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Effects of Trimebutine Maleate on Delayed Rectifier K^+ Currents in Guinea-pig Ventricular Myocytes

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Abstract

The effects of trimebutine maleate, a drug commonly used to regulate motility in the gastrointestinal tract, on the delayed rectifier K^+ current (I_K) were evaluated in guinea-pig ventricular myocytes to determine whether the drug has a proarrhythmic effect through blockade of I_K.

Trimebutine decreased I_K in a concentration-dependent manner. To investigate the effects of trimebutine on two components of I_K (I_{Kr} and I_{Ks}; rapidly activated and slowly activated components, respectively), we performed the envelope-of-tails test. Trimebutine-sensitive I_K was determined by digital subtraction of I_K during exposure to trimebutine from control I_K for each duration of the test pulse over the range 50 ms–2 s. The ratio of $\Delta I_{K,tail}/\Delta I_K$ plotted against pulse duration for trimebutine-sensitive I_K gradually decreased to a steady-state value as the duration of the test pulse was lengthened. This finding suggested a weak inhibitory effect of trimebutine on both I_{Kr} and I_{Ks}. The effects of trimebutine on the inward rectifier K^+ current (I_{K1}) responsible for the resting potential and final repolarization phase of the action potential were investigated by applying voltage clamp ramps over a broad range of potentials. No significant effects were observed at 10 or 100 μM. We next investigated the effects of the drug on the L-type Ca^{2+} current (I_{Ca}). Significant inhibition of I_{Ca} was observed at trimebutine concentrations greater than 10 μM.

These results suggested that trimebutine maleate has weak inhibitory effects on I_{Kr}, I_{Ks} and I_{Ca} at concentrations much higher than those in clinical use.

Trimebutine maleate has been shown to regulate motility in the gastrointestinal tract through direct effects on smooth muscle (Nagasaki et al 1993a, b; Xue et al 1995). Consequently, the drug is widely used for several symptoms of non-ulcer dyspepsia and irritable bowel syndrome. Recent reports have drawn attention to a rare, though important, adverse effect of cisapride, a drug which acts on the regulation of motility in the gastrointestinal tract (Drolet et al 1998), as well as terfenadine (Rampe et al 1993; Woosley et al 1993), a non-sedating antihistamine. Cisapride promotes motility of the gastrointestinal tract and lengthens the QT interval of the electrocardiogram resulting in major cardiac arrhythmias, such as polymorphic ventricular tachycardia (torsades de pointes), in some patients (Olsson & Edwards 1992; Bran et al 1995; Wysowski & Bacsanyi 1996). Torsades de pointes is also observed as a proarrhythmic effect of anti-arrhythmic drugs that prolong the action potential duration through block of the delayed K^+ current (I_K). I_K in the heart is made up of two components: rapidly activated I_{Kr}, and slowly activated I_{Ks} (Sanguinetti & Jurkiewics 1990, 1991). The mechanisms underlying the proarrhythmic effect of cisapride are attributable to blockade of I_{Kr} (Drolet et al 1998). Trimebutine has been shown to inhibit the Ca^{2+} current in rabbit sino-atrial node cells (Kotake et al 1987), and to suppress the fast and slow action potentials of guinea-pig papillary muscles, without prolonging the action potential duration (Igawa et al 1989). These observations suggest that trimebutine suppresses Ca^{2+} currents in cardiac tissue at relatively high concentrations, but has no prolongation effect on the action potential duration of ventricular muscles. Both Ca^{2+} current and I_K affect action potential duration, but in the opposite direction. However, the actual

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effect of trimebutine on cardiac I_K has not been examined in detail. Recent electrophysiological studies of trimebutine in gastric (Xue et al 1995) and ileal smooth muscle cells (Nagasaki et al 1993a, b) have shown an inhibitory action of trimebutine (10 μM) on the K^+ and Ca^{2+} currents in smooth muscle.

In this study, we evaluated the effect of trimebutine maleate on I_K, L-type Ca^{2+} current (I_{Ca}) and inward rectifier K^+ current, all of which potentially affect the action potential duration in guinea-pig ventricular myocytes.

Materials and Methods

Experiments were performed in accordance with the guidelines for animal use in research of Tottori University.

Cell preparation and solutions

Male guinea-pigs, 250–400 g, were anaesthetized with intraperitoneal injection of sodium pentobarbital. The hearts were quickly excised, cannulated via the ascending aorta and retrogradedly perfused with Ca^{2+}-free Tyrode solution at 37°C. The hearts were then perfused with the same solution containing collagenase (300 units mL^{-1}, Type I , Sigma Chemical, St Louis, MO) for 17–20 min. The ventricles were dissected to disperse the cells in a high-K^+ low-Cl^- Kraft-Brühe solution. All solutions used during the cell isolation procedure were oxygenated and maintained at 37°C. The cells were filtered through stainless steel mesh and stored in dMEM solution (Gibco) before use.

Normal Tyrode solution contained (mM): 140 NaCl, 5·4 KCl, 1·8 $CaCl_2$, 0·5 $MgCl_2$, 0·33 NaH_2PO_4, 5·5 glucose and 5·0 HEPES; pH was adjusted to 7·4 with NaOH. The nominally Ca^{2+}-free Tyrode solution used for the cell isolation procedure was prepared by simply omitting $CaCl_2$ from the normal Tyrode solution. The Kraft-Brühe solution for cell preparation contained (mM): 70 potassium glutamate, 30 KCl, 10 KH_2PO_4, 1 $MgCl_2$, 20 taurine, 0·3 EGTA, 10 glucose and 10 HEPES (pH adjusted to 7·2 with KOH). The external solution for the measurement of whole-cell I_K was normal Tyrode solution plus 0·4 μM nisoldipine.

The pipette solution contained (mM): 70 potassium aspartate, 50 KCl, 10 KH_2PO_4, 3 $MgCl_2$, 3 Na_2-ATP, 0·1 Li_2-GTP, 5 EGTA and 5 HEPES (pH adjusted to 7·2 with KOH).

Trimebutine maleate (Tanabe Seiyaku, Japan) was dissolved in normal Tyrode solution before use. Nisoldipine (a generous gift from Bayer, Germany) was prepared as a 1 mM stock solution in ethanol and then added to the normal Tyrode solution to give a final concentration of 0·4 μM.

Electrical measurement, data acquisition and analysis

A small sample of cells was placed in a 0·24-mL chamber mounted on the stage of an inverted microscope (model IX-70, Olympus, Japan). Cells were allowed to settle on the glass bottom of the chamber and were then superfused continuously at a rate of 2 mL min^{-1} with normal Tyrode solution at 37°C.

All currents were recorded in the whole-cell, voltage-clamp configuration of the patch clamp technique using an EPC-9 patch-clamp amplifier (HEKA Electronic, Germany). Patch pipettes were made from glass capillaries (1·5 mm o.d., 1·0 mm i.d.; Narishige Scientific Instrument Laboratory, Japan) using a horizontal pipette puller (model P-97, Sutter Instrument, USA). Patch pipettes had a resistance of 2·0–2·5 M when filled with the pipette solution. Pulse and PulseFit software (HEKA Electronic, Germany) were used to generate voltage-pulse protocols and to record and analyse data with computer (Power Macintosh G3 DT266, Apple computer, USA).

I_K was measured during the depolarizing test pulses, under conditions where the Na^+ channels were inactivated by setting the holding potential at −50 mV, and the L-type Ca^{2+} channels were blocked by adding 0·4 μM nisoldipine to the Tyrode solution (Sanguinetti & Jurkiewics 1991). Under these experimental conditions, I_K was monitored by measuring the magnitude of time-dependent outward current activated during a 2250-ms depolarizing step to various potentials and the amplitudes of outward tail currents elicited on repolarization to a holding potential of −50 mV after depolarizing steps. The amplitude of the tail current was determined by subtraction of the steady-state current level from the peak current level measured after the step to a holding potential. Sufficient time between test pulses was allowed for full deactivation of tail currents before application of another pulse. I_{K1} (inward rectifier) was measured by applying voltage clamp ramps from a holding potential of −50 mV first to +50 mV at a rate of 40 mV s^{-1}, then to −120 mV at a rate of −40 mV s^{-1}. I_{Ca} was elicited by a series of depolarization steps of 10-mV increments and 500 ms duration applied from a holding potential of −50 mV in normal Tyrode solution. The amplitude of I_{Ca} was measured as the

difference in current between the initial peak and the current at 100 ms after the depolarizing pulse.

Data are expressed as mean ± s.e.m., and the differences between the mean values were assessed by Student's *t*-test. $P < 0{\cdot}05$ was considered significant.

Results

Effects of trimebutine on I_K

Figure 1 shows the effects of trimebutine on I_K elicited by depolarizing test pulses from a holding potential of −50 mV in guinea-pig ventricular myocytes. Trimebutine decreased the amplitudes of the time-dependent activating and tail currents in a concentration-dependent manner.

To investigate the effects of trimebutine maleate on two components of I_K (I_{Kr} and I_{Ks}) of guinea-pig ventricular myocytes, we performed the envelope-of-tails test in the absence and presence of the drug. As shown in Figure 2A, the cell was held at a holding potential of −50 mV and depolarized to +40 mV for a duration varying between 50 ms and 2 s. Current traces were obtained before and after exposure to 10 μM trimebutine (Figure 2A). The trimebutine-sensitive I_K was then obtained by digital subtraction of I_K recorded during exposure to trimebutine from that under control conditions for each duration of test pulse ranging from 50 ms to 2 s (trace not shown). Drug effects on I_K were analysed by comparing the magnitude of the time-dependent outward current activated during depolarizing test pulses (ΔI_K) with that of the tail current elicited upon return to a holding potential of −50 mV ($\Delta I_{K,tail}$) of trimebutine-sensitive I_K for each in a series of depolarizing pulses. As shown in Figure 2B, the ratio of $\Delta I_{K,tail}/\Delta I_K$ plotted against the test pulse duration for trimebutine-sensitive I_K gradually decreased to a steady-state value as the duration of the test pulse was prolonged (means ± s.e.m., 4 experiments). If one component of I_K was suppressed by trimebutine, then the ratio of $\Delta I_{K,tail}/\Delta I_K$ should remain almost constant regardless of the pulse duration. This finding strongly suggested that trimebutine suppresses not only I_{Ks} but also I_{Kr} at concentrations greater than 10 μM.

Effects of trimebutine on I_{K1}

The I_{K1}, or inward rectifier, is the K^+ current responsible for the resting potential and final repolarization phase of the action potential. The effects of trimebutine on I_{K1} were investigated by applying voltage clamp ramps at a speed of

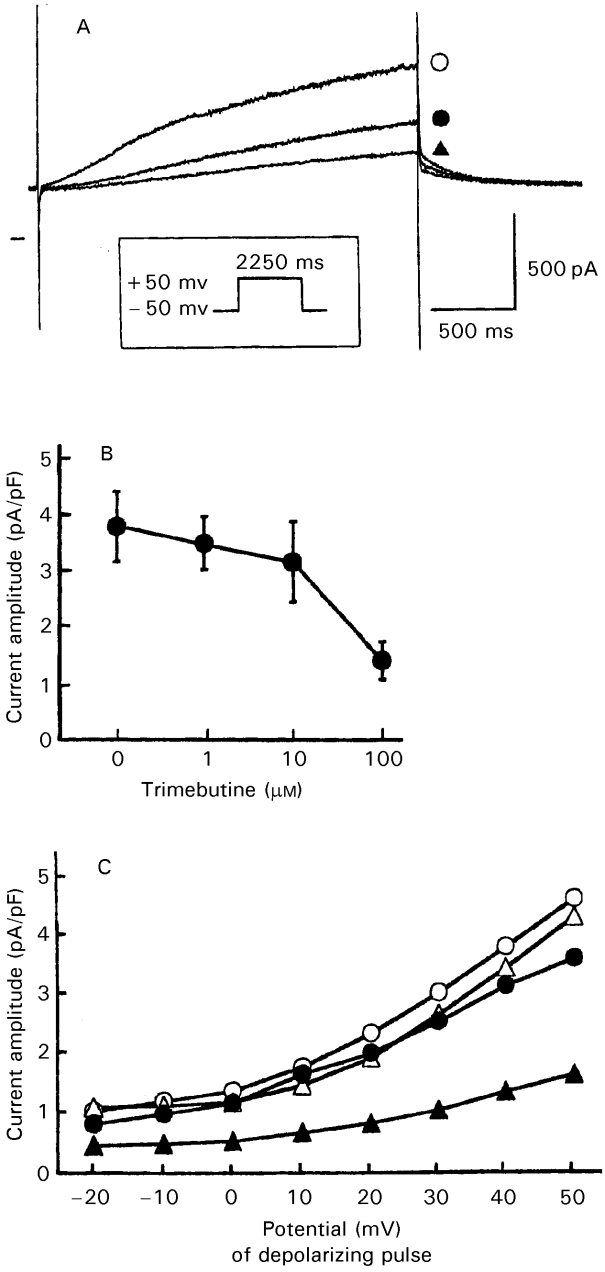

Figure 1. Effects of trimebutine on I_K elicited by depolarizing test pulses from a holding potential of −50 mV in guinea-pig ventricular myocytes. The current records in response to 2250-ms test pulses to +50 mV under control conditions (○), during exposures to 10 M (●) and 100 μM (▲) trimebutine are superimposed in A. The short horizontal line preceding the current traces indicate the zero current level. B. The amplitudes of outward tail currents in response to test pulses to +50 mV plotted against trimebutine concentration. The amplitude of the tail current was determined by subtraction of the steady-state current level from the peak current level measured after the step to a holding potential. C. Typical series of tail current amplitudes against potential level of the depolarizing pulse for each concentration. Current amplitudes during exposure to 1 μM drug (△). Data are expressed as mean ± s.e.m., n = 4–8

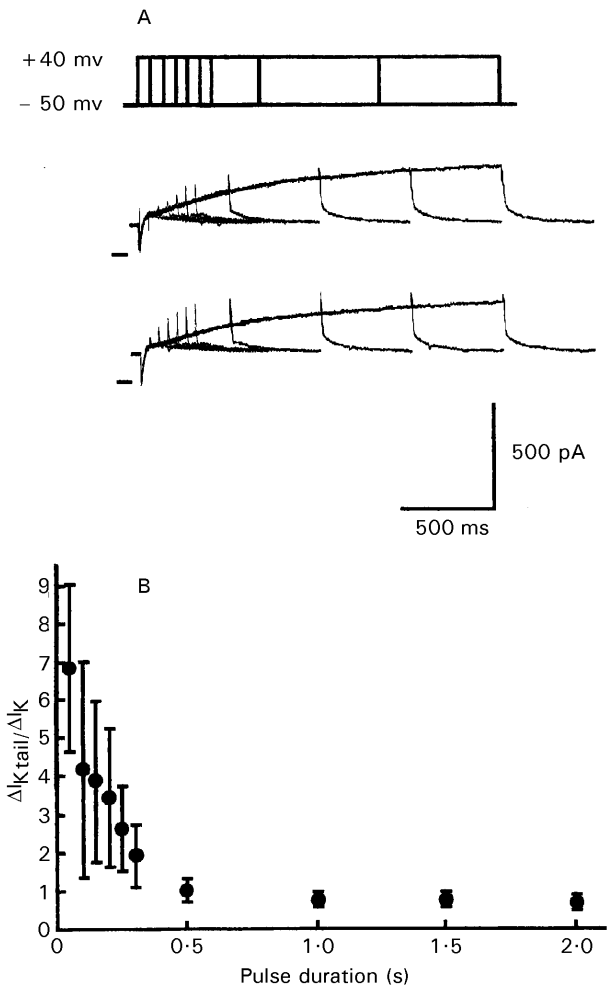

Figure 2. Envelope-of-tails test for I_K under control conditions and during exposure to trimebutine in guinea-pig ventricular myocytes. A. The upper trace shows the voltage protocol. Superimposed current traces during the depolarization steps to +40 mV for various durations followed by repolarization to a holding potential of −50 mV under control condition (middle traces), and in the presence of 10 μM trimebutine (lower traces are shown). The short horizontal lines preceding the current traces indicate the zero current level. B. Ratio of $\Delta I_{K,tail}/\Delta I_K$ plotted against the test pulse duration for trimebutine-sensitive I_K obtained by digital subtraction of I_K recorded in the presence of trimebutine from that of control I_K. Data are expressed as mean ± s.e.m., 4 experiments.

40 mV s^{-1} over a broad range of potentials, as shown in Figure 3. At 10 and 100 μM, no significant effects were seen from −120 to +50 mV. Similar results were obtained in 4 experiments.

Effects of trimebutine on I_{Ca}

We investigated the effects of trimebutine on L-type Ca^{2+} current (I_{Ca}). The cells were superfused with Tyrode solution containing 1·8 mM Ca^{2+} but without nisoldipine. The cells were maintained at a holding potential of −50 mV and voltage steps of 10-mV increments and 500 ms duration were

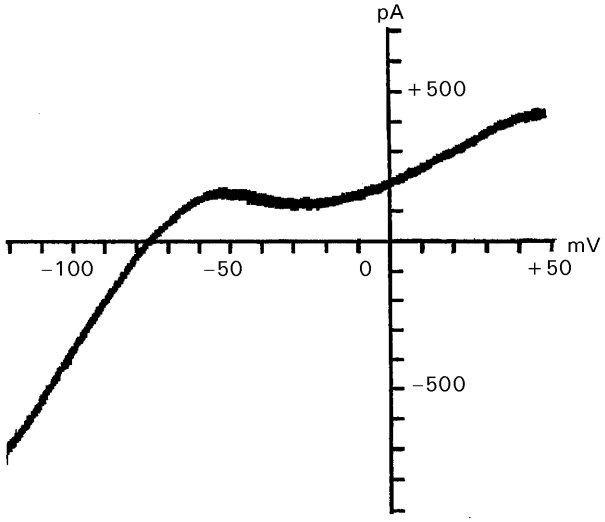

Figure 3. Effects of trimebutine on the inward rectifier, I_{K1}, in guinea-pig ventricular myocytes. Ramp voltage clamps were applied at a speed of 40 mV s^{-1} starting from a holding potential of −50 mV going to +50 mV and then to −120 mV. Current records in the absence and presence of 1 and 10 μM trimebutine were superimposed.

applied at a rate of 0·2 Hz. Figure 4A shows representative traces of peak I_{Ca} elicited by the clamp pulse to −10 mV from a holding potential of −50 mV in control and in the presence of trimebutine (10 μM) and again in normal solution (washout). Figure 4B shows the effects of trimebutine on the current–voltage (I–V) relationship of I_{Ca}.

In 3 experiments, after a 500-ms clamp from a holding potential of −50 mV to −10 mV, 10 μM trimebutine decreased the peak amplitude of I_{Ca} (−79·5%).

Discussion

Early studies (Kotake et al 1987; Igawa et al 1989) have shown trimebutine to inhibit the Ca^{2+} current in rabbit sino-atrial node cells, and to suppress the fast and slow action potentials of guinea-pig papillary muscles, without prolonging the action potential duration. This data suggests that trimebutine suppresses the Na^+ and Ca^{2+} currents in cardiac tissue at relatively high concentrations, but has no prolongation effect on the action potential duration of ventricular muscles. Both I_{Ca} and I_K affect action potential duration but in the opposite direction. However, the actual effect of trimebutine on cardiac I_K has not been well examined.

Recent electrophysiologic studies of trimebutine in rabbit ileal smooth muscle cells (Nagasaki et al 1993a, b) have shown an inhibitory action of trimebutine on the depolarization-induced, Ca^{2+}-independent and Ca^{2+}-dependent K^+ currents (with an IC50 value of 7·6 and 23·5 μM, respectively) and voltage-dependent Ca^{2+} current (IC50 7–36 μM).

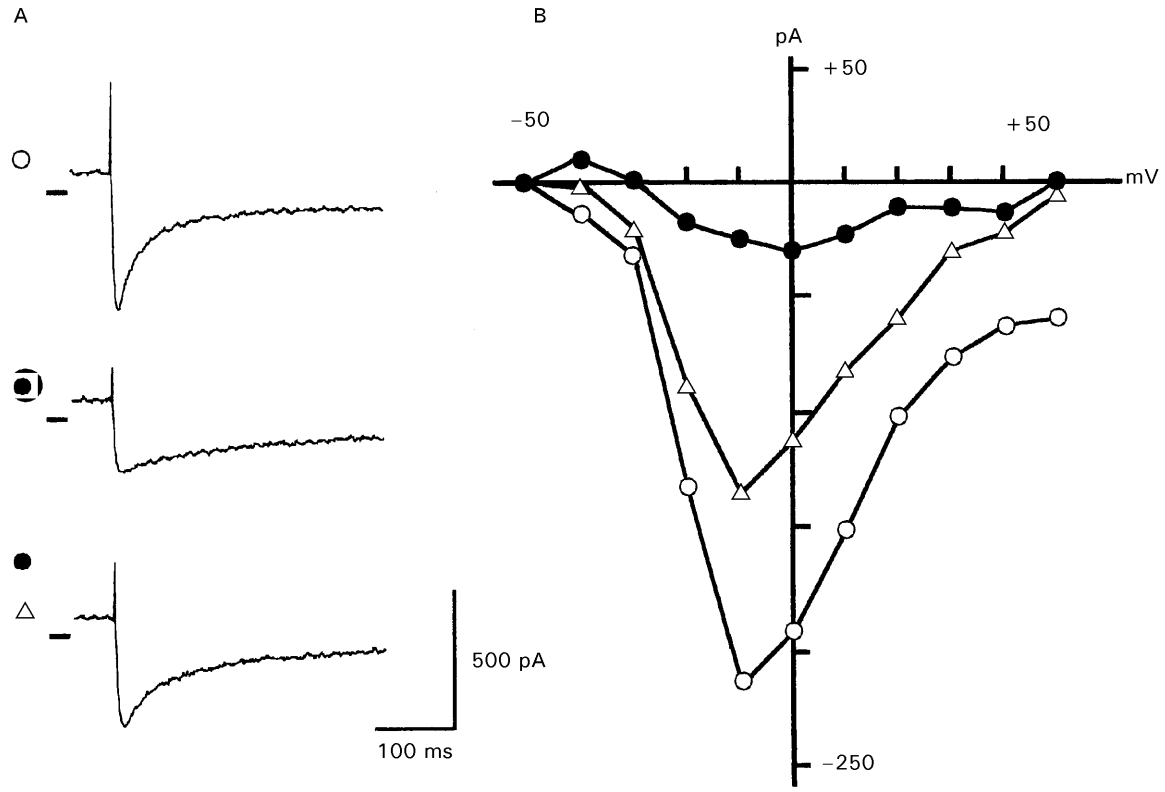

Figure 4. Effects of trimebutine on I_{Ca}. A. I_{Ca} elicited by the clamp pulse to −10 mV was recorded in the same cell before trimebutine administration (○), after 10 μM trimebutine superfusion (●) and after washout (△). The short horizontal lines preceeding the current traces indicate the zero current level. B. Effects of trimebutine on the current–voltage (I–V) relationship. I_{Ca} was elicited by a series of depolarization steps of 10-mV increments and 500 ms duration applied from a holding potential of −50 mV. The amplitude of the current (ordinate) was measured as the difference in current between the initial peak and the current at 100 ms after the depolarizing pulse. Control (○);10 μM trimebutine (●); washout (△).

Furthermore, cisapride, which promotes motility in the gastrointestinal tract, has been shown to exert inhibitory effects on I_K, especially I_{Kr}, resulting in prolongation of the electrocardiogram QT interval, and arrhythmia (Drolet et al 1998). The contributions of I_K and I_{K1} to the action potential duration are well established, and reduction in I_K or I_{K1} will cause a prolongation of action potential duration and the electrocardiogram QT interval (Giles & Imaizumi 1988; Sanguinetti & Jurkiewics 1990; Surawicz 1992). I_K has been reported to consist of two components, I_{Kr} and I_{Ks}, in many species (Sanguinetti & Jurkiewics 1990; Gintant 1996; Li et al 1996). Drugs that prolong the QT interval by inhibition of cardiac I_K, especially I_{Kr}, are often clinically associated with lethal ventricular arrhythmia, torsades de pointes (Roden et al 1986; Carlsson et al 1990; Follmer & Colatsky 1990; Woosley 1996; Drolet et al 1998).

The major finding of this study was that trimebutine, which is commonly prescribed to regulate motility of the gastrointestinal tract, has a weak inhibitory effect on both components of the delayed rectifier current, I_{Kr} and I_{Ks}. Xue et al (1995) showed the effects of trimebutine on the electrical properties of gastric smooth muscle in rat at concentrations of 0·01–0·1 μM. Clinical pharmacological studies have indicated that the maximum serum concentration of trimebutine is about 0·09 ± 0·02 μM (data file, Tanabe Seiyaku, Japan). In this study, the effects of trimebutine on I_K and I_{Ca} were observed at concentrations greater than 10 μM. The weak potency, in addition to the nonspecific effect on I_{Kr} in the electrophysiological properties of cardiac cells, provide efficacy without severe proarrhythmic properties.

Acknowledgements

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