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The Modulated Receptor Hypothesis Revisited from the Viewpoint of Myocardial Interstitial Potential

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Time- and voltage-dependent interaction of antiarrhythmic agents with target cardiac ion channels is termed the modulated receptor hypothesis. Actually class I agents suppress the maximum upstroke rate ($\dot{V}_{max}$) of intracellular potential (V_{ic}) depending on the pacing cycle length (PCL) and external potassium concentration ($[K^+]_e$). We examined this concept from the aspect of interstitial potential (V_{is}), since V_{is} reflects the second time derivative of V_{ic}. V_{ic} and V_{is} were recorded sequentially using standard microelectrode applied to the paced and superfused guinea pig papillary muscles. In the steady state, the greatest negative deflection of V_{is} ($\dot{V}_{min}$) was suppressed by quinidine (10 μM) in both PCL- and $[K^+]_e$-dependent manner, just like $\dot{V}_{max}$. However, quinidine-induced greater inhibition of $\dot{V}_{min}$ than $\dot{V}_{max}$ was evident at shorter PCL and greater $[K^+]_e$. Based on the sequential alteration of PCL and exposure to ouabain (10 μM), different quinidine sensitivity between $\dot{V}_{max}$ and $\dot{V}_{min}$ is most likely accounted for by the activity-dependent K^+ efflux and Na^+-K^+ pump-mediated K^+ uptake (i. e., $[K^+]_e$ fluctuation). Thus, the modulated receptor hypothesis is concluded to be valid in terms of V_{is}.
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Key words : Intracellular potential, Interstitial potential, Modulated receptor hypothesis

The time- and voltage-dependent inhibitory effects of antiarrhythmic agents on the target cardiac ion channels are explained by the modulated receptor hypothesis (Hondeghem & Katzung, 1977, 1984). This hypothesis is important in basic research and clinical medicine because it provides information about the drug-channel interaction and prognostic implications of the antiarrhythmic drug treatment. Class I antiarrhythmic agents of Vaughan Williams classification suppress the maximum upstroke rate ($\dot{V}_{max}$) of the myocardial intracellular potential (V_{ic}), which is indicative of the target sodium (Na) channel availability, depending on the pacing cycle length (PCL) and the external potassium concentration ($[K^+]_e$). Short PCL and elevated $[K^+]_e$ cause a greater suppression of $\dot{V}_{max}$ and Na current induced by the numerous class I agents, such as quinidine (Chen & Gettes, 1976), disopyramide (Campbell, 1983) [class Ia], lidocaine (Chen & Gettes, 1976 ; Gilliam, Starmer & Grant, 1989), mexiletine (Campbell, 1983) [class Ib], flecainide (Campbell & Vaughan Williams, 1983) and encainide (Campbell, 1983) [class Ic]. This implies that these agents block Na channels preferentially in the activated or inactivated state rather than the closed state.

Class I antiarrhythmic agents suppress the electrical propagation as well as $\dot{V}_{max}$ and Na current (Cascio *et al.* 1987 ; Davis *et al.* 1986 ; Gang *et al.* 1985). This finding is not surprising, since $\dot{V}_{max}$ is a major determinant of conduction velocity (Fozzard, 1990). In the activation wavefront, closed electrical circuit is theoretically completed by the transmembrane inward ionic current, outward capacity cur-

rent, and cytoplasmic and interstitial currents (local circuit theory). These currents flow along this circuit in such a way that the electrical charge is assumed to be neither accumulated nor depleted at any given point of the closed circuit (charge conservation theory). If these two theories are allowed to be applied to the actual conduction, class I agents are thought to affect the local circuit current *per se* during conduction. Although the modulated receptor hypothesis has already been confirmed for $\dot{V}_{max}$ (Campbell, 1983; Campbell & Vaughn Williams, 1983; Chen & Gettes, 1976) and Na current (Gilliam *et al.* 1989), little is known about the validity of this hypothesis in terms of the local circuit current itself.

The densely packed myocardium has restricted and complex interstitium (Frank & Langer, 1974; Kline, 1990; Polimeni, 1974). This myocardial interstitial space with relatively high impedance is reported to show a biphasic interstitial potential (V_{is}) during conduction (Spach *et al.* 1972). V_{is} is roughly simulated by the second time derivative of V_{ic} (d^2V_{ic}/dt^2) at any given point along a one-dimensional tissue (Geselowitz *et al.* 1982; Plonsey & Bar, 1987; Spach *et al.* 1972). For this reason, biphasic V_{is} is assumed to be influenced by the upstroke configuration of V_{ic}. We hypothesized that the dependence of V_{is} on V_{ic} would result in a PCL- and $[K^+]_e$-dependent suppression of V_{is}, as well as that of V_{ic}, by class I antiarrhythmic agents. Thus our objective in this article is to reconfirm the modulated receptor hypothesis mainly from the viewpoint of V_{is}, using a standard microelectrode applied to the interstitium and myocytes of the superfused guinea pig papillary muscles.

MATERIALS and METHODS

Guinea pigs weighing 300 to 400 g were studied. All of them were taken care of according to the Guiding Principles for the Care and Use of Animals approved by the Council of the Physiological Society of Japan. They were anesthetized with an intraperitoneal injection of sodium pentobarbital (20 to 30 mg). The hearts were rapidly removed following thoracotomy, and the papillary muscles were excised from the right ventricles. Twenty right ventricular papillary muscles were used. They were gently stretched by about 10 % of their slack length, mounted in a tissue bath, and superfused with Tyrode's solution containing (in mM) 125 NaCl, 4.5 KCl, 1.8 $CaCl_2$, 1.05 $MgCl_2$, 24 $NaHCO_3$, 0.42 NaH_2PO_4, and 5.0 glucose. Solutions with elevated $[K^+]_e$ (up to 15.0 mM) were prepared by adding KCl to the control Tyrode's solution. Solutions were gassed with 95 % O_2 and 5 % CO_2 to adjust the pH to 7.4, then warmed to 36 ℃. Ouabain and quinidine (Sigma Co. Ltd., St Louis, MO) were dissolved in distilled water and then diluted by the Tyrode's solution.

Preparations were approximately 5 to 8 mm in length and 3 to 4 mm in diameter under the binocular measurement. They were stimulated with various PCL by rectangular pulses with 1.5 msec duration and twice the end diastolic threshold strength using bipolar extracellular electrodes, which were positioned at the cut end and isolated except at the tips. A microelectrode filled with 3M KCl having a tip resistance of 15 to 25 MΩ was positioned on the preparation about 2 to 3 mm away from the stimulation site. A reference Ag-AgCl electrode was immersed in the Tyrode's solution. The DC offset of the high input impedance amplifier (MEZ-7101, Nihon-Kohden, Tokyo, Japan) was adjusted to cancel the offset potential at the microelectrode tip immersed in the Tyrode's solution before penetrating the muscle. The capacitative compensation of that amplifier was adjusted before the penetration to produce a clear right angle voltage configuration by a 10 nA square pulse passed through the microelectrode tip immersed in the Tyrode's solution. The microelectrode was then lowered vertically until the acceptable V_{ic} was recorded. The microelectrode was then pulled vertically until the stable V_{is} was recorded. An acceptable V_{is} was characterized by the same membrane potential levels (within ±1.0 mV) before and after the biphasic

configuration, as described previously (Knisley, Maruyama & Buchanan, 1991) [Fig. 1C]. This maneuver yielded a sequential, although not simultaneous, recording of V_{ic} and V_{is} in the most proximity. Once the position of the microelectrode tip was determined, it was not changed during the experiment except for the transition from the V_{ic} to the V_{is} recording. The unstable penetration to either myocytes or interstitium was not accepted.

Potentials were passed through the amplifier and displayed on a dual beam memory oscilloscope (VC-10, Nihon-Kohden, Tokyo, Japan). Signals were stored on digital audio tapes using a data recorder (RD-101T PCM, TEAC, Tokyo, Japan) and analyzed subsequently by computer (PC-9801, NEC, Tokyo, Japan) using a commercial analysis software (DSS 98-SV, Canops, Kobe, Japan) with time- and voltage-resolutions of 0.02 msec and 0.02 mV, respectively. This program allows to retrieve the voltage signal and its first time derivative simultaneously. So the intracellular recording yields V_{ic} and $\dot{V}_{max}$ automatically, whereas interstitial recording yields V_{is} and the greatest negative deflection of the differentiated V_{is} ($\dot{V}_{min}$). Actual data were plotted with an X-Y plotter (MP4300, Graphtec, Tokyo, Japan). Experimental protocols were designed to assess the followings: 1) the effects of alterations of $[K^+]_e$, 2) the effects of various PCL, and 3) the effects of quinidine or ouabain on these potentials.

Data are expressed as mean±S. D. Comparisons between groups were conducted using a paired or non-paired Student's *t*-test with Bonferroni's correction, as appropriate (Wallenstein, Zucker & Fleiss, 1980). In the case of non-linear fitting to the Boltzman's equation, least square method was used and the coefficient of correlation (r) was calculated (Motulsky & Ransnas, 1987). In both cases, a level of $p<0.05$ was accepted as statistically significant.

RESULTS

Experiments on alteration of $[K^+]_e$ and PCL

The sequential V_{ic} and V_{is} recordings were conducted in a series of five experiments. Resting membrane potential (RMP) and $\dot{V}_{max}$ were measured routinely in the steady state V_{ic} recording. The transition from V_{ic} to V_{is} during potential recording was abrupt (Fig. 1A). V_{is} showed a biphasic deflection and the differentiated V_{is} (dV_{is}/dt) showed an intrinsic negative deflection in all the preparations examined, corresponding to the upstroke of V_{ic} (Fig. 1B). Therefore, the electrophysiological parameters of V_{is} indicated in Fig. 1C were routinely measured. The resting V_{is} remained constant at $+3.9\pm0.8$ mV ($n=5$) positive to the conducting medium (Fig. 1C). The resting V_{is} was not significantly influenced by the sequential alterations of $[K^+]_e$ and PCL.

As shown in Fig. 2, the graded elevation of $[K^+]_e$ from 3.0 to 15.0 mM was undertaken with a fixed PCL (either 3.0 or 0.5 sec). During the steady state of the V_{is} recording, peak-to-peak amplitude of V_{is} as well as $\dot{V}_{min}$ decreased gradually as $[K^+]_e$ increased. Although the $[K^+]_e$-dependent alterations in V_{is} and $\dot{V}_{min}$ were observed with PCL of both 3.0 and 0.5 sec, the magnitudes of V_{is} and $\dot{V}_{min}$ were smaller with a PCL of 0.5 sec than that of 3.0 sec at each $[K^+]_e$.

The relationship between $\dot{V}_{min}$ and peak-to-peak amplitude of V_{is} was assessed at various $[K^+]_e$ and PCL. $\dot{V}_{min}$ was plotted as a function of peak-to-peak amplitude of V_{is} in two preparations as shown in Fig. 3. With either increase in $[K^+]_e$ or a decrease in PCL, $\dot{V}_{min}$ and the peak-to-peak amplitude of V_{is} were simultaneously dissipated. As a result, a significant positive correlation between these two parameters was maintained. The other three preparations showed a similar linearity. Based on the proportional relationship between these two parameters, $\dot{V}_{min}$ was used as a representative measure and was compared with $\dot{V}_{max}$.

Experiments with quinidine

Ten experiments were conducted using an independent series of preparations subjected to quinidine (10 μM) at different $[K^+]_e$ and PCL. Fig.

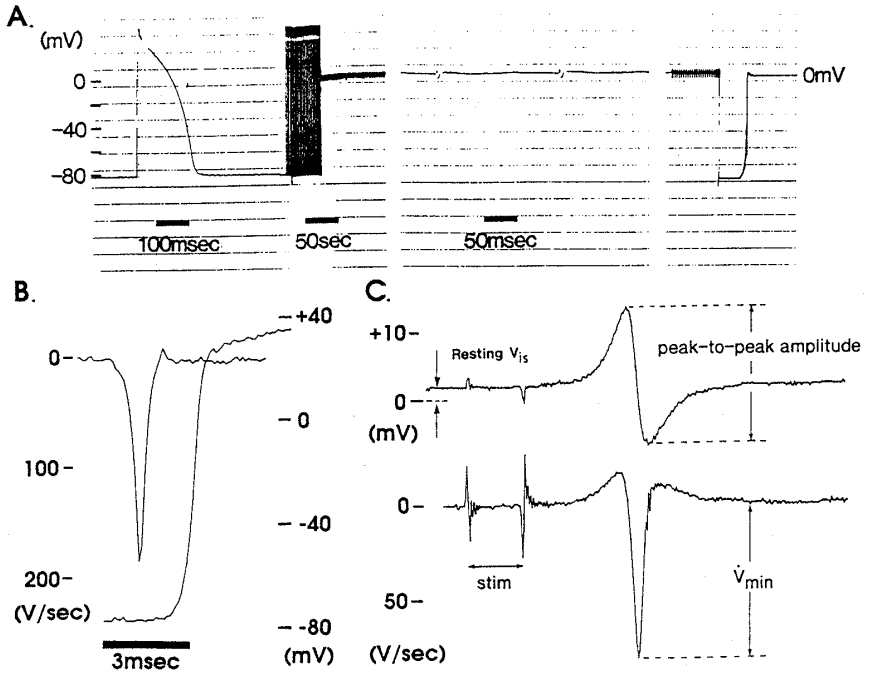

Fig. 1 **A**; Transitional potential recordings from intracellular to interstitial impalement. The time scale was altered arbitrarily. **B**; Actual recording of activation part of intracellular potential (V_{ic}) and the differentiated V_{ic} (V_{max}). The polarity of the differentiated V_{ic} is reversed and the time point is shifted for clarity. **C**; The recording of interstitial potential (V_{is}; upper) and the first time derivative of V_{is} (lower). The time scale in **C** is the same as in **B**.

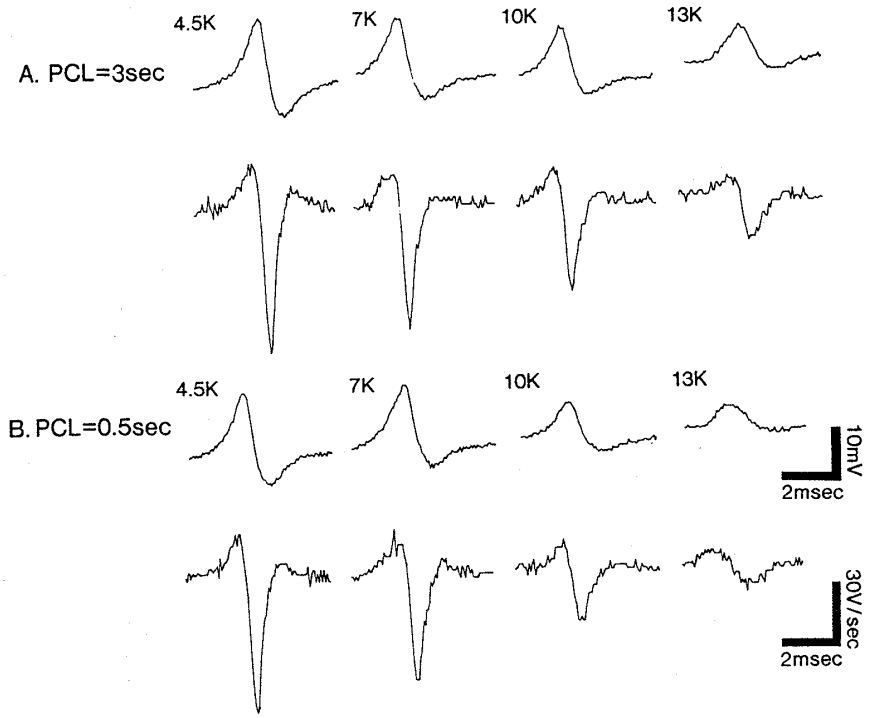

Fig. 2 Steady state effects of the alterations of the pacing cycle length (PCL) and the external potassium concentration ($[K^+]_e$) on V_{is} (upper) and the differentiated V_{is} (lower). PCL is either 3.0 (**A**) or 0.5 sec (**B**). These results were obtained from a single experiment.

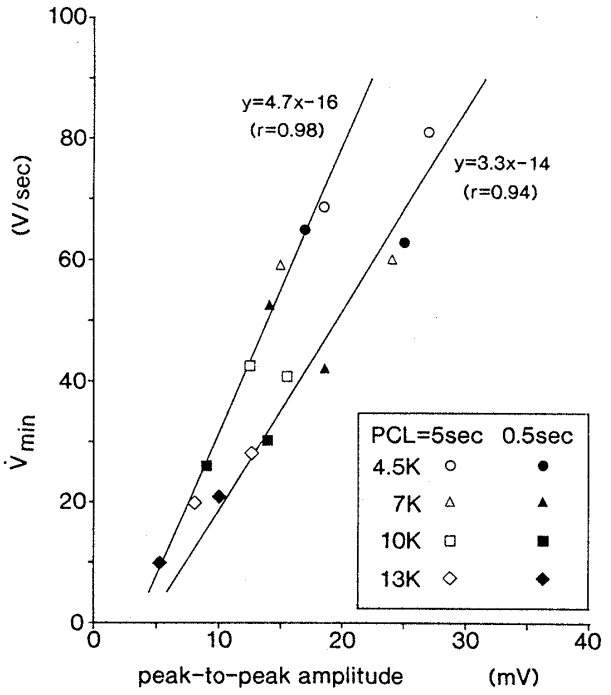

Fig. 3 The greatest negative deflection of the first time derivative of V_{ls} ($\dot{V}_{min}$) as a function of peak-to-peak amplitude of V_{ls} at different PCL and $[K^+]_e$. Each regression line indicates a single experiment. Each experiment showed a significant positive correlation ($r=0.94$ and 0.98, respectively). Data points of the experiment shown in Fig. 2 are included. Different symbols indicate the alterations of PCL and $[K^+]_e$, as shown in inset.

4 presents the PCL- and $[K^+]_e$-dependent suppression of $\dot{V}_{max}$ caused by quinidine obtained by a single experiment in the steady state. The continuous recording of $\dot{V}_{max}$ was indicated in each panel of Fig. 4 under the sequential alteration of PCL of 3.0, 1.0, 0.5 and again 3.0 sec. As PCL was shortened, $\dot{V}_{max}$ was suppressed in each panel. This effect was apt to be greater at 8.0 mM than at 4.5 mM $[K^+]_e$, and also in the presence than the absence of quinidine. Measured RMP in the steady state was significantly more negative at the PCL of 3.0 sec than 0.5 sec in each $[K^+]_e$ (-78.8 ± 0.7 vs. -75.2 ± 0.9 mV in 4.5 mM and -71.9 ± 0.6 vs. -69.7 ± 0.8 mV in 8.0 mM $[K^+]_e$, $p<0.05$). However, the treatment with quinidine did not affect the RMP at the fixed PCL and $[K^+]_e$. These findings agreed with those of previous studies (Chen & Gettes, 1976).

Fig. 5 presents the steady state reduction of $\dot{V}_{min}$ under the same pacing protocol as used in Fig. 4. In general, PCL-dependent decrease in $\dot{V}_{min}$ tended to be greater at 8.0 mM than at 4.5 mM $[K^+]_e$, and in the quinidine-treated condition as compared with the control. At 4.5 mM $[K^+]_e$, $\dot{V}_{min}$ was reduced first by the graded short PCL. Then, it was increased above the precontrol level by the abrupt return to the initial PCL (i. e., 3.0 sec). This rebound recovery at 4.5 mM $[K^+]_e$ was not observed in the case of $\dot{V}_{max}$ (Fig. 4), but was obvious in $\dot{V}_{min}$ regardless of the treatment with quinidine. At 8.0 mM $[K^+]_e$, $\dot{V}_{min}$ was reduced by a short PCL as well but to a greater extent than at 4.5 mM $[K^+]_e$. However, the rebound recovery of $\dot{V}_{min}$ was not observed at 8.0 mM $[K^+]_e$.

Fig. 6 summarizes the results of the experiments shown in Figs. 4 and 5 on the comparative suppression of $\dot{V}_{max}$ and $\dot{V}_{min}$ caused by quinidine. In the controls without quinidine (open column), the percent reduction induced by an elevated $[K^+]_e$ or a short PCL was greater in $\dot{V}_{min}$ than in $\dot{V}_{max}$. This was also the case in the presence of quinidine (closed column). The quinidine-induced suppression of $\dot{V}_{min}$ and $\dot{V}_{max}$ was indicated as a percentage in Fig. 6. As a whole, quinidine suppressed both $\dot{V}_{min}$ and $\dot{V}_{max}$ significantly in all the four settings with different $[K^+]_e$ and PCL (in the range of $p<0.01$ to 0.05). The difference of the quinidine-induced percent reduction between $\dot{V}_{min}$ and $\dot{V}_{max}$ was analyzed under the corresponding four conditions. This was significant ($p<0.01$) at a PCL of 0.5 sec (i. e., 57 vs. 91% at 10 mM $[K^+]_e$; 67 vs. 94% at 4.5 mM $[K^+]_e$). A similar trend was noted at the PCL of 3.0 sec and 10 mM $[K^+]_e$ (i. e., 86 vs. 93% ; $p<0.05$) (n=5).

Experiments of assumed $[K^+]_e$ fluctuation

To investigate the greater sensitivity of $\dot{V}_{min}$ than $\dot{V}_{max}$ to quinidine, $\dot{V}_{max}$ and $\dot{V}_{min}$ were plotted comparatively as a function of $[K^+]_e$ in a typical experiment. As $[K^+]_e$ rose from 3.0 to 15.0 mM, $\dot{V}_{max}$ decreased sigmoidally at any given PCL (Fig. 7A). $\dot{V}_{max}$ was suppressed along the ordinate by 4% but it did not shift along the abscissa at the short PCL. An

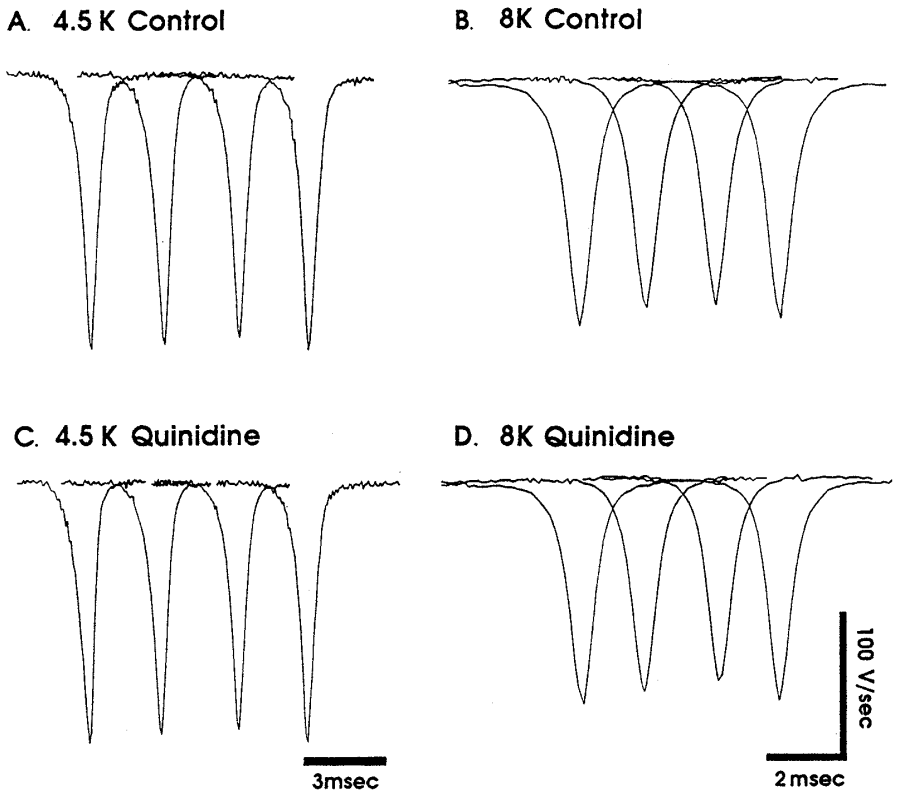

Fig. 4 The inhibitory effects of quinidine (10 μM) on the maximum upstroke rate of the differentiated V_{ic} (V_{max}) under the various PCL and $[K^+]_e$. $[K^+]_e$ is either 4.5 or 8.0 mM, as indicated. PCL was altered in each panel from 3.0 to 1.0, 0.5 and back to 3.0 sec from left to right. All the polarities of V_{max} are reversed. The time scale in **B** & **D** was different from that in **A** & **C**.

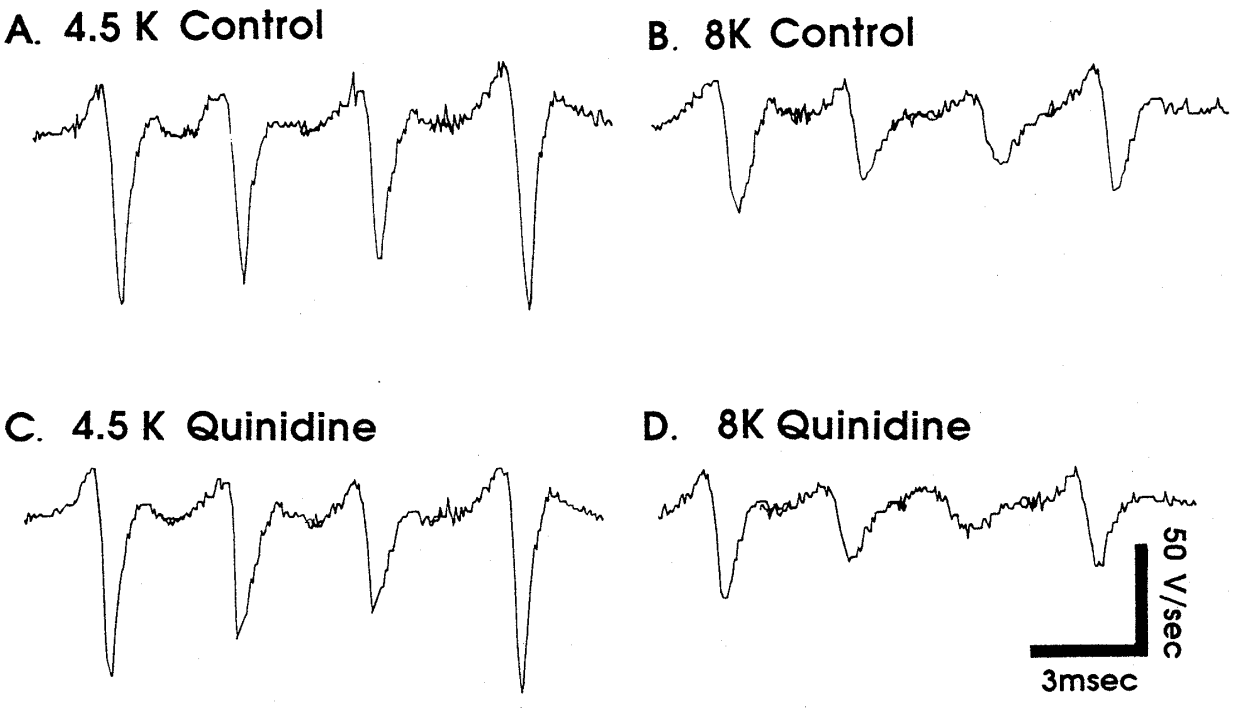

Fig. 5 PCL- and $[K^+]_e$-dependent inhibitory effects of quinidine (10 μM) on $\dot{V}_{min}$. The values used for PCL- and $[K^+]_e$-alterations were the same as in Fig. 4.

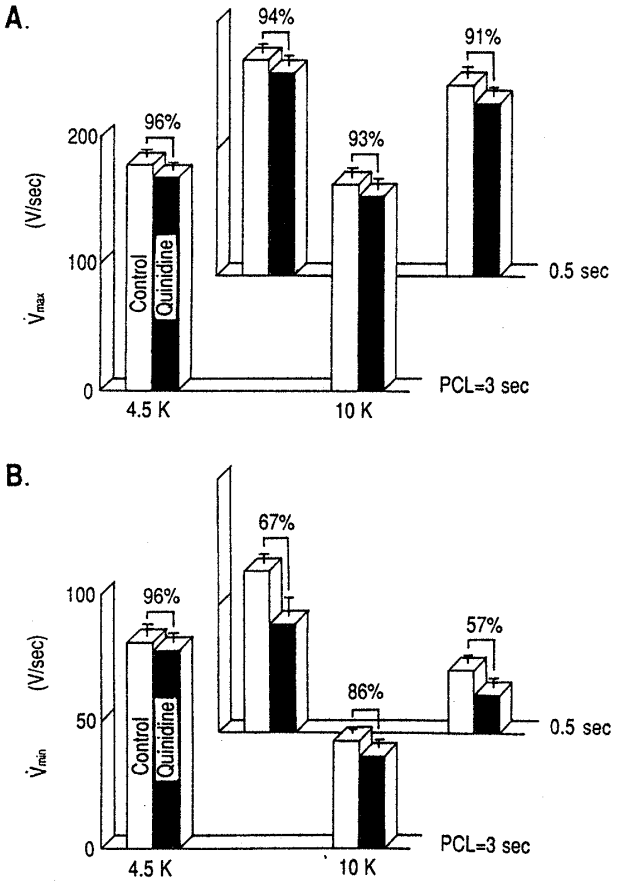

Fig. 6 The comparative quinidine-induced inhibition of $\dot{V}_{max}$ (**A**) and $\dot{V}_{min}$ (**B**) at four settings of different PCL and $[K^+]_e$. Each column and bar indicate the mean±S.D. Either an elevated $[K^+]_e$ or a short PCL commonly resulted in a significant fall in $\dot{V}_{max}$ and $\dot{V}_{min}$ regardless of the quinidine treatment ($p<0.05$ for $\dot{V}_{max}$ in the controls, $p<0.01$ for $\dot{V}_{max}$ in the presence of quinidine and for $\dot{V}_{min}$) [n=5]. The percent reduction induced by either maneuver of an elevated $[K^+]_e$ or a short PCL tended to be greater in $\dot{V}_{min}$ than in $\dot{V}_{max}$ both in the controls (open column) and the presence of quinidine (closed column). Thus the difference between the quinidine-induced suppression of $\dot{V}_{min}$ and $\dot{V}_{max}$ (indicated as percentage) exerted to be significant by either maneuver (67 vs. 94%, $p<0.01$ and 86 vs. 93%, $p<0.05$) and by the combined maneuvers (57 vs. 91%, $p<0.01$) [n=5]. Note that the scale of $\dot{V}_{min}$ is different from that of $\dot{V}_{max}$.

abrupt return to the initial PCL resumed $\dot{V}_{max}$. $\dot{V}_{min}$ was also reduced in a sigmoidal fashion as $[K^+]_e$ was elevated (Fig. 7B). Rapid pacing suppressed this sigmoidal curve by 10% and, moreover, shifted it to the left. By the termination of rapid pacing $\dot{V}_{min}$ was augmented and shifted to the right of the initial control curve. This corresponds to the rebound recovery of $\dot{V}_{min}$ observed in Fig. 5.

The parallel shift of the sigmoidal curve in $\dot{V}_{min}$ but not in $\dot{V}_{max}$ along the abscissa indicates that the apparent sensitivity of $\dot{V}_{min}$ to $[K^+]_e$ varies depending on the PCL. In other words, the actual $[K^+]_e$ is variable depending on the PCL and this is assumed to be some what affects the RMP and $\dot{V}_{min}$ regardless of the fixed nominal $[K^+]_e$. If this PCL-dependent $[K^+]_e$ fluctuation exists in our experimental condition, the parallel shift to the right of the initial control curve at the rebound recovery in $\dot{V}_{min}$ would be accounted for by the interstitial $[K^+]_e$ depletion.

To verify the above assumption and to examine the role of the Na^+-K^+ pump on the K^+-uptake and interstitial K^+-depletion, the preparation was exposed to 10 μM ouabain at 4.5 mM $[K^+]_e$ in another series of five experiments. Since K^+-depletion depends, mainly but not solely, on the Na^+-K^+ pump activity (Kline, 1990). Fig. 8 presents the results of a typical experiment using a sequential pacing protocol. This concentration of ouabain produced a greater fall in $\dot{V}_{min}$ induced by rapid pacing, and also eliminated the rebound recovery observed after the cessation of rapid pacing in the control. The other four preparations tested under the same protocol exhibited the same tendency, and the percent decrease in $\dot{V}_{min}$ induced by a short PCL was significantly greater in the ouabain-treated preparations than in the controls (62±4 vs. 73±10%, $p<0.05$) [n=5]. These results indicate that the rebound recovery following the termination of rapid pacing in 4.5 mM $[K^+]_e$ is explained, at least in part, by the K^+-uptake mediated by the Na^+-K^+ pump activated by the preceding rapid pacing. Taken together, ouabain-sensitive rebound recovery in $\dot{V}_{min}$ and the shift in the $\dot{V}_{min}$ curve depending on the PCL suggested the interstitial $[K^+]_e$ fluctuation, which may lead to the greater sensitivity of $\dot{V}_{min}$ than $\dot{V}_{max}$ to quinidine.

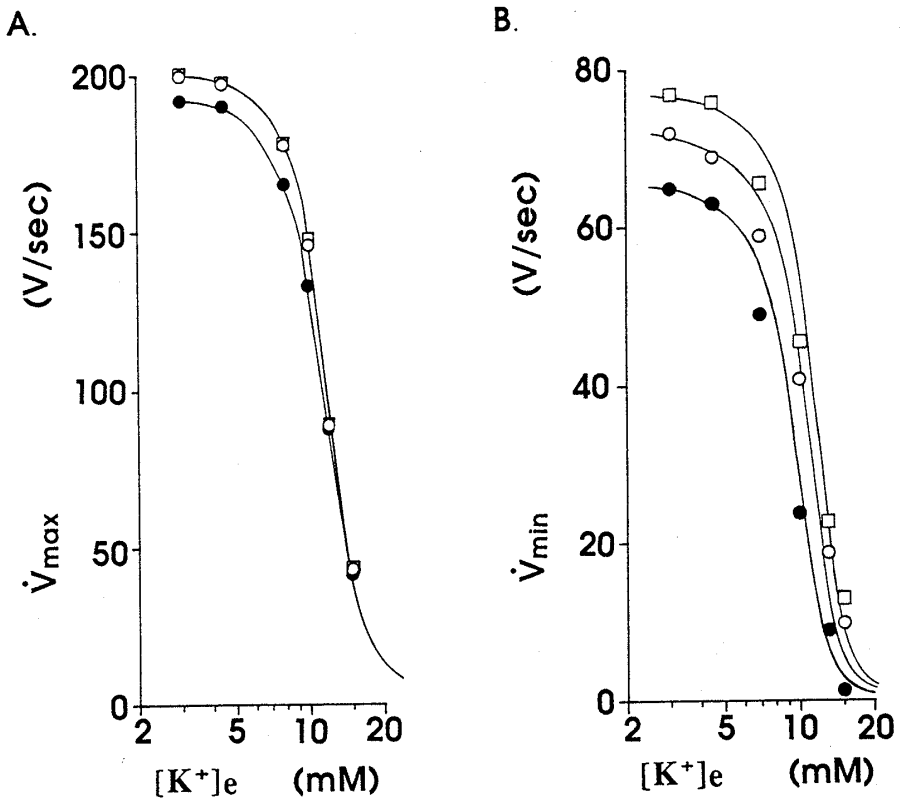

Fig. 7 $\dot{V}_{max}$ (**A**) and $\dot{V}_{min}$ (**B**) plotted as a function of $[K^+]_e$ under the conditions of gradual short PCL and the return to the initial PCL. These sigmoidal curves fit Boltzman's equation:

$$\dot{V}/\bar{\dot{V}}=(1+\exp[([K^+]_e-K_m)/k])^{-1}$$

where $\bar{\dot{V}}$ is the saturated value of either $\dot{V}_{max}$ or $\dot{V}_{min}$, K_m is $[K^+]_e$ at half inhibition, and k is a slope factor. When $\dot{V}=\dot{V}_{max}$, $\bar{\dot{V}}$ is 198 for the control (○; PCL=3.0 sec), 190 for rapid pacing (●; PCL=0.5 sec) and 199 mV for the return to the control (□), and K_m is 11.7 mM for all the three conditions, indicating a slight alteration of $\dot{V}_{max}$ along the ordinate. When $\dot{V}=\dot{V}_{min}$, $\bar{\dot{V}}$ is 72 for the control (○), 65 for short PCL (●) and 77 mV for the return to the control (□). K_m is 10.9, 9.7 and 11.4 mM for the respective three conditions. The sigmoidal curve for $\dot{V}_{min}$ is shifted along the abscissa.

DISCUSSION

In this study, we found that $\dot{V}_{min}$ was more sensitive than $\dot{V}_{max}$ to the PCL- and $[K^+]_e$-dependent quinidine-induced inhibition. This greater sensitivity could be explained most readily by the suggested interstitial $[K^+]_e$ fluctuation, which is balanced mainly by the electrical activity and Na^+-K^+ pumping. Therefore, the modulated receptor hypothesis was concluded to be valid in terms of not only transmembrane but also interstitial part of the local circuit loop.

The time- and voltage-dependent interactions of class I antiarrhythmic drug molecules and Na channels were first summarized as a modulated receptor hypothesis by Hondeghem and Katzung (1977 & 1984). Previous works concerning the antiarrhythmic drug effects in the settings of altered PCL and RMP showed that quinidine exhibited an enhanced inhibitory effects on $\dot{V}_{max}$ in the depolarized and rapidly paced fiber (Chen & Gettes, 1976). This indicates that the quinidine binding to and blockade of Na channels are dependent on the channel state, i. e., this inhibition is augmented by the K^+-depolarization causing a partial inactivation of the Na

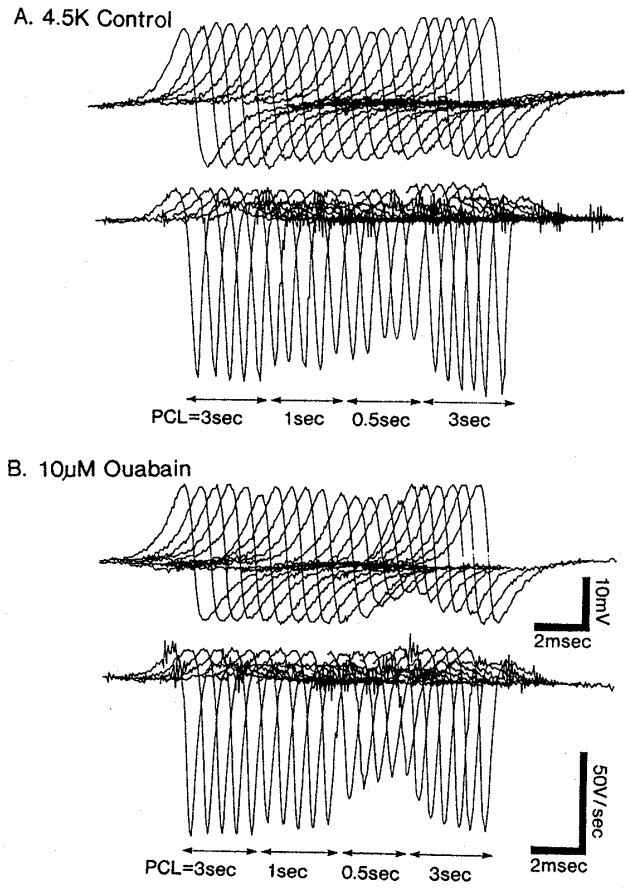

Fig. 8 Effects of 10 μM ouabain on kinetic alterations of V_{is} (upper) and the first time derivative of V_{is} (lower) during the sequential pacing protocol indicated. $[K^+]_e$ is 4.5 mM throughout the experiment. Note that the rebound recovery of $\dot{V}_{min}$ after the termination of rapid pacing in the control (**A**) is eliminated by the pretreatment with ouabain (**B**).

channels, and is attenuated by repolarization which restores the Na channel availability.

In the present study, $\dot{V}_{min}$ responded to quinidine as just $\dot{V}_{max}$, but to a greater extent (Fig. 6). If V_{is} were simply proportional to the d^2V_{ic}/dt^2, the extent of the quinidine-induced inhibition of V_{is} and V_{ic} would be identical. However in the present study this was not the case, which suggests that V_{is} is governed by d^2V_{ic}/dt^2 and, moreover, variable interstitial conductance. PCL-dependent change in the interstitial conductance is most likely accounted for by the interstitial $[K^+]_e$ fluctuation as reported in the literatures (Frank & Langer, 1974; Ilebekk, Andersen & Sejersted, 1986; Kline, 1990; Kunze, 1977). The interstitial K^+ accumulation produced by rapid activity is thought to increase the interstitial conductance and hence to decrease both $\dot{V}_{min}$ and the amplitude of V_{is}, whereas the interstitial K^+ depletion in the quiescence has the opposite effects. The assumed interstitial $[K^+]_e$ fluctuation is equilibrated by an activity-dependent K^+ efflux and an Na^+-K^+ pump-mediated K^+ uptake. This fluctuation is of special importance in various clinical facets such as tachycardia (Ilebekk *et al.* 1986; Kunze, 1977), myocardial ischemia (Hill & Gettes, 1980), cardiac automaticity (Vassalle, 1970) and so on. Although a transsarcolemmal flux during electrical activity has been reported with physiologically relevant cations such as Na^+ (Cohen, Fozzard & Sheu, 1982) and Ca^{2+} (Hilgemann, Delay & Langer, 1983), the flux of Ca^{2+} and Na^+ counteracts that of K^+ during electrical activity and hence can not explain the PCL-dependent kinetic change in $\dot{V}_{min}$ (Fig. 5).

Vassalle (1970) attributed the K^+ depletion to the K^+ uptake mediated by the Na^+-K^+ pump, which is activated by the cytosolic Na^+ accumulation induced by the preceding rapid pacing. A net K^+ release occurs until the rate-dependent K^+ efflux is compensated for by the Na^+-K^+ pump activation. A study of ouabain binding using the same preparation as in this study reported the following steady state relationship between the relative Na^+-K^+ pump activity $[T/T_{max}]$ and the stimulation rate [S; Hz] (Herzig *et al.* 1988):

$$T/T_{max}=8.8\ S+15.6$$

This equation gives the steady rate of 9.6 Hz, beyond which frequency K^+ loss is predicted to exceed the reserve capacity of the Na^+-K^+ pump in the aerobic myocardium. Although strict experimental conditions may differ, this calculation shows that the preparation has sufficient Na^+-K^+ pump capacity at the PCL employed in this study. It is, therefore, likely that this pump activation is what turns an accumulation into a depletion of interstitial

K^+ after the termination of rapid pacing.

During the electrical activity, V_{is} is most evident at the activation wavefront in both theoretical (Henriquez, Trayanova & Plonsey, 1988 ; Plonsey & Bar, 1987 ; Roth, 1988) and experimental (Knisley, Maruyama & Buchanan, 1991 ; Spach *et al*. 1972) studies (i. e., the greatest voltage gradient exists at the activation wavefront in the interstitial as well as intracellular domain). The peak-to-peak amplitude of V_{is} during longitudinal propagation is reported to be depth-dependent (Knisley, Maruyama & Buchanan, 1991 ; Plonsey & Bar, 1987) and to have a value of 20 to 25 mV at a depth of 0.5 mm (Henriquez, Trayanova & Plonsey, 1988 ; Roth, 1988). The amplitude of V_{is} observed in this study was within this range (Fig. 1B). On the other hand, in the resting state, Parent and Caillé (1985) reported that the resting V_{is} of quiescent rabbit papillary muscles is negative to the reference by 5.7 mV due to the abundant interstitial ground substance charged negatively at a normal pH (Frank & Langer, 1974 ; Haljamäe, Linde & Amundson, 1974 ; Polimeni, 1974). These investigators measured sequential interstitial and intracellular potentials using a microelectrode covered with hydrophobic material and evaluated the resting V_{is} immediately preceding and following the intracellular impalement. In the present study, resting V_{is} was measured after the stable interstitial impalement during electrical stimulation. This maneuver may have kept the microelectrode tip in the center of the interstitial space, explaining the positive resting V_{is} observed in this study. This positive value was presumably due to a difference in the ionic composition of the interstitial and bathing solutions ; i. e., the former being richer in Na^+ and K^+ and poorer in Cl^- than the latter, due to the polyanionic interstitial matrix (Haljamäe, Linde & Amundson, 1974). Therefore, our maneuvers may have evaluated the different ionic composition between the interstitial and bathing solutions, whereas those of Parent and Caillé estimated the interstitial fixed charge itself. Anyway, the polarity of the measured resting V_{is} appeared to be governed by the subtle change in the position of the microelectrode tip in the large interstitial potential gradient.

The main limitation of our study is that the actual interstitial $[K^+]_e$ was not measured. The PCL-dependent K^+ balance has been assessed by several investigators (Ilebekk, Andersen & Sejersted, 1986 ; Kline, 1990 ; Kunze, 1977) using the K^+-selective microelectrodes. In most cases, $[K^+]_e$ was measured at the surface of the preparations to avoid contamination with cytosolic K^+ in the interstitial $[K^+]_e$ measurement. However, it may be inaccurate to extrapolate interstitial $[K^+]_e$ from surface $[K^+]_e$ in the presence of $[K^+]_e$ gradient in the radial direction of the cardiac fiber (Cascio, Yan & Kléber, 1992). The second limitation is that we did not observe the PCL- and $[K^+]_e$-dependent effects of quinidine on the whole loop of local circuit. We observed only the interstitial and transmembrane portions of the local circuit loop. Intracellular axial current flow influenced mainly by gap junctional conductance (g_j) was not evaluated at all. However, g_j is not influenced by the PCL at least in the range of this study under the aerobic condition of this preparation (Hiramatsu *et al*. 1988). Moreover, 9 mM $[K^+]_e$ (about half of the maximum $[K^+]_e$ in this study) has no effects on g_j (Hiramatsu *et al*. 1989) whereas it caused to halve $\dot{V}_{max}$ and $\dot{V}_{min}$ in this study (Fig. 7). These suggest the experimental setting of various PCL and $[K^+]_e$ in this study has little effects on g_j and hence the intracellular axial current flow.

In conclusion, modulated receptor hypothesis was reconfirmed in terms of V_{is} as well as V_{ic}. We found V_{is} more sensitive to quinidine than V_{ic}, because the suggested interstitial $[K^+]_e$ fluctuation affected V_{is} directly. Therefore, V_{is} is supposed to be influenced more than V_{ic} by the various clinical settings such as myocardial ischemia, ischemia-related arrhythmia, and antiarrhythmic drug treatment.

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