to the arm of a strain gauge transducer with silk thread. The composition of the PSS was as follows: 136.8 mM NaCl, 5.4 mM KCl, 2.5 mM CaCl_2 , 1.0 mM MgCl_2 , 11.9 mM NaHCO_3 and 5.5 mM glucose. The bathing solution was bubbled with a gas mixture of 95% O_2 and 5% CO_2 and had a pH of 7.2 at 37°C. A hypoxic condition was produced by bubbling the bathing medium with a gas mixture of 95% N_2 and 5% CO_2 . Hyperosmotic 65.4 mM K^+ (Hyper 60 K^+) solution was prepared by adding an appropriate volume of 3 M KCl stock solution. Isosmotic 60 or 70 mM K^+ (Iso 60 or 70 K^+) solution was made by substituting an appropriate amount of Na^+ with equimolar K^+ in the above solution.

Measurement of muscle tension

The contractile tension of the muscle strip was recorded isometrically. Isometric tension changes were recorded with force transducers connected to a polygraph (Nihon Kohden, SB-1TA and RJG-4008). Resting tension was maintained at 1.0 g. The maximum tension of the contraction induced by the second application of the high K^+ solution was regarded as a reference response (100%), except where otherwise stated.

In the contraction induced by the addition of Ca^{2+} (a Ca^{2+} -induced contraction), the muscle preparations were previously incubated in Ca^{2+} -free solution for 30 min and then in Ca^{2+} -free 65.4 mM K^+ solution for 30 min. A concentration-response curve was made by a cumulative addition of Ca^{2+} , ranging from 0.25 mM to 10 mM, to the Ca^{2+} -free, Hyper 60 K^+ solution. After the wash-out period of 30 min, the test drugs were pretreated for 15 min, and the second set of concentration-response curves for Ca^{2+} was obtained.

Measurement of membrane potential

Membrane potential of the smooth muscle

of taenia coli was measured by a single sucrose gap method, as described by Bülbring and Burnstock (4).

Simultaneous recording of muscle tension and $[\text{Ca}^{2+}]_{\text{cyt}}$ level

The tension development and the fluorescent light representing Ca^{2+} signals were measured simultaneously in the ileal longitudinal smooth muscle. The muscle strips were about 5 mm in width and 10 mm in length. The muscle strips were treated with PSS containing 5 μM fura 2/AM and 0.25% cremophor for 6 hr at room temperature by the method described by Himpens and Somlyo (5). One end of the muscle strip was attached to an isometric strain gauge transducer (Nihon Kohden, SB-1TA), and the other end exposed to excitation light was fixed in the chamber with stainless-steel pins to avoid movement artifacts. The changes in the surface fluorescence of living muscle were measured by a fluorimeter (Nihon Spectroscopic, CAF-100) as described Ozaki et al. (6). As the light source, a xenon lamp of 75 W was used, and the excitation light was obtained by placing a rotating filter wheel (48 Hz) containing 340 and 380 nm $\pm$ (5.5 nm) interference filters over the lamp, and emitted light from the muscle was collected to a photomultiplier through a 500 nm $\pm$ (10 nm) bandpass filter. The ratio of the fluorescence by excitation at 340 nm and 380 nm (F340/F380) was calculated from the successive excitation period. In this experiment, EDTA (10 μM) and N,N,N',N'-tetrakis(2-pyridilmethyl)ethylenediamine (TPEN, 10 nM) were added to the PSS.

Determination of intracellular Na^+ and K^+ contents

Intracellular Na^+ and K^+ contents of taenia coli were determined by the La^{3+} -method, which was developed by van Breemen et al. (7). After incubation with a test solution, the muscle was exposed to a La^{3+} -solution (294.9 mM sucrose, 1.1 mM LaCl_3 and 11.9 mM Tris-HCl) at 0.5°C. After a 30-min wash-out of the tissues, they were gently blotted between filter

papers, weighed, and ashed in a quartz tube with 0.5 ml of a mixture (1:1) of HNO₃ (61%) and HClO₄ (60%), for 16 hr after at 180°C. The dried samples were then dissolved in 0.01 N HCl solution. CsCl (1 g/l) was added to standard and sample solutions to inhibit the mutual interference of Na⁺ and K⁺. The ion concentration of the diluted sample was measured with an atomic absorption spectrophotometer (Varian Type, AA-275).

Skinned fiber preparation

The effect of drug was also tested on a saponized muscle of taenia coli. A small bundle of the muscle fibers, 100–150 μ m in width and about 5 mm in length, was cut under a microscope in the PSS. One end of the fiber was connected to a holder with silk thread and the other end was connected to a strain-gauge transducer (Nihon Kohden, SB-1TA); then isometric tension was recorded. After a Hyper 60 K⁺-induced contraction was recorded, the solution was changed to a "standard relaxing solution" containing 130 mM KCl, 20 mM Tris-maleate, 5 mM MgCl₂, 5 mM ATP-Na₂ and 2 mM EGTA (pH 6.8). Saponin treatment was then carried out by keeping the muscle fibers for 20 min in the standard relaxing solution containing 100 μ g/ml saponin. These procedures were almost the same as those described by Saida and Nonomura (8).

Chemicals

The drug used were tiamulin (Nihon Zen-yaku Pharmaceutical Co., Kohriyama); monensin and verapamil (Eisai Co., Tokyo); trifluoperazine (TFP) (Sigma Chemical Co., St. Louis, MO, U.S.A.); fura 2/AM and TPEN (Dojindo, Kumamoto); and saponin (ICN, U.S.A.).

Statistical analyses

The results were expressed as mean values $\pm$ S.E.M. Student's paired and unpaired *t*-tests were used for statistical analyses of the results, and a *P* value of less than 0.05 was taken to be significant.

RESULTS

Inhibitory effect of tiamulin on Hyper 60 K⁺-induced contraction

Pre-treatment with tiamulin: A low concentration of tiamulin (2×10^{-6} M) decreased the amplitude of spontaneous contractions of taenia coli but increased their frequency. However, a higher concentration of tiamulin (2×10^{-5} M) decreased both parameters of the spontaneous contractions (Fig. 1). An application of Hyper 60 K⁺ solution resulted in a rapid (phasic) contraction (7–10 g) followed

Fig. 1. Effects of tiamulin (2×10^{-6} or 2×10^{-5} M) and verapamil (5×10^{-7} M) on the contractions induced by an application of Hyper 60 K⁺ solution in taenia coli. Hyper 60 K⁺ solution was applied for 15 min, and the wash period was 15 min.

by a sustained (tonic) one (8–11 g). Tiamulin at 2×10^{-6} M decreased the phasic and tonic components of the Hyper 60 K⁺-induced contraction within 15 min; and 2×10^{-5} M tiamulin decreased the phasic component of Hyper 60 K⁺-induced contraction, but almost completely inhibited the tonic one. However, verapamil (5×10^{-7} M) preferentially inhibited the tonic contraction but partially inhibited the phasic one (Fig. 1). The concentration-response curves for the pre-treatment with tiamulin and verapamil are shown in Fig. 2. The IC₅₀s of tiamulin and verapamil were 1.7×10^{-6} M and 2×10^{-8} M, respectively. The potency of verapamil in the inhibitory action was 100-fold greater than that of tiamulin.

Post-treatment with tiamulin: Tiamulin at various concentrations from 2×10^{-7} M to 2×10^{-4} M was added to the medium at 30 min after an application of Hyper 60 K⁺ solution. Tiamulin (2×10^{-6} M) inhibited the tonic contraction induced by Hyper 60 K⁺ solution and then reached a new steady level within 15 min; and the drug, at concentrations above $2 \times$

Fig. 2. Inhibition of Hyper 60 K⁺-induced contraction by various concentrations of tiamulin (●) and verapamil (○) in taenia coli. Ordinate: relative tension (%) to the control (second application of the Hyper 60 K⁺-induced contraction in the absence of drug). Abscissa: logarithmic concentration (–log M) of tiamulin or verapamil. The mean values $\pm$ S.E.M. for 5–6 experiments are given.

10^{-5} M, completely inhibited the tonic contraction (Fig. 3).

The Ca²⁺-induced contraction: Inhibitory effects of tiamulin and verapamil were investigated on the Ca²⁺-induced contraction in Hyper 60 K⁺-depolarized muscle. The muscle was fully relaxed after 2-hr incubation with the Ca²⁺-free, Hyper 60 K⁺ solution. A cumulative addition of Ca²⁺ (0.1–10 mM) produced stepwise contractions in the depolarized muscle. Pre-treatment with tiamulin for 30 min inhibited the Ca²⁺-induced contraction in a concentration-dependent manner (Fig. 4A). The mode of inhibition by tiamulin or verapamil was estimated by Lineweaver-Burk plots of the reciprocal of the rate of the Ca²⁺-induced contraction against the reciprocal of the external Ca²⁺ concentration. The values in the figure gave straight lines in the absence and presence of tiamulin (2×10^{-6} M, 6×10^{-6} M and 2×10^{-5} M). These lines intersected at a single point on the ordinate, indicating that the antagonism of tiamulin to external Ca²⁺ is typically competitive (Fig. 4B).

Fig. 3. Inhibition of Hyper 60 K⁺-induced contraction by the post-treatment with various concentrations of tiamulin in taenia coli. Ordinate: Relative tension (%) to the control. Abscissa: Time (min) of exposure to various concentrations of tiamulin. The mean values $\pm$ S.E.M. for 5–6 experiments are given. Tiamulin: ●, 2×10^{-7} M; ▲, 2×10^{-6} M; ◆, 6×10^{-6} M; ■, 2×10^{-5} M; ▼, 2×10^{-4} M.

Effect of tiamulin on the high K⁺-induced contraction under the hypoxic condition

Phasic contraction: The muscle treated with Hyper 60 K⁺ solution produced a large phasic contraction followed by a very small tonic contraction under the hypoxic condition. Repeated application of Hyper 60 K⁺ solution reached almost a steady level in the phasic response under the hypoxia (Fig. 5). The phasic contraction was decreased to $76.5 \pm 2.01\%$ ($n = 4$), $46.1 \pm 4.42\%$ ($n = 4$) and $65.1 \pm 7.60\%$ ($n = 4$) of the control by 2×10^{-6} M tiamulin, 2×10^{-5} M tiamulin, and 6×10^{-7} M verapamil, respectively. There was no remarkable difference between the inhibitory effect of tiamulin and that of verapamil on the high K⁺-induced phasic contraction under hypoxia.

Tonic contraction: The relative tension of the tonic contraction under the hypoxic condition at 30 min later was $22.1 \pm 1.7\%$ ($n = 12$) of that under the aerobic condition. When 40 mM glucose was added to the medium, the

muscle tension gradually increased and reached a new steady level corresponding to $45.6 \pm 5.3\%$ ($n = 8$) of the control within 20 min. Such a contraction maintained under hypoxia was more resistant to the inhibitory effect of monensin (10^{-7} – 10^{-5} M) than that under normoxia (9); however, the contraction was strongly inhibited by tiamulin (2×10^{-7} – 2×10^{-4} M). The concentration-inhibition curve for monensin was shifted to the right by hypoxia, while the curves for tiamulin under the normal and hypoxic conditions were almost identical (Fig. 6).

Effect of tiamulin on the carbachol-induced phasic contraction in the depolarized muscle

A carbachol-induced phasic contraction in taenia was obtained by the modified method described by Ohashi et al. (10). After exposure of muscle strip to Ca²⁺ free, Iso-60 K⁺ solution at 24°C for 15 min, 1.0 mM Ca²⁺ was added for 15 min to load Ca²⁺ to store sites. Following a 15-min wash with Ca²⁺ free, Iso-

Fig. 4. Effect of tiamulin on the contractions induced by cumulative application of Ca²⁺ in Hyper 60 K⁺-depolarized taenia coli. (A) The magnitude of the contraction in response to Hyper 60 K⁺ solution in the presence of 2.5 mM Ca²⁺ was taken as the reference response (100%). (B) Lineweaver-Burk plot of data obtained from inhibition of the Ca²⁺-induced contraction by tiamulin. Ordinate: Reciprocal of the rate of Ca²⁺-induced contraction, 1/(tension/min), Abscissa: Reciprocal of the external Ca²⁺ concentration, 1/Ca²⁺ (mM⁻¹). Data were obtained at 15 min after tiamulin application. Each point represents the values from 5–6 experiments. Control (○), Tiamulin (▲, 2×10^{-6} M; ◆, 6×10^{-6} M; ■, 2×10^{-5} M).

Fig. 5. Effects of tiamulin (2×10^{-6} and 2×10^{-5} M) on the phasic contractions induced by repeated applications of Hyper 60 K⁺ solution under the hypoxic condition in taenia coli.

60 K⁺ solution, 10^{-4} M carbachol was added for 2 min. After washing with Ca²⁺-free, Iso-60 K⁺ solution for another 15 min, 1.0 mM Ca²⁺ was again added for Ca²⁺ loading. This procedure was repeated until a steady phasic contraction by carbachol was obtained (Fig. 7). Tiamulin at 2×10^{-6} M added for 15 min before the application of carbachol decreased the carbachol-induced phasic contraction to $89.5 \pm 6.1\%$ ($n = 4$), which was not significantly different from the control. However, 2×10^{-5} M tiamulin remarkably decreased the phasic contraction to $67.5 \pm 5.6\%$ ($n = 4$). An application of 6×10^{-7} M verapamil did not affect the carbachol-induced phasic contraction.

Fig. 6. Comparison of the tiamulin (A) and monensin (B) induced relaxation under normoxic and hypoxic conditions in taenia coli. Tiamulin or monensin was added cumulatively to the muscle in the presence of Hyper 60 K⁺ solution under normoxia (opened circles) or in the presence of Hyper 60 K⁺ solution and 40 mM glucose under hypoxia (closed circles). Exposure to various concentrations of tiamulin. Ordinate: inhibition of tension (%) and the magnitude of the contraction induced by Hyper 60 K⁺ solution was taken as the reference response (100%). Abscissa: logarithmic concentration ($-\log M$) of tiamulin and monensin. Mean values $\pm$ S.E.M. are given for 5–6 experiments.

Effect of tiamulin on membrane potential

The membrane potential and muscle tension of taenia coli were simultaneously measured at 32°C by the single sucrose-gap method. The muscle spontaneously depolarized the membrane accompanied by spike discharges and rhythmic contractions as shown Fig. 8A. An application of 2×10^{-5} M tiamulin decreased spike discharge almost concomitantly with the loss of mechanical activity, but it scarcely

Fig. 7. Effect of tiamulin (2×10^{-5} M) on the carbachol-induced phasic contraction in Ca²⁺ free, Iso-60 K⁺ solution at 24°C in taenia coli. Ca²⁺-depleted muscle was incubated with 1 mM Ca²⁺ for 15 min (Ca²⁺ loading), washed with Ca²⁺-free solution (0.5 mM EGTA) for 15 min and challenged by 10⁻⁴ M carbachol in the presence of Iso-60 K⁺ solution.

Fig. 8. Effect of tiamulin (2×10^{-5} M) on changes in membrane activity of guinea pig taenia coli. In A and B, different strips were employed. In the sucrose-gap apparatus, the flow rate was 3 ml/min.

affected the resting membrane potential. An application of 30 mM K^+ caused depolarization of approximately 12 mV and increased the frequency of spike discharge. When tiamulin (2×10^{-5} M) was added during the depolarization, it decreased the frequency of spike discharge, but had little effect on the membrane depolarization (Fig. 8B)

Effect of tiamulin on $[Ca^{2+}]_{cyt}$ and contraction induced by high K^+ solution

In the ileal muscle loaded with fura-2/AM, changes in the ratio of F340/F380, an indicator of $[Ca^{2+}]_{cyt}$, corresponded with the spontaneous contraction. In the presence of Iso-70 K^+ , an application of 2×10^{-5} M tiamulin lowered the elevated ratio of F340/380 almost concomitantly with the lowering of the developed muscle tension. An application of EGTA (2 mM) decreased both the $[Ca^{2+}]_{cyt}$ and muscle tension to below the resting level (Fig. 9).

Effect of tiamulin on intracellular Na^+ and K^+ contents

In taenia coli, the intracellular Na^+ or K^+ content was 8.29 ± 0.41 mmol/kg wet wt. ($n = 4$) or 62.68 ± 3.56 mmol/kg wet wt. ($n = 4$), respectively. A 60-min exposure to tiamulin (2×10^{-6} – 2×10^{-4} M) did not affect them. After 30 min in the Hyper 60 K^+ solution, the intracellular Na^+ content of taenia coli increased up to 15.3 ± 2.21 mmol/kg wet wt. ($n = 4$), but the intracellular K^+ content did not change (64.33 ± 3.95 mmol/kg wet wt. $n = 4$). Tiamulin (2×10^{-5} and 2×10^{-4} M) did not significantly change the increased intracellular Na^+ contents and the intracellular K^+ contents of the muscle in the Hyper 60 K^+ solution at 15 min or 30 min after the tiamulin application.

Effect of tiamulin on Ca^{2+} -induced contraction in the chemically skinned taenia preparations

An application of Ca^{2+} (10^{-6} M) developed tension to about 0.1–0.15 g. Tiamulin (2×10^{-5} M) did not significantly inhibit the Ca^{2+} -induced contraction. Tiamulin at 2×10^{-4} M

Fig. 9. Effect of tiamulin (2×10^{-5} M) on Iso-70 K^+ induced increase of the $[Ca^{2+}]_{cyt}$ level and tension in guinea pig ileal longitudinal smooth muscle. Fluorescences, the fluorescent ratio and muscle tension were simultaneously measured in the muscle strips loaded with fura-2. The external solution (PSS) was replaced by Iso-70 K^+ solution, and then 2×10^{-5} M tiamulin or 2 mM EGTA was applied. The muscles were excited at 340 or 380 nm and the emission at 500 nm was monitored; the ratio of F340 and F380 is also shown (Ratio). The vertical bars show a change in fluorescence, taking the fluorescence without excitation lights as 0% and that with 340 nm or 380 nm excitation light in resting muscle as 100%.

slightly increased the contraction, but trifluoperazine at 10^{-4} M caused a complete inhibition (figure is not shown).

DISCUSSION

In the present paper, we have investigated the tiamulin inhibition of the phasic and tonic contraction of the Hyper 60 K⁺-induced contraction. The Hyper 60 K⁺-induced tonic contraction in the polarized smooth muscle or the Ca²⁺-induced contraction in the depolarized muscle is likely to be caused by an increase in cytoplasmic Ca²⁺ that entered from the external medium through a voltage-dependent Ca²⁺ channel (11). An organic Ca²⁺ antagonist like verapamil (12, 13) or cellular Na⁺ accumulated by ouabain (14) or vanadate (15) inhibited the entrance of Ca²⁺ through the channel. In the experiment on the Ca²⁺-induced contraction, the inhibitory effects of tiamulin at the 2×10^{-6} – 2×10^{-5} M as well as those verapamil (10^{-8} – 10^{-5} M) were competitively antagonized by raising the external Ca²⁺ concentration. A number of investigators have suggested that extracellular Ca²⁺ can be utilized during the tonic component of contraction and that the phasic contraction is mainly due to a Ca²⁺ release from an intracellular store. Recently, simultaneous measurements of tension development and intracellular Ca²⁺ concentration with fura 2 have been done with some kinds of tissues, but not with taenia coli. Rink and Pozzan (16) reported that the guinea pig taenia coli probably do not have the esterase to catalyze the conversion of fura 2/AM in the cytoplasm. For this reason, we were obliged to measure the tension development and [Ca²⁺]_{cyt} level in the strip of guinea pig ileal longitudinal muscle instead of using taenia coli; the results were similar to those observed in tiamulin inhibition in taenia coli (S. Nakajyo et al., unpublished data). Though the isolated preparation differ, tiamulin still inhibited the high K⁺-induced contraction by decreasing the [Ca²⁺]_{cyt} level. Judging from these results, it is suggested that tiamulin inhibits the high K⁺-induced tonic contraction possibly by preventing the Ca²⁺ influx through a voltage-dependent Ca²⁺ channel. Moreover, tiamulin at the higher concentration (2×10^{-5} M) de-

creased the frequency of spike-discharges accompanied by rhythmic contraction corresponding to the absence or presence of high K⁺, which was sensitive to Ca²⁺ antagonists (12, 13). This result is compatible with the concept that tiamulin inhibits the Ca²⁺-influx. As tiamulin did not change the intracellular Na⁺ and K⁺ contents of the depolarized muscle, it seems to have no influence on the permeability of monovalent cations through the smooth muscle cell membrane or on the Na⁺ pump. Thus, it is likely that tiamulin inhibits the Ca²⁺ entry through the voltage-dependent Ca²⁺ channel without changing intracellular Na⁺ content. Meanwhile, metabolic inhibiting factors such as glucose depletion (17, 18), hypoxia (18, 19), and application of monensin (9) decreased the tonic tension of the depolarized muscle. That is, the tonic component of the high K⁺-induced contraction under an aerobic condition requires energy which is produced by ATP synthesized in the mitochondria of the smooth muscle cell (20, 21). In the present paper, the concentration-inhibition curve for monensin was shifted to the right by hypoxia as reported by Kishimoto et al. (9). However, the curves for tiamulin under normoxia and hypoxic conditions were almost the same. The result may exclude the possibility that tiamulin inhibits the tonic contraction induced by high K⁺ by depressing the ATP synthesis in mitochondria. In the subsequent series of experiments, tiamulin also did not affect the Ca²⁺-induced contraction in the chemically skinned smooth muscle which was inhibited by trifluoperazine, a calmodulin inhibitor. Accordingly, tiamulin may not inhibit the high K⁺-induced tonic contraction directly by the contractile and regulatory proteins of smooth muscle.

It is generally accepted that the high K⁺-induced contraction consists of two components in the intestinal muscle: One is a phasic component which is likely to be caused by the released Ca²⁺ from the sarcoplasmic reticulum, and the other is a tonic component which is related to the Ca²⁺ entering from the external medium through the voltage-dependent Ca²⁺

channel. Therefore, Ca^{2+} antagonists, i.e., verapamil selectively inhibits the tonic response of the high K^{+} -induced contraction but only inhibits the phasic response slightly. Tiamulin, as well as verapamil, had less effect on the high K^{+} -induced phasic contraction under hypoxia and the carbachol-induced phasic contraction which is likely to be caused by $[\text{Ca}^{2+}]_{\text{cyt}}$ released from the sarcoplasmic reticulum (10). However, tiamulin considerably inhibited the rapid response of the high K^{+} -induced contraction, in contrast to verapamil (Fig. 1). We can not explain this discrepancy between the effect of tiamulin and that of verapamil on the phasic contraction by the present data. In summary, these results suggest that the inhibitory action of tiamulin on the high K^{+} -induced tonic contraction is possibly due to the competitive inhibition of Ca^{2+} entry through the voltage-dependent Ca^{2+} channel of the intestinal smooth muscle cell.

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