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Inhibitory Effects of Tiamulin on Contractile and Electrical Responses in Isolated Thoracic Aorta and Cardiac Muscle of Guinea-pigs

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Abstract—The inhibitory effect of tiamulin, an antibiotic produced by *Pleurotus mutilis*, on contractile and electrical responses in isolated thoracic aorta and cardiac muscle of guinea-pigs was studied. In the thoracic aorta, tiamulin with an IC50 of $9{\cdot}7 \times 10^{-6}$ M inhibited sustained contractions induced by isosmotically added 60 mM KCl. The inhibitory effect of tiamulin on a Ca^{2+}-induced contraction in a depolarized muscle was competitively antagonized by raising external Ca^{2+} concentration. Bay K 8644 (10^{-7} M) antagonized tiamulin's inhibition of the Ca^{2+}-induced contraction. Tiamulin (2×10^{-5} M) decreased the elevated cytoplasmic Ca^{2+} level measured by the fura 2 AM method in the depolarized muscle. In high K^+-isoprenaline-treated left atria, tiamulin (2×10^{-5}–2×10^{-4} M) produced negative inotropic effects. On the other hand in the membrane action potential of papillary muscles, tiamulin (2×10^{-6}–2×10^{-4} M) produced decreases in action potential and durations and 2×10^{-4} M tiamulin depressed the slow response action potential in depolarized muscles. Tiamulin produced prolongations of the PR interval in ECG, negative chrono- and inotropic effects, and an increase in perfusion flow in guinea-pig isolated and perfused hearts. These effects of tiamulin on the aorta or cardiac muscle were similar to those of verapamil and nifedipine. These results suggest that both the inhibitory action of tiamulin on the high K^+-induced contraction in the aorta and the negative inotropic effect of tiamulin on the cardiac muscle are due to an inhibition of Ca^{2+} entry through the voltage-dependent Ca^{2+} channels of cells of both these muscles.

A number of clinically useful antibiotic drugs are known to have pharmacological actions on the cardiovascular system (Cohen et al 1970) and various types of smooth muscle (Tamargo et al 1982). That is, macrolides produce adverse cardiovascular reaction (Chapelle-Groz & Athias 1988), and aminoglycosides cause the depression of action potential and blockage of the slow Ca^{2+} channels in atrial myocardium (Adams & Goodman 1975; Adams & Durentt 1978). Tiamulin (14-deoxy-14[(2-diethyl-aminoethyl)-mercapto-acetoxy] dehydro mutilin) is produced by culture of the bassidomycete (Arigoni 1962; Hodgin & Hognauer 1974). This antibiotic is a potent pressor lowering agent in laboratory animals and in high doses disturbs repolarization of the cardiac muscle in dogs (Stevens et al 1982). However, the effect of tiamulin on the cardiovascular system is not yet fully understood. In the present study, we have investigated the effects of tiamulin on thoracic aorta and cardiac muscle of guinea-pigs.

Materials and Methods

Vascular smooth muscle preparations

The thoracic aortae were removed from male guinea-pigs, 250–400 g, and dissected free from fat and connective tissues. Helically cut strips, approximately 2 mm wide and 15 mm length, were prepared as described by Furchgott (1960). The adventitial and endothelial layers were removed from the media-intimal layer to avoid the effects of pharmacologically active endogenous substances. Muscle strips were maintained in physiological salt solution (PSS) and bubbled with 95% O_2–5% CO_2 at 37°C. The composition of PSS was as follows (mM): NaCl 136·9, KCl 5·4, $CaCl_2$ 2·5, $MgCl_2$ 1·0, $NaHCO_3$ 23·8 and glucose 5·5. Isosmotic 65·4 K^+ (Iso-60 K^+) or 75·4 mM K^+ (Iso-70 K^+) solution was made by replacing 60 or 70 mM NaCl in the above solution with equimolar KCl. The muscle strips were suspended in an organ bath (15 mL) under a resting tension of 0·5 g and contraction was recorded isometrically with a strain gauge transducer (Nihon Kohden). The tension of maximum contraction in the second application of Iso-60 K^+ solution was considered as the reference response (100%), except where otherwise stated. Effects of the drug were also investigated on contractions induced by adding Ca^{2+} to the muscle in Ca^{2+}-free, Iso-60 K^+ solution (Ca^{2+}-induced contraction). A concentration-response curve was made by a cumulative addition of Ca^{2+} ranging from 0·25 to 10 mM. Antagonists were applied 15 min before the Ca^{2+} addition. The Ca^{2+}-free solution was made by omitting $CaCl_2$ from the original medium without any substitution for the change in osmolarity.

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

The tension development and the fluorescent light representing Ca^{2+} signals were measured simultaneously in the thoracic aorta smooth muscle. The muscle strips were treated with PSS containing 5 μM of the acetoxymethyl ester of fura 2 and 0·25% cremophor (non-cytoxic detergent) for 6 h at room temperature (24°C) by the method described by Himpens & Somlyo (1988). One end of the muscle strip was connected to an isometric strain gauge transducer (Nihon Kohden, SB-1TA), and the other end was pinned to an

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excitation light and the surface fluorescence of the living muscle was measured using a fluorimeter (Nihon Spectroscopic, CAF-100) as described by Ozaki et al (1987). A part of the muscle strip was excited by light obtained from a xenon lamp (75 W) equipped with a rotating filter wheel (48 Hz) containing 340 and 380 nm (±5·5 nm) interference filters, and the emitted light from the muscle was collected by a photomultiplier through a 500 nm (±10 nm) bandpass filter. The ratio of the fluorescence due to excitation at 340 and 380 nm (F340/F380) was calculated from the successive excitation periods. In this experiment, EDTA (0·2 mM) and *N,N,N',N'*-tetrakis(2-pyridyl-methyl) ethylendiamine (TPEN, 10 nM) were added to the PSS.

Mechanical response of left atria

Preparations were suspended vertically in the organ bath containing 30 mL of Tyrode solution saturated with 95% O_2–5% CO_2 at 30°C with the following composition (mM): NaCl 140·0, KCl 3·0, $MgCl_2$ 1·0, $CaCl_2$ 1·8, NaH_2PO_4 0·4, $NaHCO_3$ 12·0 and glucose 5·0. Isometric contractile tension (CT) was measured with a force displacement transducer (Nihon Kohden, TB-651G). Maximum rate of tension development (dT/dt_{max}) was obtained by electrically differentiating contractile tension by using a differentiator (Nihon Kohden, ED-601G). The resting tension was adjusted to 0·5 g and the preparation was electrically stimulated, by square wave pulse of 3 ms duration at a frequency of 2 Hz and an intensity of 20% above the threshold, through a bipolar platinum electronic stimulator (Nihon Kohden, SEN-7203) through an isolation unit (Nihon Kohden, SS-302J). To investigate slow contractile response, fast Na^+ channels were inactivated by increasing the external potassium concentration, $[K^+]$, to 22 mM. Stimulation frequency was then decreased to 0·5 Hz and (-)isoprenaline was added to the solution to yield a final concentration of 10^{-6} M.

Transmembrane action potential of papillary muscles

For the electrical experiments, papillary muscles were horizontally pinned down on the plate of cork placed in a 20 mL organ bath containing Tyrode solution of the above composition bubbled with 95% O_2–5% CO_2. The preparation was electrically stimulated by square wave pulses, of 3 ms duration at a frequency of 1 Hz and an intensity of 20% above the threshold through bipolar platinum electrodes. Transmembrane potentials were recorded using glass microelectrodes filled with 3 M KCl and with a tip resistance of 10 to 50 Mohm. A microelectrode amplifier providing capacity composition (Nihon Kohden, MEZ-7101) was used to record the transmembrane electrical responses. The maximum rate of action potential ($\dot{V}_{max}$) was obtained by differentiation using an RC circuit with a time constant of 50 μs. Both membrane potential and $\dot{V}_{max}$ were displayed on an oscilloscope (Nihon Kohden, VC-10) and simultaneously recorded on a data recorder (TEAC, MR-10). To investigate the slow response action potential, the fast Na^+ channels were inactivated by increasing $[K^+]$ to 22 mM. The stimulation frequency was descreased to 0·2 Hz and histamine was added to the solution to yield a final concentration of 3×10^{-5} M. The preparations were allowed to equilibrate for at least 1 h before the start of experiments. In order to examine concentration-response relationships, drugs were cumulatively applied at intervals of 20 min.

Langendorff's guinea-pig isolated heart preparation

For the isolated and perfused hearts, Langendorff's technique, as modified by Sakai & Shiraki (1978) was employed. Using a glass cannula filled with the 95% O_2–5% CO_2 saturated Krebs-Henseleit solution, the heart was mounted. The hearts were perfused via the aorta at a perfusion pressure of 60 mm Hg. Krebs-Henseleit solution as the perfusate contained the following (mM): NaCl 118·4, KCl 4·7, $MgSO_4$ 1·2, $CaCl_2$ 2·5, KH_2PO_4 1·2, $NaHCO_3$ 25·0 and glucose 10·6. An electrocardiogram (ECG) was taken by means of a bipolar lead from two (different and indifferent) silver-wire electrodes touching the origin of the aorta and from one touching the left intra-ventricular wall. The bioelectric amplifier (Nihon Kohden, AB-621G) was set. Heart rate (HR) was measured with a cardiotachometer (Nihon Kohden, AT-601G) or calculated from the R-R or S-S interval of the ECG. For measurement of left ventricular cavity pressure (LVP), a glass cannula was inserted via the left atrium into the left ventricle. Another glass cannula was filled with water and connected by a water-filled tube to a pressure transducer (Nihon Kohden, TP-200T).

The maximum first derivative of left ventricular pressure (LV dP/dt_{max}) was obtained using a differentiating circuit (Nihon Kohden, EQ-601G). Perfusion flow (PF) was measured with an electric flow meter (Nihon Kohden, MFV-2100). A glass cannula inserted via the aorta, was connected by a catheter for measurement of perfusion flow. All parameters were recorded on a multipurpose polygraph (Nihon Kohden, RM-6000). ECG signals were also recorded on a data recorder (Nihon Kohden, RMG-5204). Drugs were injected in a volume of 0·1 mL in 10 s into the aortic bulb of the heart.

Chemicals

Drugs used were tiamulin hydrochloride (Nihon Zenyaku, Japan), verapamil hydrochloride (Eisai, Japan), nifedipine (Bayer, Germany), Bay K 8644 (Wako, Japan), fura 2 AM (Dojin, Japan), TPEN (*N,N,N',N'* tetrakis(2-pyridyl-methyl)ethylenediamine) (Dojin, Japan), saponin (ICN, USA), histamine hydrochloride (Sigma, USA) and (−)-isoprenaline (Wako, Japan).

Statistical analysis

The values presented in Figs and Tables are expressed as the mean ± s.e. For comparing two groups of results, statistical analyses were performed by Student's *t*-test.

Results

Effect of tiamulin on Iso-60 K^+-induced contraction of the thoracic aorta

The vascular relaxing effect of tiamulin was examined in the guinea-pig thoracic aorta contracted by Iso-60 K^+. The effect of tiamulin on the Iso-60 K^+-induced contraction of the guinea-pig aorta was compared with the effects of verapamil and nifedipine, both calcium antagonists (Fig. 1). Cumulative application of tiamulin, verapamil or nifedipine inhibited the sustained contraction induced by Iso-60 K^+ in guinea-pig aorta. The concentration curves for verapamil

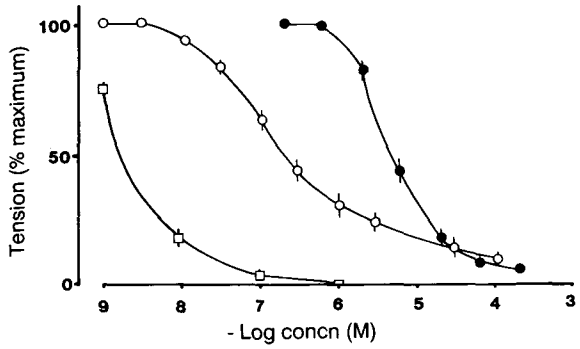

FIG. 1. Inhibition of Iso-60 K^+-induced contraction by various concentrations of tiamulin (●), verapamil (○) and nifedipine (□) in guinea-pig thoracic aorta. Each point represents the mean value ± s.e. for 5–6 experiments. Iso-60 K^+; hyperosmotically added 60 mM K^+.

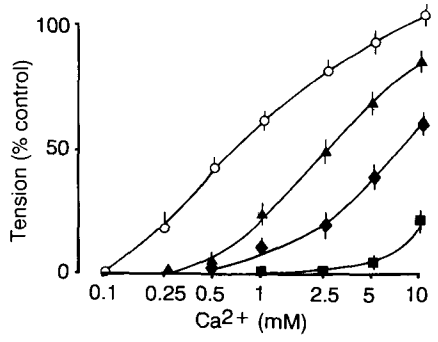

FIG. 2. Effects of tiamulin on the contractions induced by cumulative application of Ca^{2+} in 60 mM K^+-depolarized guinea-pig thoracic aorta in Ca^{2+}-free medium. The magnitude of the contraction in response to Iso-60 K^+ in the presence of 2·5 mM Ca^{2+} was taken as the reference response (100%). Control ○, tiamulin 2×10^{-6} ▲, 6×10^{-6} ◆ and 2×10^{-5} M ■.

and nifedipine were shifted in parallel, while the curve for tiamulin was not parallel and had a steep slope. IC50 values for tiamulin, verapamil or nifedipine calculated from the concentration-response curves, were $9{\cdot}7 \times 10^{-6}$, $3{\cdot}0 \times 10^{-7}$ and $3{\cdot}2 \times 10^{-9}$ M, respectively. The potency of nifedipine needed for the inhibitory action was 300-fold that of tiamulin (Table 1).

Table 1. IC50 values (M) of tiamulin, verapamil and nifedipine.

	Aorta	Left atria
Tiamulin	$9{\cdot}7 \times 10^{-6}$	$6{\cdot}1 \times 10^{-5}$
Verapamil	$3{\cdot}0 \times 10^{-7}$	$5{\cdot}4 \times 10^{-7}$
Nifedipine	$3{\cdot}2 \times 10^{-9}$	$6{\cdot}1 \times 10^{-7}$

Effect of tiamulin on the Ca^{2+}-induced contraction

Cumulative addition of Ca^{2+} (0·25–10 mM) produced a stepwise contraction in the depolarized muscle of the aortic preparation. Inhibitory effect of tiamulin was further investigated on Ca^{2+}-induced contraction. Pretreatment with tiamulin or verapamil for 30 min inhibited the Ca^{2+}-induced contraction, in a concentration-dependent manner. The concentration-response curves were shifted to the right by verapamil and by tiamulin. The mode of tiamulin- or verapamil-inhibition was analysed by Lineweaver–Burk plots of the reciprocal of the external Ca^{2+} concentration. The values in Fig. 3a, b gave straight lines in the presence or absence of 2×10^{-6} M tiamulin, intersecting at a single point on the ordinate. However, in the presence of 10^{-7} M Bay K 8644, verapamil and tiamulin slightly inhibited these Ca^{2+}-induced contractions (Figs 2, 3).

Simultaneous measurement of Ca^{2+} signal and tension development in the isolated thoracic aorta

In the fura 2 AM-loaded aortic strip, the basal level of either F340 or F380 showed a slow and steady decrease, although the ratio (R340/380) remained constant. Iso-70 K^+ solution, which induced a maximal response in muscle tension, rapidly increased F340, decreased F380 and increased the ratio which indicates the relative $[Ca^{2+}]_{cyt}$. The addition of tiamulin (2×10^{-5} M) rapidly decreased the elevated $[Ca^{2+}]_{cyt}$ and decreased muscle tension. The application of EDTA (0·4 mM) decreased both the $[Ca^{2+}]_{cyt}$ and muscle tension to the resting level (Fig. 4).

Effects of tiamulin on the mechanical response of left atria

A low concentration of tiamulin (2×10^{-6}–6×10^{-6} M) produced a slight increase in contractile tension (CT) and the

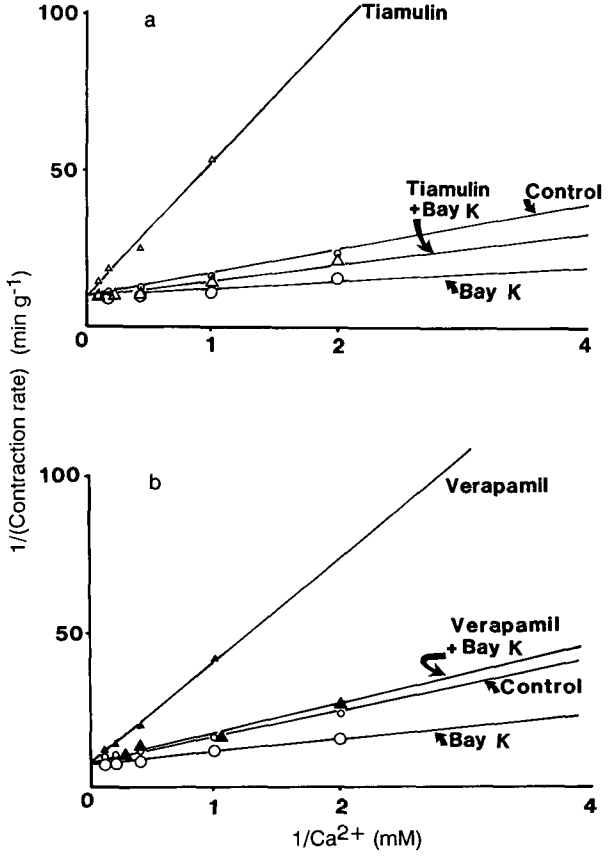

FIG. 3. Lineweaver–Burk plot of data obtained from inhibition of Ca^{2+}-induced contraction by tiamulin, verapamil and Bay K 8644 (10^{-7} M). The ordinate is the reciprocal of rate of Ca^{2+}-induced contraction, 1/(tension min^{-1}), the abscissa is the reciprocal of external Ca^{2+} concentration, $1/Ca^{2+}$ (mM). Data were obtained 15 min after tiamulin or verapamil application. Each point represents the values from 5–6 experiments.

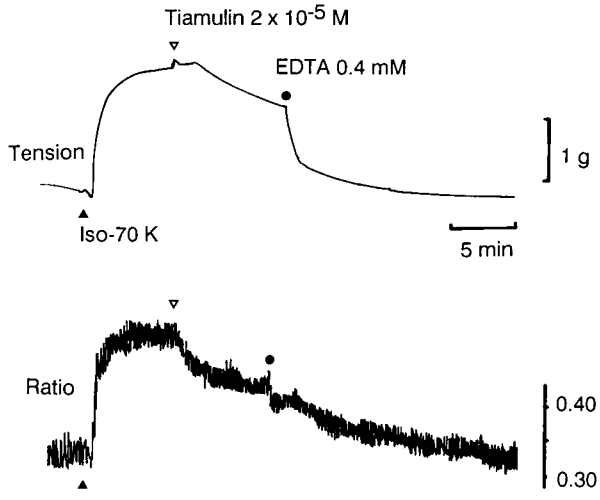

FIG. 4. Effect of tiamulin (2×10^{-5} M) on Iso-70 K^+-induced increase of the Ca^{2+} concentration and tension in guinea-pig thoracic aorta. Fluorescence, the fluorescent ratio and muscle tension were simultaneously measured in the muscle strips loaded with fura 2 AM. The external solution was replaced by Iso-70 K^+ solution, then 2×10^{-5} M tiamulin or 0·4 mM EDTA was applied. The muscles were excited at 340 or 380 nm and the emission at 500 nm was monitored (F340 and F380); the ratio of F340 to F380 is also shown (Ratio). The vertical bars represent a change in fluorescence, taking the fluorescence without excitation as 0% and that with 340 or 380 nm excitation in resting muscle as 1·00.

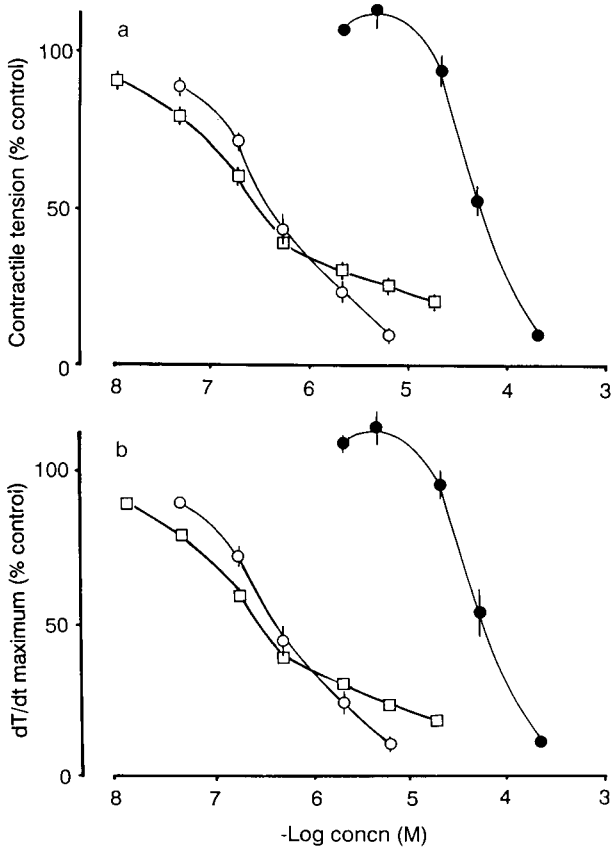

FIG. 5. Effects of tiamulin (●), verapamil (○) and nifedipine (□) on isometric contractile tension (a) and the maximum rate of tension development (dT/dt_{max}) (b) in electrically-driven left atria of guinea-pigs. Each point value is the mean ± s.e. of data from atria bathed in K^+ (3·0 mM) Tyrode solution.

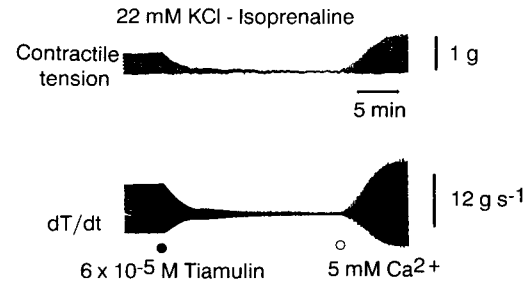

FIG. 6. Depressant effects of tiamulin (6×10^{-5} M) on slow contractile responses induced in guinea-pig atria bathed with 22 mM K^+-Tyrode solution plus 10^{-6} M isoprenaline. The negative inotropic effect of tiamulin was reversed by increasing the Ca^{2+} concentration to 5 mM in the bathing medium. dT/dt, differentiated contractile tension.

maximum rate of tension development (dT/dt_{max}) but a high concentration of tiamulin (2×10^{-5}–2×10^{-4} M) produced a concentration-dependent decrease in CT and dT/dt_{max} to 11·3 ± 2·3 and 12·5 ± 2·2%, respectively. Verapamil (6×10^{-6}–6×10^{-5} M) produced a concentration-dependent decrease in CT and dT/dt_{max} to 11·4 ± 2·8 and 12·4 ± 2·6%, as did nifedipine (2×10^{-8}–2×10^{-5} M) to 22·3 ± 2·0 and 20·0 ± 1·4%, respectively (Fig. 5). In high K^+-isoprenaline-treated atria, tiamulin (6×10^{-6} – 10^{-4} M) concentration-dependently decreased CT and dT/dt_{max}. The IC50 values of tiamulin, verapamil and nifedipine were $6{\cdot}1 \times 10^{-5}$, $5{\cdot}4 \times 10^{-7}$ and $6{\cdot}1 \times 10^{-7}$ M, respectively (Table 1). The effects were reversed by increasing the calcium concentration (Fig. 6).

Effects on the transmembrane action potentials of papillary muscle

At a low concentration, tiamulin (2×10^{-7} M) caused no significant influence on the resting potential (RP) and action potential amplitude (APA); when the concentration of tiamulin was increased to 2×10^{-6} and 2×10^{-5} M, RP and APA significantly decreased. The change in repolarization (APD50) was concentration-dependent, but the change in action potential duration at the APD was not concentration-dependent. However, over the concentration range of 2×10^{-7}–2×10^{-5} M tiamulin, there was no significant change in the maximum upstroke velocity ($\dot{V}_{max}$). On the other hand, verapamil (2×10^{-7}–2×10^{-5} M) produced a concentration-dependent decrease in APA, APD50, APD90 and $\dot{V}_{max}$, but no significant decrease in the RP (Table 2).

Slow response action potentials were elicited in the preparation bathed in 22 mM K^+-Tyrode solution containing 3×10^{-5} M histamine. High concentrations of tiamulin (2×10^{-4} M) depressed the slow response (Fig. 7).

Effects on the isolated and perfused hearts

Effects of tiamulin and verapamil on the guinea-pig isolated and perfused hearts were expressed as percentages of the respective basal values. Tiamulin, at a dose of more than 10 or 100 μg, dose-dependently produced a prolongation of PR interval, QRS duration and QT interval, a decrease in HR, LVP and LV dP/dt_{max} and an increase in PF. Verapamil, at a dose of more than 0·3 μg produced a prolongation of PR interval, a shortening of QT interval, a decrease in LVP and

Table 2. Effect of tiamulin and verapamil on the transmembrane action potential of guinea-pig papillary muscle.

Drugs (M)	RP	APA	APD50	APD90	$\dot{V}_{max}$
Tiamulin					
2×10^{-7}	98·6±1·2	98·2±1·2	88·7±4·5*	91·7±3·5*	100·8±2·7
2×10^{-6}	96·1±1·2**	96·3±1·2**	84·7±7·1*	90·3±5·5	98·4±3·4
2×10^{-5}	94·7±1·0**	94·7±2·0*	79·6±8·5*	91·1±5·5	99·0±4·7
Verapamil					
2×10^{-7}	101·4±0·8	95·5±0·5**	92·2±2·3*	93·1±4·6	100·5±4·0
2×10^{-6}	99·2±1·1	93·0±2·3*	86·2±3·3*	91·6±5·4	89·3±3·8*
2×10^{-5}	97·7±1·2	88·4±3·5*	69·0±4·7**	79·7±3·9**	77·3±5·9*

RP, resting potential; APA, action potential amplitude; APD50, action potential duration at 50% repolarization; APD90, action potential duration at 90% repolarization; $\dot{V}_{max}$, the maximum upstroke velocity. % of control (mean ± s.e.) $*P<0.005$, $**P<0.01$.

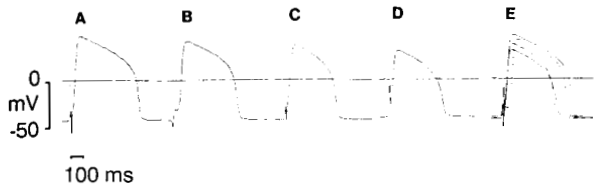

FIG. 7. Effect of tiamulin (2×10^{-4} M) on slow response action potential induced by histamine (3×10^{-5} M) in depolarized papillary muscles under high $[K^+]_o$. A: before the application of tiamulin. B–D: 3, 10, 30 min after the addition of tiamulin, respectively. E: superimposition of A–D.

LV dP/dt_{max}, and an increase in PF. However, verapamil had no affect on QRS duration or QT interval or on HR, even at higher doses of 1 or 3 μg. Moreover, it was not affected by the effective dosage of verapamil (Fig. 8). These results indicated that both tiamulin and verapamil produce a prolongation of PR interval, a decrease in LVP and LV dP/dt_{max}, and an increase in PF, and that tiamulin, unlike verapamil, produces a prolongation of QRS duration and QT interval, and a decrease in HR.

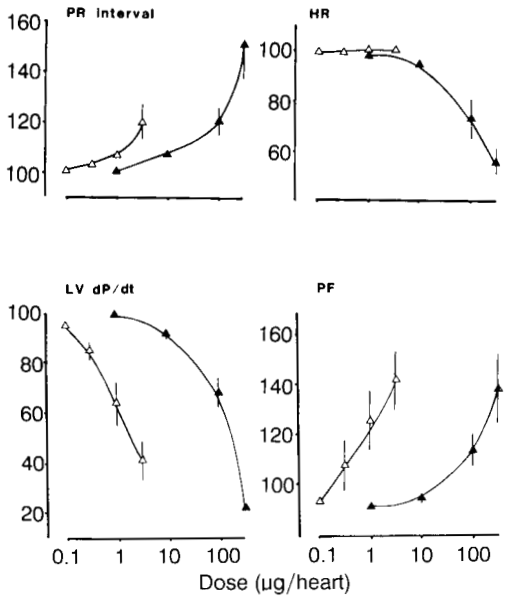

FIG. 8. Effects of tiamulin (▲) and verapamil (△) on guinea-pig isolated hearts. HR: heart rate; LV dP/dt_{max}: maximum first derivative of left ventricular pressure; PF: perfusion flow. Each value represents the mean percentage and s.e. (n = 5–6).

Discussion

Tiamulin, which is produced in cultures of the bassidomycete, *Pleurotus mutilis*, is a pleuromutilin derivative, 14-deoxy-14[(2-diethyl-aminoethyl)-mercaptoacetoxy]dihydro mutilin (Arigoni 1962; Hodgin & Hognauer 1974), and it shows antibacterial activity. In the present experiment, tiamulin dose-dependently inhibited the Iso-60 K^+-induced contraction in guinea-pig thoracic aorta. As judged by IC50 values, tiamulin was less potent than verapamil and nifedipine. In another series of experiments, the inhibitory effect of tiamulin or verapamil on the Ca^{2+}-induced contraction was competitively antagonized by raising the external Ca^{2+} concentration. Moreover, Bay K 8644 increased the IC50 value of tiamulin and verapamil on the Ca^{2+}-induced contraction. This will lead us to the conclusion that contraction by tiamulin was a Ca^{2+}-dependent process. Recently, the fluorescent Ca^{2+} indicator fura 2 AM has been used to measure the $[Ca^{2+}]_{cyt}$ in smooth muscle simultaneously with the tension development (Grynkiewicz et al 1985; Himpens & Somlyo 1988). We measured the simultaneous tension development and $[Ca^{2+}]_{cyt}$ level in the guinea-pig thoracic aorta muscle strip using the apparatus devised by Ozaki et al (1987). Tiamulin inhibited the Iso-70 K^+-induced sustained contraction by decreasing the $[Ca^{2+}]_{cyt}$ level elevated by Iso-70 K^+ solution. These data suggest that tiamulin inhibits the Iso-70 K^+-induced sustained contraction possibly by inhibiting the Ca^{2+} influx through a voltage-dependent Ca^{2+} channel in the rat aorta. The concentration-inhibition curves (Fig. 1) were parallel for verapamil and nifedipine, but not for tiamulin. However, results now presented seem to suggest the possibility that tiamulin affects a different site. We do not yet have an explanation for this inconsistency.

In left atria isolated from guinea-pig, tiamulin induced negative inotropic effects (decrease in CT and dT/dt_{max}) in a concentration-dependent manner, although these effects were less potent than those of verapamil and nifedipine. Moreover, tiamulin induced a decreased slow response contractile tension in the high K^+-isoprenaline treated atria, which was reversed by increasing the external Ca^{2+} concentration, a shortened APD50 of normal action potential, and a suppressed slow action potential. These data indicate that

tiamulin inhibited the Ca^{2+} influx through the voltage-dependent Ca^{2+} channel in cardiac muscles as well as vascular smooth muscles as described above. Among Ca^{2+} antagonists, verapamil and D-600, which inhibit the slow inward current (I_{si}, mainly consisting of Ca^{2+} current), inhibit the fast Na^+ current (I_{Na}) to decrease $\dot{V}_{max}$ at high concentrations (Fleckenstein 1972). Unlike these drugs, dihydropyridines such as nifedipine selectively inhibit I_{is} but not I_{Na} (Kohlhardt et al 1972). Since tiamulin did not affect $\dot{V}_{max}$ of normal action potential, it appears to be similar to the latter drugs in this respect. On the contrary, there is the possibility that tiamulin has other modes of action, for example, reducing K^+ conductance, besides Ca^{2+} entry blockade, as judged by its slightly positive inotropic effects at lower concentrations, slightly decreased resting membrane potential, and concentration-independently shortened APD90, unlike APD50. Although it has been shown that low concentrations of dihydropyridine calcium channel antagonists can display agonist effects (Brown et al 1986), mechanisms of positive inotropic effects of tiamulin may be different from those of dihydropyridines because tiamulin shortened APD50 in normal action potential even at concentrations at which it induced positive inotropic effects in left atria.

In the isolated and perfused heart, tiamulin as well as verapamil dose-dependently produced prolongation of the PR interval in the ECG, decrease in LV dP/dt_{max} and an increase in PF, indicating that tiamulin induced suppression of atrioventricular conductivity, the decrease in contractile tension of cardiac muscle and the relaxation (dilution) of coronary arteries through the Ca^{2+}-entry blocking action. However, tiamulin, unlike verapamil, induces a marked decrease in heart rate and prolongation of QRS and QT intervals, probably due to the change in heart rate.

It has been reported that among Ca^{2+}-entry blockers, there are tissue differences in the potency of Ca^{2+}-entry blocking action. For example, nifedipine was found to be more selective in vascular smooth muscle than in cardiac muscle (Raschack 1976; Triggle & Swamy 1980). On the basis of our data, nifedipine can be said to be more effective than verapamil or tiamulin for vascular smooth muscle. The inhibition of electrical or contractile response by tiamulin in the cardiac muscles was qualitatively similar to that by verapamil. From these observations, it is likely that tiamulin is the same type of Ca^{2+} antagonist as verapamil rather than the dihydropyridines.

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