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Electrophysiological effects of OPC-88117, a new antiarrhythmic agent on papillary muscles and single ventricular myocytes isolated from guinea-pig hearts

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1 The effects of OPC-88117, a new antiarrhythmic agent, on transmembrane action potentials were examined in right ventricular papillary muscles and in single ventricular myocytes isolated from guinea-pig hearts.

2 In papillary muscles, OPC-88117 above 3×10^{-6} M caused a dose-dependent prolongation of action potential duration (APD).

3 OPC-88117 above 3×10^{-5} M caused a significant decrease in the maximum upstroke velocity ($\dot{V}_{max}$) of the action potential without affecting the resting membrane potential. The inhibition of $\dot{V}_{max}$ was enhanced at higher stimulation frequencies.

4 In the presence of OPC-88117, trains of stimuli at rates $\geqslant 1.0$ Hz led to a use-dependent inhibition of $\dot{V}_{max}$ with rapid onset. The time constant for the recovery of $\dot{V}_{max}$ from the use-dependent block was 456 ms.

5 The curves relating membrane potential and $\dot{V}_{max}$ were shifted by OPC-88117 to the direction of more negative potentials (9 mV at 10^{-4} M).

6 In single ventricular myocytes treated with OPC-88117 ($1–3 \times 10^{-4}$ M), the $\dot{V}_{max}$ of test action potentials preceded by conditioning clamp pulses to 0 mV was decreased progressively as the clamp pulse duration was prolonged.

7 These findings suggest that the primary electrophysiological effect of OPC-88117 on the cardiac muscle cell is prolongation of APD (Class III action) and that at high concentrations, it may also possess a lignocaine-like sodium channel inhibitory effect (Class I action).

Introduction

OPC-88117 (8-methyl-3-(4-methyl-1-piperazinyl)-2(1H)-quinolinone hydrochloride) is a newly synthesized antiarrhythmic compound (Figure 1). *In vivo* experiments in anaesthetized dogs have demonstrated that OPC-88117 given intravenously has a potent inhibitory action against ventricular arrhythmias induced by catecholamines or cardiac glycosides. The antiarrhythmic potency of this substance is comparable to or higher than lignocaine, procainamide, disopyramide or mexiletine (unpublished data). Preliminary experiments in guinea-pig isolated ventricular muscles have shown that OPC-88117 prolongs the action potential duration and suppresses the maximum upstroke velocity ($\dot{V}_{max}$) of the action potential without affecting the resting membrane potential. These findings seem to suggest that OPC-88117 is a unique antiarrhythmic drug having characteristics both of Class I and Class III drugs (Vaughan-Williams, 1970; 1984; Hauswirth & Singh, 1979). However the precise mode of action of OPC-88117 on cardiac muscle cells in relation to its antiarrhythmic activity remains to be elucidated.

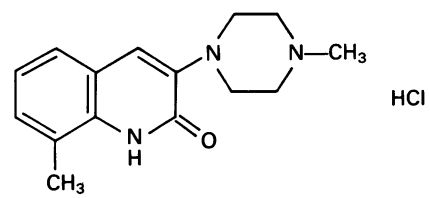

Figure 1 Chemical structure of OPC-88117.

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In the present study, the effects of OPC-88117 on the transmembrane action potential were investigated in right ventricular papillary muscles, as well as in single ventricular myocytes isolated from guina-pig hearts. The modulation of drug-induced $\dot{V}_{max}$ inhibition by stimulation frequencies or by membrane potential level was studied most extensively to compare the characteristics of its sodium channel blocking action with other Class I drugs.

Methods

Papillary muscles

Guinea-pigs of either sex weighing 200–250 g were killed by a blow on the head and the hearts were quickly removed. Papillary muscles 2 to 3 mm in length and 0.3 to 0.4 mm in diameter were dissected from the right ventricle. The preparation was mounted in the tissue bath and superfused continuously with Krebs-Ringer solution kept at 34°C and gassed with 95% O_2 and 5% CO_2. The composition of the solution was as follows (in mM): NaCl 120.3, KCl 4.0, $CaCl_2$ 1.2, $MgSO_4$ 1.3, $NaHCO_3$ 25.2 and glucose 5.5 (pH 7.4). Equipment for stimulation and for recording transmembrane action potentials was the same as described by us previously (Kodama *et al.*, 1985; Toyama *et al.*, 1987).

To study the use-dependent effects of OPC-88117 on the maximum upstroke velocity ($\dot{V}_{max}$) of action potentials, the preparation was stimulated repetitively at varying rates ranging from 0.2 to 2.5 Hz. Rest periods of 30 s, which were sufficient to ensure full recovery from the rate-dependent decrease in $\dot{V}_{max}$ were interposed between the trains of stimuli. This experimental protocol is able to detect the existence of two types of $\dot{V}_{max}$ inhibition by OPC-88117, tonic and use-dependent block. The former is defined by the decrease of $\dot{V}_{max}$ of the first action potential preceded by the rest period, and the latter is the decrease of $\dot{V}_{max}$ during the train (from the value of first action potential to the new steady-state level).

The recovery of $\dot{V}_{max}$ from the use-dependent block was studied by applying a single test stimulus at various coupling intervals following a stimulation train for 20 s at 1.0 Hz. The intensity of the test stimuli was adjusted to obtain a constant latency from the stimulus artifact to the initiation of the action potential upstroke.

To determine the relationship between membrane potential and $\dot{V}_{max}$, the papillary muscle was stimulated at an interstimulus interval of 30 s and the resting potential was made less negative by adding KCl to the medium in 1–2 mM steps up to a final K^+ concentration of 20 mM. After an equilibration period of 7 to 8 min for each concentration of K^+, an action potential was recorded to measure the $\dot{V}_{max}$.

Single ventricular myocytes

Single ventricular myocytes were isolated enzymatically by a procedure similar to that described by Watanabe *et al.* (1985). In brief, hearts were quickly removed from guinea-pigs and perfused on a Langendorff apparatus with the following solutions in sequence: (1) Ca^{2+}-free Krebs solution for 5 min, (2) enzyme solution containing 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 single myocytes. A few drops of cell suspension were placed in a recording chamber attached to an inverted microscope. The chamber was perfused at a rate of 2 ml min^{-1} with normal Krebs solution of the following composition (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; pH was adjusted to 7.4 by adding NaOH, and the solution was equilibrated by 100% O_2. The temperature was maintained at 35°C.

Following the increase in calcium concentration of the medium to 1.8 mM (normal Krebs solution), 30 to 40% of myocytes deteriorated into round-shaped cells due to irreversible contracture. The remaining cells were tolerant to calcium, their intact rod-shape was maintained without spontaneous beating, and the experiments were carried out with these myocytes.

Single suction pipette electrodes were used for membrane potential recording as well as for whole-cell voltage clamp. The pipettes were fire polished to a tip diameter of 1.5 μm and filled with internal solution to give a resistance ranging from 2 to 3 MΩ. The pipette solution consisted of (mM): KCl 120.0, NaH_2PO_4 10.0, EGTA 1.0, MgATP 5.0 and HEPES 10.0; pH was adjusted to 7.2 by adding KOH. Action potentials were recorded by the current-clamp mode by passing a short stimulus current (less than 4 ms in duration) through the electrodes. 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 protocols are given under Results.

Drugs and data analysis

OPC-88117 (Otsuka Pharmaceutical Co. Ltd., Osaka, Japan) was dissolved in deionized water and diluted with superfusate (Krebs solutions) to achieve the final concentration required.

Values are presented as the means ± s.e. unless otherwise stated. Data were analysed by t test, analysis of variance, Dunnett's test, and regression analysis. Differences were considered significant at $P < 0.05$.

Results

Action potentials of papillary muscle

Effects of OPC-88117 (3×10^{-6}–3×10^{-4} M) on the membrane action potential configuration were examined in eight papillary muscles constantly stimulated at 1.0 Hz (Figure 2, Table 1). Following exposure to OPC-88117 at 3×10^{-6} M for 30 min, no significant changes were observed. OPC-88117 at 10^{-5} M caused a significant prolongation of action potential duration at 80% repolarization (APD_{80}), while other parameters were still unaffected. At 3×10^{-5} M, APD_{80} was prolonged further, and a prolongation of action potential duration at 30% repolarization (APD_{30}) was also observed. In addition, the maximum upstroke velocity ($\dot{V}_{max}$) was decreased significantly. At the higher concentration, the prolongation of APD_{80} and decrease in $\dot{V}_{max}$ were enhanced in a dose-dependent manner, whereas APD_{30} was shortened to a level below control. The resting membrane potential (RP) was unchanged even at 3×10^{-4} M OPC-88117. IC_{20} of $\dot{V}_{max}$ inhibition by OPC-88117, which was obtained by interpolation in a graph of log molar drug concentrations versus responses, was 9.4×10^{-5} M.

Effects of OPC-88117 on the contour of membrane action potentials were also examined at a low (0.5 Hz) and a high (2.0 Hz) stimulation frequency and compared with those at 1.0 Hz. The results obtained are summarized in Figure 3. The prolongation of APD_{80} by OPC-88117 was similar in its extent at the three different stimulation frequencies. In contrast, the $\dot{V}_{max}$ inhibition was largely influenced by the stimulation frequency. Thus, at 0.5 Hz OPC-88117 caused no significant decrease in $\dot{V}_{max}$ even at 10^{-4} M, whereas at 2.0 Hz the OPC-88117-induced reduction of $\dot{V}_{max}$ was much more marked than that observed at 1.0 Hz.

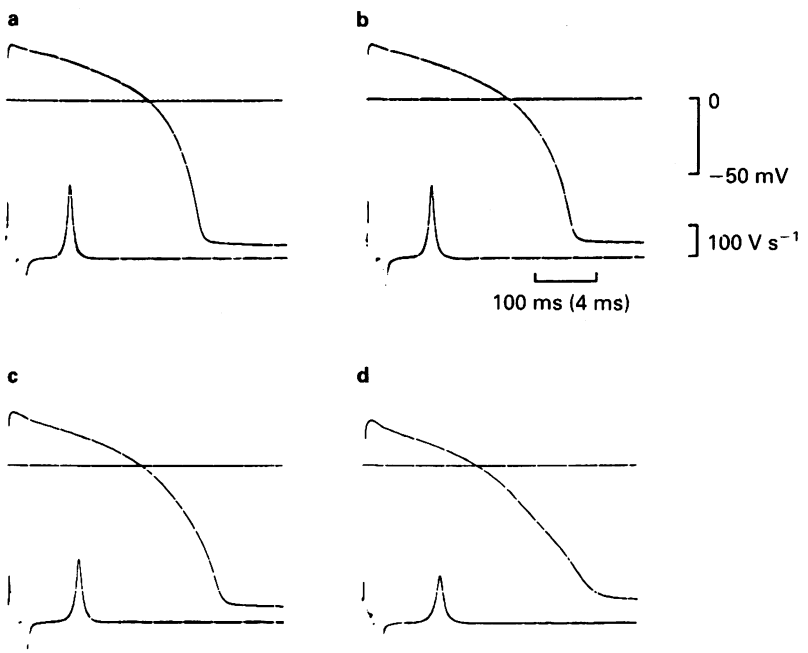

Figure 2 Effects of OPC-88117 on transmembrane action potentials of papillary muscles. (a), Control; (b) OPC-88117 (3×10^{-5} M); (c) OPC-88117 (10^{-4} M); (d) OPC-88117 (3×10^{-4} M). Upper trace is membrane action potential and lower trace is the differentiated upstroke spike of the action potential ($\dot{V}_{max}$). $\dot{V}_{max}$ was recorded at a faster sweep velocity. The preparation was constantly stimulated at 1.0 Hz. Membrane potential was recorded just before and 30 min after the drug application at each concentration.

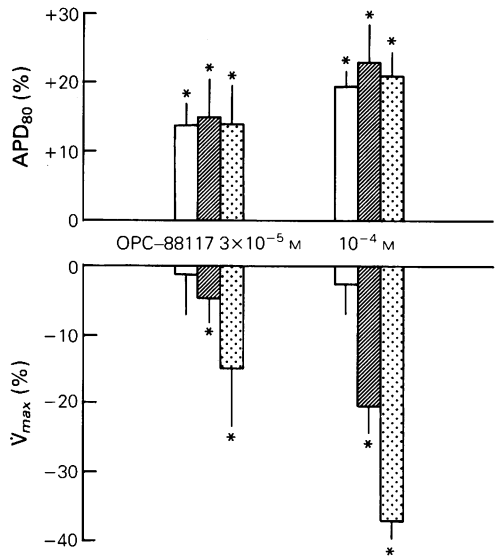

Figure 3 Influence of stimulation frequency on the effects of OPC-88117 in papillary muscles. Effects of OPC-88117 on the action potential duration at 80% repolarization (APD_{80}) and on the maximum upstroke velocity ($\dot{V}_{max}$) at three different stimulation frequencies (0.5 Hz (open columns), 1.0 Hz (hatched columns) and 2.0 Hz (stippled columns)) were compared. Values are presented as % change from control (means ± s.e., $n = 5$). *Significantly different from control at $P < 0.05$.

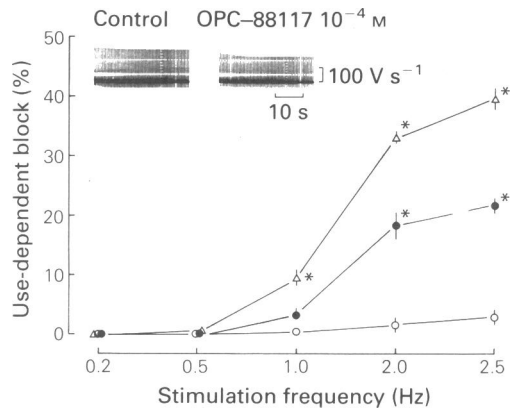

Figure 4 Use-dependent block of $\dot{V}_{max}$ by OPC-88117 in papillary muscles. The traces show differentiated upstroke spikes of action potential during stimulation trains at 2.0 Hz in previously quiescent tissue. The records were obtained before and 30 min after application of OPC-88117 10^{-4} M.

The graph shows the relationship between stimulation frequency and the intensity of the use-dependent block. Ordinate scale: percentage decrease of $\dot{V}_{max}$ from the first action potential of stimulation trains to the new steady state level. Data were obtained before (○), and 30 min after application of OPC-88117 at 3×10^{-5} M (●) and at 10^{-4} M (△). Values are presented as means of five preparations; vertical lines show s.e.mean. *The change was statistically significant at $P < 0.05$.

Use-dependent effects on $\dot{V}_{max}$

The frequency-dependent inhibition of $\dot{V}_{max}$ by OPC-88117 was tested more extensively by trains of stimulation at different frequencies, which were separated from each other by a rest period of 30 s. In untreated papillary muscles, the value of $\dot{V}_{max}$ was almost unchanged at rates of stimulation trains from 0.2 to 2.5 Hz. After the treatment with OPC-88117 for 30 min, $\dot{V}_{max}$ of the first action potential in each train was slightly decreased; there was a tonic block of $\dot{V}_{max}$ by 3.0 ± 0.8% at 3×10^{-5} M ($n = 5$) and 5.1 ± 1.3% at 10^{-4} M ($n = 5$). During stimulation by trains at rates ⩾1.0 Hz, a rapid decrease in $\dot{V}_{max}$ approaching a new steady level within the 10th to 15th beats (use-dependent block) was observed. The use-dependent block of $\dot{V}_{max}$ by OPC-88117 was greater at higher stimulation frequencies and at higher drug concentrations (Figure 4).

The recovery of $\dot{V}_{max}$ from the use-dependent block was studied by adding a single test stimulus at various coupling intervals following a stimulation train for 20 s at 1.0 Hz. Before the application of OPC-88117, $\dot{V}_{max}$ of the test action potential recovered almost completely within 100 ms of the diastolic interval (the interval from the end of the last action potential to the beginning of the test action potential). After treatment with OPC-88117 (10^{-4} M), much slower $\dot{V}_{max}$ recovery was observed. Representative results are shown in Figure 5, where fractional reduction of $\dot{V}_{max}$ of test action potentials was plotted against the diastolic interval in a semilogarithmic graph. In the presence of OPC-88117, the recovery time course of $\dot{V}_{max}$ with a diastolic interval longer than 100 ms was approximated by a single exponential function. The average time constant was calculated to be 456 ± 28 ms ($n = 5$).

Voltage-dependent effects on $\dot{V}_{max}$

The effects of OPC-88117 on the relationship between $\dot{V}_{max}$ and the resting membrane potential from which the action potential originated were examined in five papillary muscles, driven at an interstimulus interval of 30 s. The slow rate of stimulation was used to eliminate the use-dependent

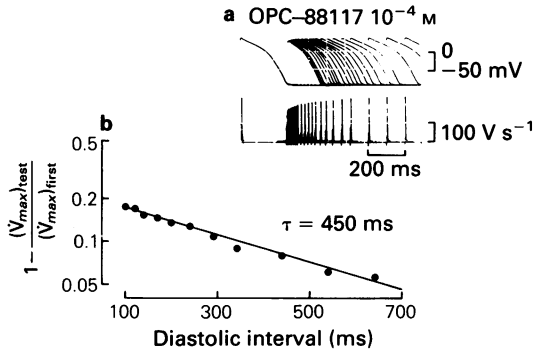

Figure 5 Offset of the use-dependent block of $\dot{V}_{max}$ by OPC-88117 in papillary muscle. (a) Superimposed records of action potentials (upper trace) and their differentiated upstroke spikes (lower trace) 30 min after application of OPC-88117 (10^{-4} M). The preparation was stimulated at 1.0 Hz for 20 s after a rest period of 30 s. After cessation of the stimulation train, a single test stimulus was applied with various coupling intervals. (b) The recovery process of $\dot{V}_{max}$ of test action potential. Ordinate scale: fractional $\dot{V}_{max}$ reduction of test action potential as compared with $\dot{V}_{max}$ of the first action potential. Abscissa scale: diastolic interval, which was measured from the end (at 95% repolarization) of the last action potential induced by the stimulation train to the upstroke of the test action potential. The time course was approximated by single exponential function with a time constant (τ) of 450 ms.

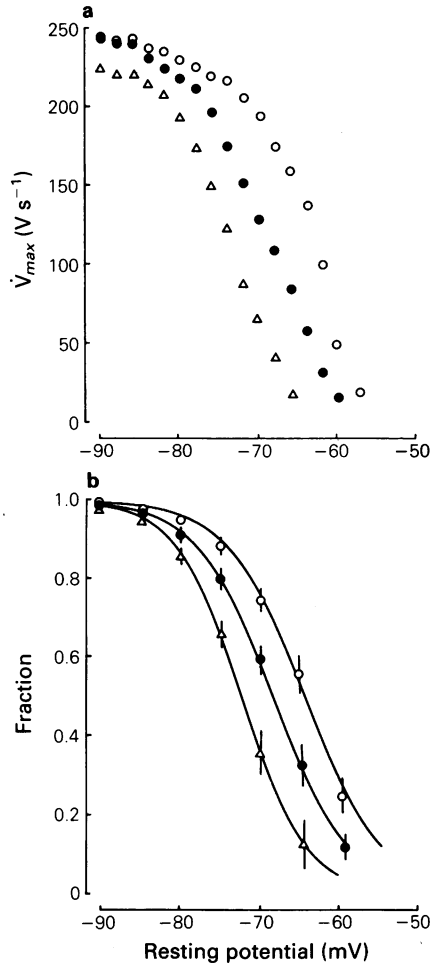

Figure 6 Effects of OPC-88117 on the relationship between resting membrane potential and $\dot{V}_{max}$ in papillary muscles driven with an interstimulus interval of 30 s. Values were obtained before (○) and 30 to 40 min after application of OPC-88117 at 3×10^{-5} M (●) and at 10^{-4} M (△). Absolute values in one experiment are shown in (a). Means of the normalized values ($n = 5$) are illustrated in (b); vertical lines indicate s.e.mean.

depression of $\dot{V}_{max}$ by OPC-88117. The membrane potential was depolarized in steps from the original resting level at 4 mM $[K^+]_0$ to about −60 mV, by increasing the K^+ concentration in the medium. The decrease of $\dot{V}_{max}$ by OPC-88117 (3×10^{-5} M, 10^{-4} M) was more pronounced at less negative membrane potentials (Figure 6a). A fraction of $\dot{V}_{max}$ was calculated in each experiment by normalizing the data with the value at 4 mM $[K^+]_0$, and means ± s.e. were obtained. As shown in Figure 6b, at a level of 50% reduction of $\dot{V}_{max}$, the average curve obtained following the drug application was shifted by 5 mV, at 3×10^{-5} M and by 9 mV, at 10^{-4} M OPC-88117, along the voltage axis in the direction of hyperpolarization.

$\dot{V}_{max}$ of single ventricular myocytes

In single ventricular myocytes, the effects of conditioning clamp pulses on $\dot{V}_{max}$ of subsequent test action potentials were examined, in order to determine whether the use-dependence of the inhibition of $\dot{V}_{max}$ by OPC-88117 is due to the blockade of an activated or an inactivated sodium channel.

Figure 7 shows the results of experiments with a single clamp pulse to 0 mV. Following a rest period of 30 s, the membrane potential was clamped up 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, the membrane potential was clamped back to the holding voltage for 100 ms, which is long enough for a drug-free channel to reactivate fully (Carmeliet & Vereecke, 1979; Ebihara &

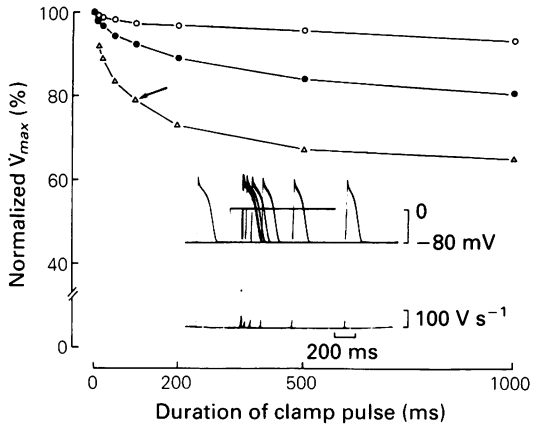

Figure 7 Effect of 0 mV conditioning clamp pulse on the inhibition of $\dot{V}_{max}$ induced by OPC-88117 in a single ventricular myocyte. Ordinate scale: $\dot{V}_{max}$ of test action potential normalized by the value of action potential without clamp pulse (reference level) in each condition. Data were obtained before (○) and 10 min after addition of OPC-88117 at 10^{-4} M (●) or at 3×10^{-4} M (△). An arrow indicates time constant (105 ms) for the exponential decay of $\dot{V}_{max}$. Inset shows superimposed records obtained 10 min after addition of OPC-88117 at 3×10^{-4} M. Action potential at extreme left was elicited without clamp pulse as a reference. Right side superimposed action potentials were preceded by 0 mV clamp pulse of 10, 20, 50, 100, 200, 500 and 1000 ms in duration with a coupling interval of 100 ms.

Johnson, 1980), but short enough so that only partial dissociation of the drug from the 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 $\dot{V}_{max}$. A clamp pulse of 1000 ms in duration decreased $\dot{V}_{max}$ by 6.1 ± 1.2% ($n = 5$) from the value of action potentials without conditioning clamp pulse (reference level). Treatment of the myocyte with OPC-88117 at 10^{-4} M or 3×10^{-4} M for 10 min did not affect the resting potential (RP). $\dot{V}_{max}$ of reference action potential was slightly (3–8%) decreased compared with the value before drug application. In such myocytes, a conditioning clamp pulse caused a progressive decrease in $\dot{V}_{max}$ of the test action potential as the clamp pulse duration was prolonged (Figure 7). At 10^{-4} M OPC-88117, the decrease in $\dot{V}_{max}$ from the reference value was significant when the clamp pulse duration was longer than 50 ms, and reached 20.1 ± 0.8% ($n = 5$) at 1000 ms. At 3×10^{-4} M OPC-88117, similar clamp pulses caused a greater decrease in $\dot{V}_{max}$. Thus, a slight but significant decrease of $\dot{V}_{max}$ (5.8 ± 1.1%, $n = 5$) from the reference value was observed even at a clamp pulse of 10 ms, and it reached an almost steady level (34 ± 2.9%, $n = 5$) at 500 ms. At this concentration, the change in $\dot{V}_{max}$ with prolongation of clamp pulse duration in each experiment was well fitted to a single exponential function. The average time constant was 106 ± 8 ms ($n = 5$).

The recovery process of $\dot{V}_{max}$ following a conditioning clamp pulse to 0 mV for 1000 ms was examined, by introducing a test action potential with various coupling intervals during which the membrane potential was clamped back at the resting level (−82 mV). Before the application of OPC-88117, $\dot{V}_{max}$ of test action potentials recovered rapidly and followed a single exponential function with a time constant of 31 ± 2 ms ($n = 5$). In the presence of OPC-88117 (10^{-4} M, 3×10^{-4} M), the recovery process of $\dot{V}_{max}$ was approximated by a dual exponential function (Figure 8). The time constant of the early fast component (τ), and the late slow component (τ_2) were calculated by using the peeling-off method (Riggs, 1963). Values of τ_1 at 10^{-4} M and 3×10^{-4} M OPC-88117 were 29 ± 2 ms ($n = 5$) and 30 ± 4 ms ($n = 4$), respectively. These values were similar to the recovery time constant of $\dot{V}_{max}$ before the drug application. On the other hand, τ_2 values at 10^{-4} M and 3×10^{-4} M OPC-88117 were 466 ± 11 ms ($n = 5$) and 461 ± 13 ms ($n = 4$), respectively. There was no statistical difference between the two values.

Discussion

The present experiments in guinea-pig papillary muscles have revealed that OPC-88117 above 3×10^{-6} M causes a dose-dependent prolongation of action potential duration (APD) at the late repolarization phase. At concentrations above 3×10^{-5} M, OPC-88117 also caused a significant decreased in the maximum upstroke velocity ($\dot{V}_{max}$) of action potential without affecting the resting membrane potential (RP). The prolongation of APD was not affected by stimulation frequencies ranging from 0.5 to 2.0 Hz, whereas the inhibition of $\dot{V}_{max}$ was enhanced at higher frequencies.

The prolongation of APD by OPC-88117 can be attributed to either an increase of inward currents or a decrease of outward currents during the repolarization phase (Carmeliet & Vereecke, 1979). The contribution of the slow calcium inward current (I_{Ca}) or

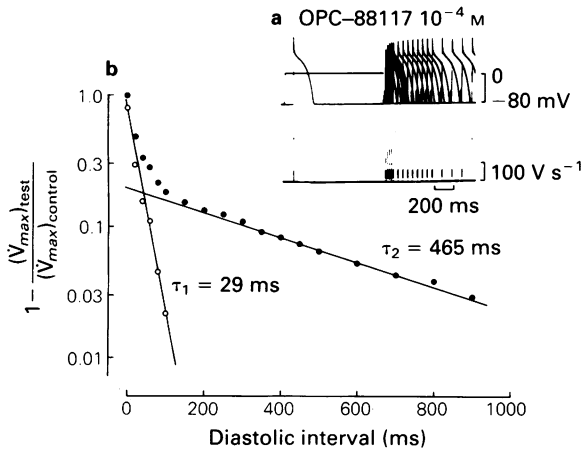

Figure 8 Recovery of $\dot{V}_{max}$ in single ventricular myocyte treated with OPC-88117. (a) Superimposed records of action potentials (upper trace) elicited after a conditioning clamp pulse of 1000 ms in duration and their differentiated upstroke spikes (lower trace) 10 min after application of OPC-88117 10^{-4} M. The coupling interval between the clamp pulse and the test action potential, during which the cell was clamped to the resting potential level (−82 mV), was varied, and each clamp pulse was preceded by a 30 s rest period. (b) Recovery process of $\dot{V}_{max}$ of test action potential. Ordinate scale, fractional $\dot{V}_{max}$ reduction of test action potential without conditioning clamp pulse (reference level). Abscissa scale, coupling interval between the clamp pulse and the test action potential. The time course was approximated by a dual exponential function with τ_1 of 29 ms and τ_2 of 465 ms.

the fast sodium inward current (I_{Na}) seems unlikely for the following reasons. An enhancement of I_{Ca} would be reflected in a prolongation of APD at the early repolarization phase (Carmeliet & Vereecke, 1979). However, the present data showed that APD_{30} was shortened by OPC-88117 at concentrations above 10^{-4} M. OPC-88117-induced prolongation of APD_{80} was preserved even when the $\dot{V}_{max}$ of the action potential, which reflects the sodium channel availability (Gintant *et al.*, 1983; Grant *et al.*, 1984), was markedly inhibited by this substance. The prolongation of APD could, therefore, be ascribed to other ionic mechanisms including a decrease of time-dependent or time-independent K^+ outward currents. Nevertheless, further experimental studies using the voltage-clamp technique are required to clarify this point.

The inhibition of $\dot{V}_{max}$ by OPC-88117 was enhanced by the higher stimulation frequency. The frequency-dependence or use-dependence of this effect can be interpreted within the framework of 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. According to this hypothesis, the reduction of I_{Na} is due to the accumulation of drug-associated nonconducting channels (blocked channels). If OPC-88117, like most of Class I antiarrhythmic drugs (Grant *et al.*, 1984; Hondeghem & Katzung, 1984), has a higher affinity for the receptor of an activated or inactivated channel than for that of a resting channel, an accumulation of blocked channels during the stimulation train leading to a use-dependent inhibition of $\dot{V}_{max}$ would be expected. The small tonic block of $\dot{V}_{max}$ by OPC-88117 in normally polarized papillary muscles is consistent with such an assumption.

The use-dependent block of $\dot{V}_{max}$ by OPC-88117 was observed during stimulation trains at rates $\geqslant$1.0 Hz, and its onset kinetics were very rapid. These characteristics are similar to those described for fast kinetic Class I drugs such as lignocaine, mexiletine and tocainide (Campbell, 1980; 1983a,b). OPC-88117 caused a slow phase of $\dot{V}_{max}$ recovery following stimulation trains, reflecting the dissociation of the drug from the inactivated or the resting sodium channels. The recovery time constant (456 ms) was comparable to those obtained for lignocaine, mexiletine and tocainide (200–500 ms) (Campbell 1980; 1983a). These observations may suggest that binding and unbinding kinetics of OPC-88117 molecules to the cardiac sodium channel receptors are similar to those of fast-kinetic Class I drugs.

The relationship of $\dot{V}_{max}$ to membrane potential was investigated in papillary muscles stimulated with an interstimulus interval of 30 s. Under such experimental conditions, a decrease in $\dot{V}_{max}$ may reflect only the tonic block by OPC-88117, while the use-dependent block may be negligible. The present results have revealed that the decrease in $\dot{V}_{max}$ as a result of tonic block by OPC-88117 is more pronounced at less negative membrane potentials. This effect could be due to a high affinity of this drug for inactivated sodium channels (Grant *et al.*, 1984).

Recently, we have shown that Class I antiarrhythmic drugs can be subdivided into two groups in terms of their sodium channel blocking phase during the conditioning clamp pulse to 0 mV; one 'transient' and one maintained (Kodama *et al.*, 1987a; 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 do so predominantly during the inactivated state which corresponds to

the plateau phase of the action potential. In the present experiments with OPC-88117, we tested such a 'state-dependency' of sodium channel block by using a suction pipette whole-cell clamp technique in single ventricular myocytes. The protocols employed were similar to those in our previous experiments using single sucrose-gap voltage clamp technique (Kodama *et al.*, 1987a,b). In the presence of OPC-88117 (10^{-4} M, 3×10^{-4} M), the $\dot{V}_{max}$ of the test action potential, which was elicited 100 ms after the termination of 0 mV clamp, decreased progressively as the preceding clamp pulse duration was prolonged. At 3×10^{-4} M OPC-88117, the decay of $\dot{V}_{max}$ was well fitted to a single exponential function with a time constant of 106 ms. These findings may indicate that OPC-88117 blocks the sodium channel primarily when it is in the inactivated state. Time constants for the onset of the inactivated channel block by OPC-88117 are comparable to those for lignocaine and mexiletine but appreciably shorter than aprindine (Kodama *et al.*, 1987a,b).

In myocytes treated with OPC-88117 (10^{-4} M, 3×10^{-4} M), the recovery of $\dot{V}_{max}$ of a test action potential, following a 1000 ms conditioning clamp to 0 mV, was expressed by a dual exponential function. The time constant for the early fast component (τ_1) was similar to that for $\dot{V}_{max}$ recovery in untreated control myocytes. It may, therefore, reflect the repriming process (recovery from inactivation) of drug-free (unblocked) sodium channels. The time constant for the late slow component (τ_2) was concordant with the recovery time constant of $\dot{V}_{max}$ from the use-dependent block by OPC-88117 in papillary muscles. Accordingly, it may reflect the dissociation of the drug molecules (unbinding) from the sodium channels.

The *in vitro* concentrations of OPC-88117 used in the present study to prolong APD_{80} of guinea-pig papillary muscles were well within the effective i.v. doses used in dog experiments (unpublished data), whereas those concentrations needed to inhibit $\dot{V}_{max}$ were somewhat higher. Accordingly, it seems reasonable to conclude that the primary electrophysiological effect of OPC-88117 on cardiac muscle is prolongation of APD (Class III action) and that, at a higher concentration, it may also possess an inhibitory effect on the fast sodium channels (Class I action) which is similar to lignocaine and mexiletine in terms of use- and channel state-dependence.

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