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Effects of propafenone on electrical and mechanical activities of single ventricular myocytes isolated from guinea-pig hearts

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1 The effects of propafenone on the transmembrane action potential and sarcomere shortening during twitch contraction were investigated in single ventricular myocytes isolated from guinea-pig hearts.

2 Propafenone at low concentrations ($3–5 \times 10^{-7}$ M) slightly lengthened action potential duration (APD), but shortened it at higher concentrations. The shortening of APD was accompanied by an attenuation of sarcomere shortening during twitch contraction.

3 Propafenone ($>10^{-6}$ M) caused a concentration-dependent decrease in the maximum upstroke velocity ($\dot{V}_{max}$) of the action potential. In the presence of propafenone (3×10^{-6} M), trains of stimuli led to an exponential decline in $\dot{V}_{max}$. A time constant for the recovery of $\dot{V}_{max}$ from the use-dependent block was 4.8 s.

4 In myocytes treated with propafenone (3×10^{-6} M), the $\dot{V}_{max}$ of test action potentials preceded by the conditioning clamp pulses to 0 mV was progressively decreased by increasing the duration of single clamp pulse or by increasing the number of multiple brief clamp pulses.

5 These findings suggest that propafenone has use-dependent inhibitory action on the sodium channel by binding to the channel during both activated and inactivated states, and that the unbinding rate is comparable to that of Class-I antiarrhythmic drugs with intermediate kinetics. Propafenone may also have an inhibitory action on calcium and potassium channels.

Introduction

Propafenone is a newly introduced antiarrhythmic drug. Clinical studies have demonstrated that propafenone given by the oral or parenteral route has potent inhibitory actions against both supraventricular and ventricular tachyarrhythmias under various pathological conditions (Seipel & Breithardt, 1980). Experiments by previous investigators have shown that propafenone reduces the maximum upstroke velocity ($\dot{V}_{max}$) of action potential without affecting resting membrane potential (Kohlhardt & Seifert, 1980; 1983; 1985; Ledda *et al.*, 1981; Dukes & Vaughan Williams, 1984). In animals *in vivo*, and in patients, propafenone has been shown to suppress conduction velocity in all cardiac tissues (Connolly *et al.*, 1983). These findings suggest that primary electrophysiological effects of propafenone in the heart are similar to those of local anaesthetic-type (Class-I) antiarrhythmic drugs: inhibition of the sodium current. However, the precise mode of action of propafenone on the cardiac sodium channel in relation to the cellular antiarrhythmic mechanism remains to be elucidated.

In the present study, the effects of propafenone on the transmembrane action potentials were investigated in single ventricular myocytes isolated from guinea-pig hearts. The modulation of drug-induced $\dot{V}_{max}$ inhibition by stimulation frequency or by various clamp pulses was studied extensively to compare with the characteristics of its sodium channel blocking action with other Class I drugs. Mechanical activity of single cells was also obtained by measurement of average single sarcomere length during twitch contractions through the use of our original image processing system (Sato *et al.*, 1988).

In experiments to assess the drug action on cardiac cellular properties, such single myocytes are considered to be more favourable than conventional

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multicellular preparations, because various influences of conduction and of ion accumulation or depletion in the narrow extracellular spaces can be eliminated.

Methods

Cell isolation

Single ventricular myocytes were enzymatically isolated by a procedure similar to that described previously (Watanabe *et al.*, 1985). In brief, hearts were quickly removed from guinea-pigs (200–300 g) and perfused by the Langendorff method with the following solutions in sequence: (1) Ca^{2+}-free Krebs solution for 5 min, (2) enzyme solution containing either collagenase (2 mg ml^{-1}, Sigma, type V), trypsin (0.01 mg ml^{-1}, Sigma, type III-S) and 4×10^{-5} M Ca^{2+}, or collagenase (2 mg ml^{-1}, Sigma, type V) and protease (0.2 mg ml^{-1}, Sigma, type XIV), for 5 min, and (3) Ca^{2+}-free Krebs solution for 5 min. The left ventricle was then cut into small pieces in Ca^{2+}-free Krebs solution to disperse myocytes. A few drops of cell suspension were placed in a recording chamber attached to an inverted microscope. The chamber was perfused with normal Krebs-bicarbonate solution at a rate of 2 ml min^{-1}. In experiments using suction pipette electrodes, Krebs-HEPES solution was employed instead of Krebs-bicarbonate solution. The temperature of the perfusate was maintained at 35°C.

Following the stepwise increase in calcium concentration of the medium to 1.8 or 2.0 mM (normal Krebs solution), 30 to 40% of myocytes had deteriorated into round-shaped cells due to irreversible contracture, whereas the remaining cells were tolerant to calcium; the cells maintained rod-shapes without spontaneous beating. The experiments were carried out only on the latter type of myocyte.

Electrophysiological set-up

Membrane potential was recorded through conventional glass microelectrodes filled with 3 M KCl (20–50 MΩ). The membrane potential was electronically differentiated to obtain the maximum upstroke velocity ($\dot{V}_{max}$) of action potential. Cells were stimulated by application of short current pulses (1 ms in duration) through the recording microelectrode. Intensity of the stimulation was adjusted to obtain a constant latency (2–4 ms).

Mechanical activities of single myocytes were characterized by measuring average single sarcomere length by the microcomputer-based image processing system. Details of the system may be obtained from our recent paper (Sato *et al.*, 1988).

The whole-cell clamp method (Hamill *et al.*, 1981) was used for voltage clamp parts of the experiments. Pipettes were fire-polished and filled with internal solution to have a resistance ranging from 2 to 3 MΩ. Action potentials were recorded in current-clamp mode through the pipette by passing short (<4 ms) stimulus current. Transition from the voltage-clamp mode to the current-clamp mode was regulated by a pulse generator through an electronic relay. Details of the experimental protocol are given in the Results section.

Solutions

Composition of the Krebs-bicarbonate solution was as follows (mM): NaCl 120, KCl 4.0, $CaCl_2$ 2.0, $MgSO_4$ 1.3, NaH_2CO_3 25.2 and glucose 5.0. The solution was equilibrated with 95% O_2:5% CO_2 to maintain a pH of 7.4. The Ca^{2+}-free Krebs solution had the same composition as that of Krebs-bicarbonate solution without $CaCl_2$. The Krebs-HEPES solution contained (mM): NaCl 136.9, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4 0.33, HEPES 5.0 and glucose 5.0. The pH was adjusted to 7.4 by adding NaOH, and the solution was equilibrated with 100% O_2. The pipette internal solution consisted of (mM): KCl 120.0, NaH_2PO_4 10.0, EGTA 1.0, MgATP 5.0 and HEPES 10.0. The pH was adjusted to 7.2 by adding KOH.

Propafenone (Yamanouchi Pharmaceutical Co. Ltd.) was dissolved in deionized water to make a stock solution (10^{-1} M), and desired drug concentrations were obtained by adding a small amount of stock solution to the perfusate.

Data analysis

Values were presented as means ± s.e. with number unless otherwise specified. Data were analyzed by use of the *t* test, analysis of variance, Dunnett's test and regression analysis. Significance level was set at $P < 0.05$.

Results

Effects of propafenone on membrane action potential and twitch contraction

The electromechanical effects of propafenone were examined in guinea-pig ventricular myocytes constantly stimulated at 1.0 Hz. Transmembrane action potential accompanied by sarcomere shortening (twitch contraction) was recorded by use of glass microelectrodes and the image-processing system (Figure 1). Control values of action potential parameters before drug application were as follows: resting potential (RP), -90.3 ± 0.3 mV; action potential duration at 75% repolarization (APD_{75}), 204 ± 6 ms; and $\dot{V}_{max}$, 262 ± 6 V s^{-1} ($n = 18$). The resting sarcomere length was 1.86 ± 0.02 μm

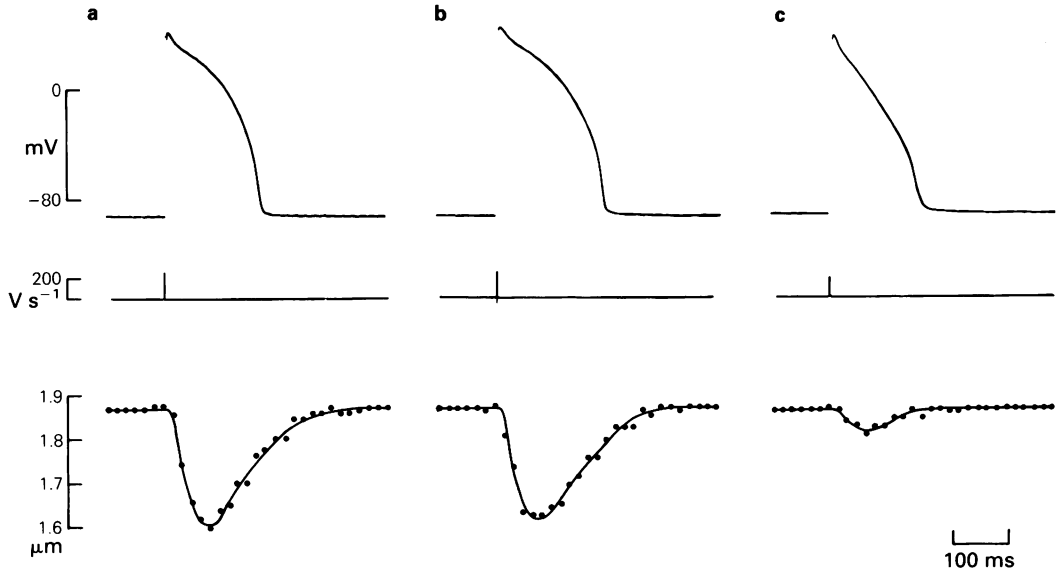

Figure 1 Effects of propafenone of the transmembrane action potential and accompanied sarcomere shortening in guinea-pig ventricular myocyte: (a) control; (b) propafenone (3×10^{-7} M); (c) propafenone (3×10^{-6} M). The top trace is the membrane potential and the middle trace is its first derivative. The bottom trace shows average single sarcomere shortening during twitch contraction accompanied by action potential. The myocyte was constantly driven at 1.0 Hz. Data were obtained before and 30 min after the drug application at each concentration.

($n = 10$). Twitch contraction was initiated soon after the fast upstroke of the action potential, and the shortest sarcomere length ($1.57 \pm 0.02\,\mu m$, $n = 10$) was achieved approximately 80–100 ms after the stimulus. Relaxation progressed slowly; and the sarcomere length returned to the resting level at 100–200 ms after full repolarization of the action potential.

Following exposure to propafenone at 10^{-7} M for 30 min, there were no significant changes in action potential configuration and sarcomere shortening traces. Propafenone (3×10^{-7} M) caused a slight prolongation of APD_{75} without affecting other parameters (Figure 1). At concentrations above 10^{-6} M, however, APD_{75} was shortened, and the $\dot{V}_{max}$ was decreased in a dose-dependent manner (Figures 1, 2). The shortening of sarcomere length during twitch contraction was also decreased. At 3×10^{-6} M, APD_{75}, $\dot{V}_{max}$ and the peak shortening of sarcomere length were decreased to 87%, 65%, and 19% of the respective control values. RP and the resting sarcomere length were unaffected even at the highest concentration tested (10^{-5} M).

Stoichiometry of drug-receptor complex was applied for the inhibition curves of $\dot{V}_{max}$ (Figure 2a) under the assumption of the law of mass action. An apparent K_D value at 1.0 Hz (a concentration required for half reduction of $\dot{V}_{max}$) was 4.4×10^{-6} M with Hill's coefficient (n_H) of 1.8. The $\dot{V}_{max}$ inhibition by propafenone was enhanced with a higher stimulation frequency (2.5 Hz) and the curve was shifted to the left with a K_D value of 2.0×10^{-6} M and n_H of 1.4.

Use-dependent block of $\dot{V}_{max}$ by propafenone

The enhancement of inhibition of $\dot{V}_{max}$ by propafenone at higher stimulation frequencies may reflect the use-dependent block of the sodium channel as with other Class-I antiarrhythmic drugs. This characteristic was investigated further by applying stimulation trains (1.0 or 2.5 Hz), which were separated from each other by a rest period of 120 s (Figure 3). In untreated control myocytes, the $\dot{V}_{max}$ was almost unchanged during such stimulation trains. Following treatment with propafenone (3×10^{-6} M), $\dot{V}_{max}$ of the first action potential in each train was slightly decreased; there was a tonic block of $\dot{V}_{max}$ by $2.3 \pm 0.2\%$ ($n = 5$). Further decline of $\dot{V}_{max}$ during the stimulation train (use-dependent block) depended on the stimulation frequency; the decrease of $\dot{V}_{max}$ from the first action potential to the new steady-state was $27.2 \pm 2.4\%$ at 1.0 Hz ($n = 5$), and $60.1 \pm 2.3\%$ at 2.5 Hz ($n = 4$).

Beat to beat decline of $\dot{V}_{max}$ during stimulation trains was expressed as a single exponential curve, so that the onset rate per action potential (AP^{-1}) at which the $\dot{V}_{max}$ fell to the new steady state level was calculated in each experiment. The average values

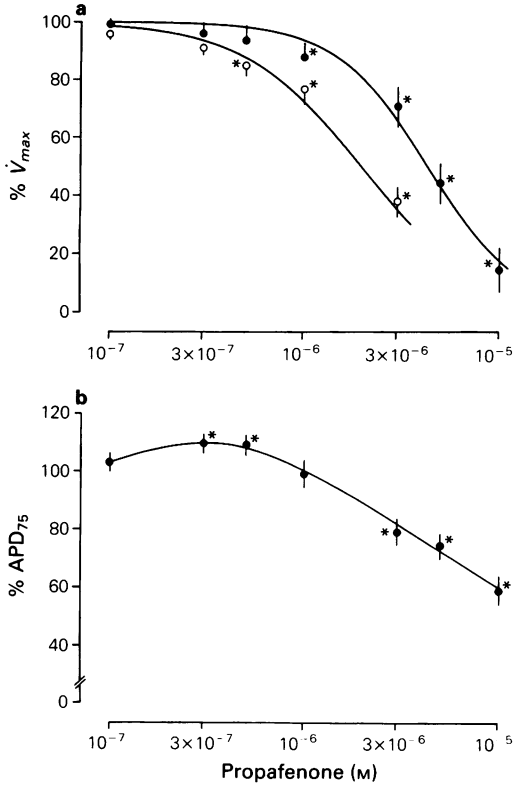

Figure 2 Concentration-dependent effects of propafenone on $\dot{V}_{max}$ (a) and APD_{75} (b). All values are normalized by the reference value under drug-free control conditions. Data were obtained before and 30 min after the drug application at each concentration. Stimulation frequencies were 1.0 Hz (●) and 2.5 Hz (○). Values are indicated as mean with s.e. shown by vertical lines ($n = 5$). *Significantly different from the reference value at $P < 0.05$. The curves in (a) represent nonlinear least-squares fits to the equation:

$$\%\dot{V}_{max} = (1 + [\text{drug}]^{nH}/K_D)^{-1}$$

where n_H is Hill's coefficient and K_D is the apparent dissociation constant. The K_D value was 4.4×10^{-6} M (n_H 1.8) at 1.0 Hz and 2.0×10^{-6} M (n_H 1.4) at 2.5 Hz.

were $0.33 \pm 0.03\,AP^{-1}$ at 1.0 Hz ($n = 5$), and $0.20 \pm 0.02\,AP^{-1}$ at 2.5 Hz ($n = 4$).

Effects of conditioning clamp pulses on $\dot{V}_{max}$ inhibition

The effects of depolarizing clamp pulses on the $\dot{V}_{max}$ of subsequent test action potentials were examined in order to determine whether the marked use-dependency of $\dot{V}_{max}$ inhibition induced by propafenone is due to blockade of the activated or inactivated sodium channel (Kodama *et al.*, 1987).

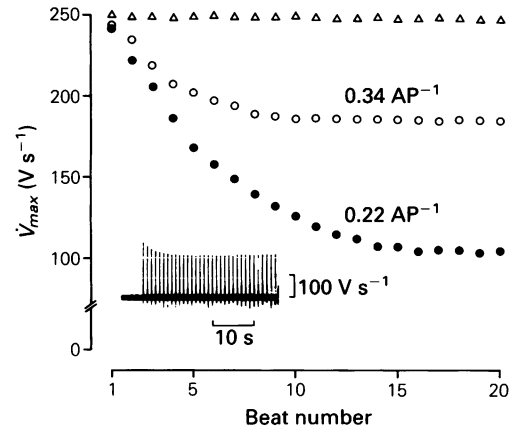

Figure 3 Rate-dependent decrease in $\dot{V}_{max}$ (use-dependent block) by propafenone. Inset shows superimposed record of differentiated upstrokes of the action potentials during stimulation train at 1.0 Hz in a previously quiescent myocyte. The record was obtained 30 min after the drug application at 3×10^{-6} M. The graph shows the beat-to-beat change in $\dot{V}_{max}$ at the start of stimulation trains. Ordinate scale indicates $\dot{V}_{max}$, and abscissa scale indicates number of beats (action potentials) from the initiation of the stimulation train. Frequencies of stimulation were 1.0 Hz under control conditions (△), and 1.0 Hz (○) and 2.5 Hz (●) in the presence of propafenone.

Figure 4 shows the experiment with a single clamp pulse of 0 mV. Following a rest period of 120 s, the membrane potential was clamped from the resting level (holding potential of −82 mV) to 0 mV for 10 to 1000 ms. At the end of the conditioning clamp pulse, membrane potential was clamped back to the holding potential for 100 ms, which is long enough for a drug-free channel to reactivate fully (Carmeliet & Vereecke, 1979), but short enough so that only partial dissociation of drugs from blocked channel occurs (Grant *et al.*, 1984). The voltage-clamp was then released, and a stimulus was applied to elicit a test action potential.

In untreated control myocytes, such a clamp pulse with a duration less than 200 ms had no significant effect on the $\dot{V}_{max}$ of the test action potential. However, further prolongation of the clamp pulse duration resulted in a slight but significant decrease in $\dot{V}_{max}$, suggesting a slow inactivation of the sodium channel. A clamp pulse of 1000 ms in duration decreased $\dot{V}_{max}$ by $6.2 \pm 1.0\%$ ($n = 5$) from the value of the action potential without conditioning clamp pulse (reference level). In myocytes treated with propafenone (3×10^{-6} M) similar clamp pulses caused more remarkable $\dot{V}_{max}$ reduction of the test action potential. Thus, a significant $\dot{V}_{max}$ decrease ($6.6 \pm 1.1\%$, $n = 5$) from the reference value was

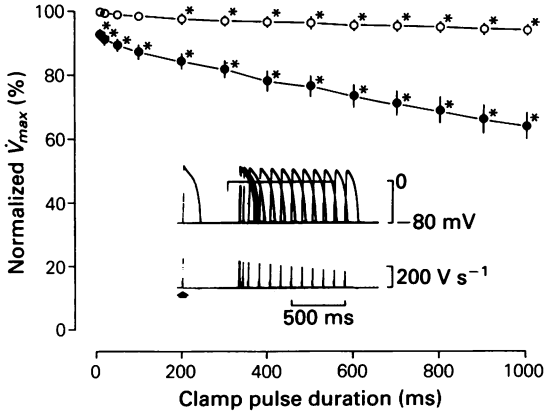

Figure 4 Influence of clamp pulse duration on $\dot{V}_{max}$ inhibition by propafenone. Inset shows superimposed record of action potentials (upper trace) and their differentiated upstroke spikes (lower trace) 30 min after addition of propafenone at 3×10^{-6} M. Action potentials were elicited without clamp pulse as a reference value of $\dot{V}_{max}$ (arrow at left) or 100 ms after a single clamp pulse to 0 mV having a duration of 10, 20, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 ms. Each conditioning clamp pulse was preceded by a 2 min rest period. The results obtained from five myocytes are summarized in the graph. Ordinate scale, $\dot{V}_{max}$ of test action potential normalized by the reference value. Abscissa scale, clamp pulse duration. Data were obtained before (○) and 30 min after the drug application (●). Values are indicated as mean with s.e. shown by vertical lines ($n = 5$). *Significantly different from the reference value at $P < 0.05$.

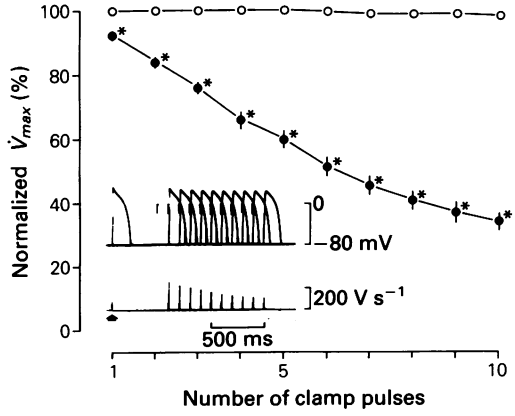

Figure 5 Influence of multiple clamp pulses on $\dot{V}_{max}$ inhibition by propafenone. Inset shows superimposed record of action potentials (upper trace) and their differentiated upstroke spikes (lower trace) 30 min after addition of propafenone at 3×10^{-6} M. Action potentials were elicited without clamp pulse as a reference value of $\dot{V}_{max}$ (arrow at left) or 100 ms after the last clamp pulse to 0 mV. The duration of each clamp pulse was 10 ms and the interval between the clamp pulses, during which the membrane potential was clamped to the resting potential level (−82 mV), was 100 ms. The number of clamp pulses was varied from one to ten. Each conditioning clamp pulse train was preceded by a 2 min rest period. The results obtained from five myocytes are summarized in the graph. Ordinate scale, $\dot{V}_{max}$ normalized by the reference value. Abscissa scale, number of clamp pulses. Data were obtained before (○) and 30 min after the drug application (●). Values are indicated as mean with s.e. shown by vertical lines ($n = 5$). *Significantly different from the reference value at $P < 0.05$.

observed even with 10 ms clamp pulse. The $\dot{V}_{max}$ reduction was further enhanced as the clamp pulse duration was prolonged and reached 36.4 ± 3.1% ($n = 5$) at 1000 ms.

Figure 5 illustrates the effects of multiple brief clamp pulses. The duration of each clamp pulse to 0 mV was 10 ms and the interval between clamp pulses, during which membrane potential was clamped at the resting level (−82 mV), was set to 100 ms. The test action potential was elicited 100 ms after termination of the last pulse. In untreated control cells, such multiple clamp pulses up to 10 had no significant effect on the $\dot{V}_{max}$ of the test action potential. In myocytes treated with propafenone at 3×10^{-6} M, the $\dot{V}_{max}$ of the test action potential was decreased progressively as the number of pulses was increased. At the 10th pulse, the decrease of $\dot{V}_{max}$ from the reference level reached 63.6 ± 2.9% ($n = 5$).

In four myocytes, the recovery process of $\dot{V}_{max}$ following the use-dependent block was examined by introducing a single test action potential following 20 clamp pulses to 0 mV (each clamp pulse duration was 10 ms and the interval between the pulses was 100 ms) with various coupling intervals. Under drug-free control conditions, the $\dot{V}_{max}$ recovered almost completely within 100 ms after the termination of the last clamp pulse. After treatment with propafenone (3×10^{-6} M), much slower $\dot{V}_{max}$ recovery was observed. Representative results are shown in Figure 6, where fractional $\dot{V}_{max}$ reduction was plotted against the coupling interval in a semilogarithmic graph. In the presence of propafenone, the recovery time course of $\dot{V}_{max}$ with a coupling interval longer than 1 s was approximated by a single exponential function. The average time constant (τ_R) was calculated as 4.8 ± 0.2 s ($n = 4$).

Discussion

The present study on guinea-pig ventricular myocytes indicates that propafenone above 10^{-6} M causes a concentration-dependent decrease in $\dot{V}_{max}$ of action potential, and that this $\dot{V}_{max}$ inhibition is

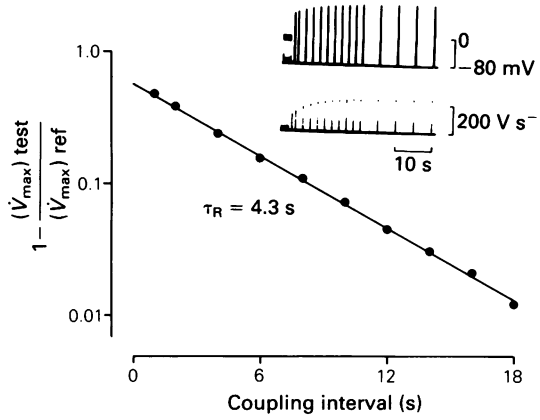

Figure 6 Recovery of $\dot{V}_{max}$ from a use-dependent block by propafenone. Inset shows superimposed record of action potentials (upper trace) and their differentiated upstroke spikes (lower trace). Following 20 conditioning clamp pulses to 0 mV (each clamp pulse duration was 10 ms and the interval between the pulses was 100 ms), a test action potential was elicited with various coupling intervals, during which the membrane potential was clamped to the resting potential (−82 mV). Each conditioning clamp pulse train was preceded by a 2 min rest period. Data were obtained 30 min after addition of propafenone at 3×10^{-6} M. The graph indicates recovery process of the $\dot{V}_{max}$ of the test action potential. Ordinate scale, fractional $\dot{V}_{max}$ reduction of test action potential compared to the $\dot{V}_{max}$ value of action potential without conditioning clamp pulse (reference value): $1 - (\dot{V}_{max})\text{test}/(\dot{V}_{max})\text{ref}$. Abscissa scale, coupling interval between the last clamp pulse and the test action potential. The time course of $\dot{V}_{max}$ recovery with a coupling interval longer than 1 s was approximated by a single exponential function with a time constant of 4.3 s.

enhanced at higher stimulation frequencies. Propafenone at low concentrations ($3–5 \times 10^{-7}$ M) slightly prolonged action potential duration (APD), but shortened that at the higher concentrations. The resting membrane potential (RP) was not affected even at 10^{-5} M. These findings are more or less in agreement with those reported previously by Kohlhardt & Seifert (1980; 1983; 1985) in guinea-pig papillary muscles. Dukes & Vaughan Williams (1984) also reported similar $\dot{V}_{max}$ inhibition in rabbit ventricular and atrial muscles as well as in Purkinje fibres.

In mammalian ventricular muscle cells, most of the ionic current flowing across the cell membrane at the time of $\dot{V}_{max}$ is the sodium current; other membrane currents make no significant contributions (Carmeliet & Vereeck, 1979). The decrease in $\dot{V}_{max}$ by propafenone without any change in RP may, therefore, reflect an inhibitory effect of this drug on the fast sodium inward current (I_{Na}). In the following discussion, we used $\dot{V}_{max}$ as an approximate index of sodium channel availability to infer changes in the drug-blocked sodium channels. For quantitative measurements, however, it seems important to note that the probably convex-shaped non-linear relationship between $\dot{V}_{max}$ and the maximal limiting Na conductance (Bean *et al.*, 1982; Sheets *et al.*, 1988) might introduce a variable error on the precise amount of g_{Na} depression so that I_{Na} block will be increasingly overestimated at higher drug concentrations.

The present experiments using stimulation trains showed a marked use-dependent block of $\dot{V}_{max}$ by propafenone with little tonic block. Such characteristics are consistent with those observed in guinea-pig papillary muscles (Kohlhardt & Seifert, 1983; 1985). According to the 'modulated receptor hypothesis' proposed by Hondeghem & Katzung (1977, 1980) to explain the interaction between local anaesthetic type (Class I) antiarrhythmic drugs and cardiac sodium channels, the reduction of I_{Na} is due to accumulation of drug-associated nonconducting channels (blocked channels). If propafenone, like most Class I antiarrhythmic drugs (Grant *et al.*, 1984; Hondeghem & Katzung, 1984) has a higher affinity for the receptor of an activated and inactivated channel than for a resting channel, the accumulation of blocked channels during the stimulation train leading to a marked use-dependent inhibition of $\dot{V}_{max}$ would be expected.

The onset rate of the use-dependent block by propafenone in the present experiments ($0.33\,AP^{-1}$ at 1.0 Hz, 3×10^{-6} M propafenone) is comparable to that observed in guinea-pig papillary muscles (Kohlhardt & Seifert, 1985) and those for quinidine, disopyramide, which have been classified as intermediate kinetic Class I drugs by Campbell (1983).

Recently, we (Kodama *et al.*, 1987) have shown that Class I antiarrhythmic drugs currently available can be divided into two groups in terms of their sodium channel blocking phase during the conditioning clamp pulse to 0 mV; one 'transient' and one 'maintained' (Courtney, 1988). The former group of drugs (quinidine and disopyramide) may block the sodium channel mainly during its activated state corresponding to the upstroke phase of the action potential, while the latter group (lignocaine, mexiletine, tocainide and aprindine) may act predominantly during the inactivated state, corresponding to the plateau phase of the action potential. In the present experiments with propafenone we examined such a 'state-dependency' of sodium channel block by using the suction pipette whole-cell clamp technique. Protocols employed are similar to our previous experiments with single sucrose-gap voltage clamp techniques (Kodama *et al.*, 1987). In the presence of propafenone (3×10^{-6} M), a single conditioning clamp to 0 mV resulted in a substantial

transient decrease in $\dot{V}_{max}$ (6.6% at 10 ms pulse) followed by an additional maintained decrease in $\dot{V}_{max}$ with further prolongation of the clamp pulse duration. It was also shown that multiple brief clamp pulses caused much more remarkable decrease of $\dot{V}_{max}$ than single prolonged clamp pulse even when the total amount of time clamped to 0 mV level of the former one was much less than the latter one. These findings suggest that propafenone may block sodium channels by binding both activated and inactivated states. In other words this drug has intermediate binding characteristics between activated channel blockers and inactivated channel blockers.

The recovery process of $\dot{V}_{max}$ from the use-dependent block following multiple clamp pulses was expressed by a single exponential function with a time constant (τ_R) of 4.8 s. This value is similar to that reported by Kohlhardt & Seifert (1985) in guinea-pig papillary muscle preparations. It is therefore suggested that the propafenone molecule may dissociate from sodium channel receptors at resting or inactivated state with rates comparable to quinidine or disopyramide (Campbell, 1983).

The biphasic change of APD may reflect multiple modes of action of propafenone on membrane ionic currents. The shortening of APD by propafenone at $>10^{-6}$ M, which was accompanied by an attenuation of sarcomere shortening during twitch contraction, may probably be explained by a decrease of slow calcium inward current (I_{Ca}), because activation of I_{Ca} is of prime importance for the genesis of the plateau phase of the action potential as well as of twitch contraction in mammalian ventricular muscle cells. In voltage clamp experiments on cat papillary muscles, Kohlhardt (1977) demonstrated that propafenone (6.5×10^{-5} M) inhibited both I_{Na} and I_{Ca}. The inhibition of I_{Ca} by propafenone was also shown in rabbit sinus node cells (Satoh & Hashimoto, 1984). These reports seem to lend support to the above explanation. However, we cannot eliminate other possible mechanisms for the shortening of APD. For instance, propafenone might decrease the slowly inactivating sodium current during the plateau phase of the action potential (tetrodotoxin-sensitive sodium window current) (Clarkson *et al.*, 1984). APD prolongation by low concentrations of propafenone might be due to a decrease of the potassium outward current by this drug. Further experimental studies are required to determine these points and to obtain a complete profile of the drug action.

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