British Journal of Pharmacology (1995) 116, 2134-2140

Voltage-dependence of Ca^{2+} agonist effect of YC-170 on cardiac L-type Ca^{2+} channels

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1 We investigated the voltage-dependence of the agonist actions of YC-170, a dihydropyridine (DHP) derivative, on cardiac L-type Ca^{2+} channels in rabbit ventricular cells, using the patch clamp technique. The characteristics of YC-170 were compared with those of other DHP Ca^{2+} agonists (Bay K 8644, CGP 28392). Ca^{2+} channel activities were elicited by depolarizing pulses to 0 mV from a holding potential (HP) of either −80 mV or −40 mV.

2 YC-170 (10 μM) increased Ca^{2+} channel activities when HP was set at −80 mV. However, decreasing HP to −40 mV abolished the agonist action. The agonist effect of Bay K 8644 (1 μM) on Ca^{2+} channels was elicited to the same extent at either HP. CGP 28392 (10 μM) also increased Ca^{2+} channel activities at both HPs, but its agonist effect was significantly larger at an HP of −80 mV than at −40 mV.

3 All of the three DHP Ca^{2+} agonists prolonged open times of Ca^{2+} channels, but the prolongation did not correspond to the voltage-dependence of Ca^{2+} agonist effects of the three DHPs.

4 YC-170 markedly reduced the closed time of the Ca^{2+} channel when the HP was at −80 mV, but prolonged it at HP of −40 mV. Bay K 8644 reduced closed times at an HP of −80 mV. At an HP of −40 mV, Bay K 8644 slightly reduced closed times. CGP 28392 reduced closed times at an HP of −80 mV and prolonged those at an HP of −40 mV. Thus the voltage-dependence of the agonist effects of these agents was in good agreement with the voltage-dependence of changes in closed times of Ca^{2+} channel.

5 Two mechanisms may be involved in the agonist action of YC-170; a prolongation of open times, and a reduction of closed times of Ca^{2+} channels, i.e. an increase in reopening. The former mechanism is not dependent on HP and the latter mechanism is highly dependent on HP. Thus, the voltage-dependence of the agonist action may be attributed to the voltage-dependence of their enhancing effect on reopening of Ca^{2+} channels.

Keywords: Dihydropyridine; Ca^{2+} agonist; L-type Ca^{2+} channel; cardiomyocyte; YC-170; BAY K 8644; CGP 28392

Introduction

L-Type Ca^{2+} channels are a major subtype of Ca^{2+} channels in heart muscles and vascular smooth muscles. 1,4-Dihydropyridine (DHP) derivatives selectively bind to the L-type Ca^{2+} channels and modulate their function (Hess, 1990). DHP Ca^{2+} agonists, which specifically activate the L-type Ca^{2+} channels, are an important pharmacological tool for electrophysiological characterization of the L-type Ca^{2+} channels. For example, Hess *et al.* (1984) discovered a unique action of Bay K 8644, a DHP Ca^{2+} agonist: initiation of long-lasting opening behaviour of the Ca^{2+} channels (also see Kokubun & Reuter, 1984; Ochi *et al.*, 1984). Nilius *et al.* (1985) used Bay K 8644 for classifying subtypes of the Ca^{2+} channels. Ohya & Sperelakis (1989) reported the capability of Bay K 8644 of preventing a run down phenomenon of the Ca^{2+} channels in vascular smooth muscle cells.

In previous studies, describing the agonist effect of Bay K 8644 on L-type Ca^{2+} channels at the macroscopic current level, it has been stressed that Ca^{2+} agonist effect of DHPs shows voltage-dependence (Sanguinetti *et al.*, 1986). The enhancement induced by DHPs of the L-type Ca^{2+} current was attenuated or reversed at less negative holding potentials (HPs). Some reports showed that Ca^{2+} agonists shifted the activation and inactivation curves of the Ca^{2+} current rightward along the voltage axis and resulted in enhancement of Ca^{2+} current (Sanguinetti *et al.*, 1986; Cohen & Lederer, 1987; Markwardt & Nilius, 1988). It was also reported that Bay K 8644 shifted an activation curve of the gating current of Ca^{2+} channels (Josephson & Sperelakis, 1990). At the single channel level, the agonist effect has been ascribed to prolongation of channel openings (Hess *et al.*, 1984; Kokubun & Reuter, 1984; Ochi *et al.*, 1984). Although Kokubun *et al.* (1986) described voltage-dependent attenuation of the agonist effect at the single channel level, there is no report investigating the relation between the long-lasting opening of channels and the voltage-dependence of the agonist action.

The voltage-dependent characteristics of DHP agonists implied a potential for the development of agents useful in the management of cardiac performance in acute myocardial infarction (AMI). At the present time, some Ca^{2+} antagonists (i.e. verapamil, diltiazem) are used to block lethal ventricular arrhythmias in AMI. However, the Ca^{2+} antagonists weaken the cardiac contractility. Therefore, a Ca^{2+} agonist, which exhibits the Ca^{2+} antagonist effect in the depolarized muscles, may be suitable for treatment of arrhythmia in AMI. If an ideal agent having a voltage-dependent agonist effect could be developed, it could block the lethal arrhythmias originating from the depolarized (deseased) muscles with protection (or improvement) of the contractility in the polarized (normal) muscles.

In previous studies, we reported strong voltage-dependence of the agonist action of YC-170, a DHP Ca^{2+} agonist (Nakaya *et al.*, 1986; Hattori *et al.*, 1986). YC-170 exhibited a Ca^{2+} agonist effect on polarized cardiac muscles although it decreased the force of contraction in depolarized cardiac muscles. These findings suggest that YC-170 may be an effective drug for AMI. Therefore, in this study, we investigated the voltage-dependent effects of YC-170 on cardiac L-type Ca channels by recording single channel activities, and comparing results with representative DHP Ca^{2+} agonists, Bay K 8644 and CGP

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28392, which are well known to produce an agonist effect both at the macroscopic current and single channel levels (Kokubun & Reuter, 1984; Brown *et al.*, 1984).

Methods

Cell preparations

Single cardiomyocytes were isolated from rabbits by enzymatic dissociation, as described previously (Tohse, 1990). Briefly, the heart was removed from the open chest rabbit, anaesthetized with pentobarbitone sodium. The excised heart was perfused in a Langendorff aparatus with 0.01% collagenase (Wako Pure Chemical Industries, Osaka, Japan) dissolved in a nominally Ca^{2+}-free Tyrode solution. After 30 min digestion, the ventricle was rinsed with a Kraftbrühe (KB) solution (Isenberg & Klöckner, 1982) and cut into small pieces. The cell suspension in KB solution was stored in a refrigerator for later use.

The composition of the Tyrode solution was (in mM): NaCl 143, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4 0.33, glucose 5.5 and HEPES-NaOH buffer (pH 7.4) 5.0. The nominally Ca^{2+}-free Tyrode solution was prepared by omitting $CaCl_2$ from the normal Tyrode solution. The composition of the KB solution was (in mM): KOH 70, L-glutamic acid 50, KCl 40, taurine 20, KH_2PO_4 20, $MgCl_2$ 3, glucose 10, EGTA 1.0 and HEPES-KOH buffer (pH 7.4) 10.

Single channel recordings

Unitary current recordings were obtained in the cell-attached configuration of the patch clamp technique (Hamill *et al.*, 1981). Single ventricular cells were placed in a recording chamber (1 ml volume) attached to an inverted microscope (Olympus IMT-2, Tokyo, Japan) and superfused with the normal Tyrode solution. The temperature of the external solution was kept at 36 ± 1.0°C. Patch pipettes with a diameter of 2–4 μm were Si-coated, heat polished and filled with a solution containing 110 mM $BaCl_2$, 5 mM HEPES (pH was adjusted to 7.4 by 2-amino-2-hydroxymethyl-1,3-propandiol, tris). Tip resistance of the pipette filled with the pipette solution was 4–8 MΩ. After a gigaohm seal was established between the patch electrode and the cell membrane in the Tyrode solution, the bath solution was changed to a depolarizing solution containing (mM): KCl 145, $MgCl_2$ 0.5, glucose 5.5, EGTA 10 and HEPES-KOH 5.5 (pH 7.4). In the depolarizing solution, the resting membrane potential of a myocyte should be 0 mV and we could control membrane potential through an electrode in the pipette. The HPs were clamped at −80 mV or −40 mV and Ca^{2+} channel activities were elicited by applying depolarizing pulses of 50 ms duration from HP to 0 mV. Current signals obtained by a patch-clamp amplifier (EPC-7, List-Medical, Darmstadt, Germany) were stored on a video tape recorder (VICTOR HR-S5800, Japan) through a pulse code modulator (SONY PCM- 501 ES, Japan) for later analysis. The stored data were filtered with a cut-off frequency of 1 kHz through a low pass filter (4-pole Bessel), digitized by a 16-bit A/D converter (Conoopus Electronics ADX-98, Japan) with a sampling frequency of 10 kHz, and stored on a personal computer (NEC PC-98). The capacitative and leak currents were digitally subtracted using blank sweeps. NP_o value was calculated and used as an index of Ca^{2+} channel activities (N: the number of channels included in the patch. P_o: probability of channel openings during depolarizing pulses). To analyse kinetics of Ca^{2+} channels, we used recordings from patches containing only a single channel.

Drugs and chemicals

YC-170 (2-(2-pyridil)ethyl 4-(o-chlorophenyl)-2,6-dimethyl-5-phenylcarbamoyl-1,4-dihydropyridine-3-carboxylate) was a kind gift from the Yamanouchi Pharmaceutical Co. (Tokyo,

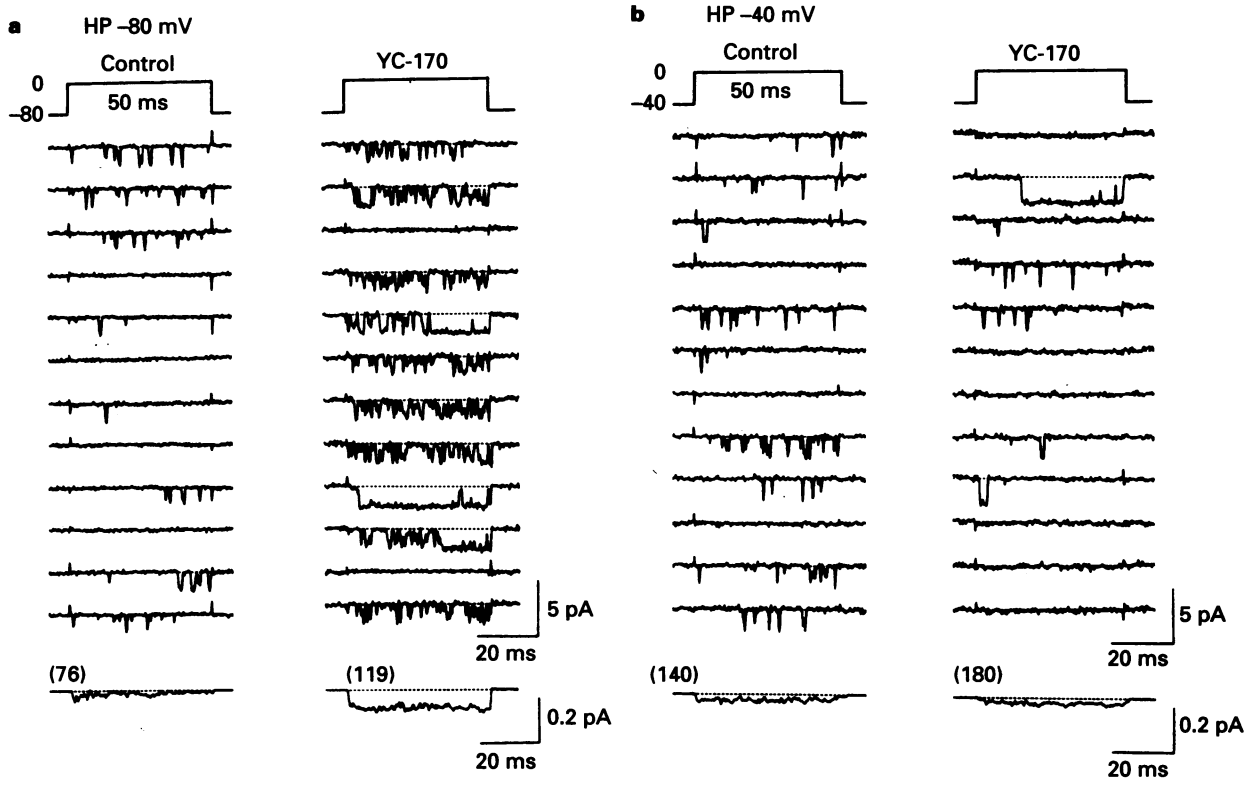

Figure 1 Effects of YC-170 on single Ca^{2+} channel currents. HPs were clamped at −80 mV (a) and −40 mV (b). Ca^{2+} channel activities were elicited by depolarizing pulses to 0 mV for a duration of 50 ms repetitively at 0.5 Hz. The left and right panels show data obtained in a control depolarizing solution and in the presence YC-170 (10 μM), respectively. The bottom traces are averaged currents calculated from the continuous current traces. Numbers in parenthesis indicate the numbers of traces used for the calculation.

Japan). Bay K 8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate) was a kind gift from Bayer AG (Leverkusen, Germany) and CGP 28392 (4-[2-(difluoromethoxyphenyl]-1,4,5,7-tetrahydro-2-methyl-5-oxofluoro[3,4-6]pyridine-3-carboxylic acid ethylester) from Ciba-Geigy AG (Basel, Switzerland). YC-170 was prepared as stock solution (5 mM) in 10% dimethyl sulphoxide with 6% 1 N HCl. Bay K 8644 and CGP 28392 were prepared in ethanol. The final concentrations of the solvents used in this study did not exceed 0.1%, which had no effects on Ca^{2+} channel activities. Although the stock solution of YC-170 contained 6% 1 N HCl, the pH of the final solution was 7.4. The concentrations we used in this study were 10 μM for YC-170, 1 μM for Bay K 8644 and 10 μM for CGP 28392 because these concentrations were found to exert maximum effects in increasing Ca^{2+} channel activities in preliminary experiments.

Statistics

All values are presented in terms of mean ± s.e. Statistical analyses were performed by Student's *t* test for paired and unpaired observations. *P* values less than 0.05 were accepted as indicating a significant difference.

Results

Effects of YC-170

Depolarizing pulses from a holding potential (HP) of −80 mV to 0 mV for 50 ms elicited openings of a Ca^{2+} channel (Figure 1), which was identified as an L-type Ca^{2+} channel because its activities were completely abolished by treatment with nifedipine (1 μM). Bath application of YC-170 (10 μM) increased sweeps with bursting of channel openings, and concomitantly evoked long lasting openings, which were rarely observed in control patches. YC-170 did not alter the amplitude of the unitary current. YC-170 produced a marked increase in the amplitude of the averaged current as depicted at the bottom of Figure 1, indicating that YC-170 increased the NP_o value in this patch. In 5 different patches, YC-170 increased NP_o to 710 ± 93% of the control value. When the HP was clamped at −40 mV, YC-170 increased the number of blank sweeps, i.e. sweeps not accompanied with openings of Ca^{2+} channels. However, long-lasting openings were always observed, when the channel activities were evoked. Therefore the averaged current did not alter markedly from control. The NP_o value was slightly decreased by YC-170 (94 ± 6.6%, $n = 5$).

Effects of Bay K 8644

Figure 2 shows typical effects of BAY K 8644 on Ca^{2+} channel activities. At a HP of −80 mV, application of Bay K 8644 (1 μM) elicited recurrently long-lasting openings of the Ca^{2+} channel, and increased an ensemble averaged current, as described in previous papers (Kokubun & Reuter, 1984; Hess *et al.*, 1984; Brown *et al.*, 1984). At HP of −40 mV, Bay K 8644 also produced frequent long-lasting openings and enhanced ensemble averaged current. The unit amplitude of the Ca^{2+} channels was not changed by Bay K 8644 at either HP. The NP_o value of the Ca^{2+} channels at HPs of −80 and −40 mV was increased to 920 ± 93% ($n = 5$) and 990 ± 85% ($n = 5$), respectively.

Effects of CGP 28392

At a HP of −80 mV, CGP 28392 (10 μM) prolonged the open time of the Ca^{2+} channel and increased ensemble averaged current (Figure 3). These results were consistent with those of previous reports (Kokubun & Reuter 1984; Brown *et al.*, 1984). CGP 28392 did not alter the unit amplitude of Ca^{2+} channels. Increase in NP_o was 350 ± 35% ($n = 5$) of control. CGP 28392 also enhanced activities of Ca^{2+} channels at an HP of −40 mV. CGP 28392 produced prolongation of channel openings and increased ensemble averaged current. The increase in NP_o value was 230 ± 9% ($n = 5$) at a HP of −40 mV. The increase was significantly smaller than that at HP of −80 mV ($P < 0.01$).

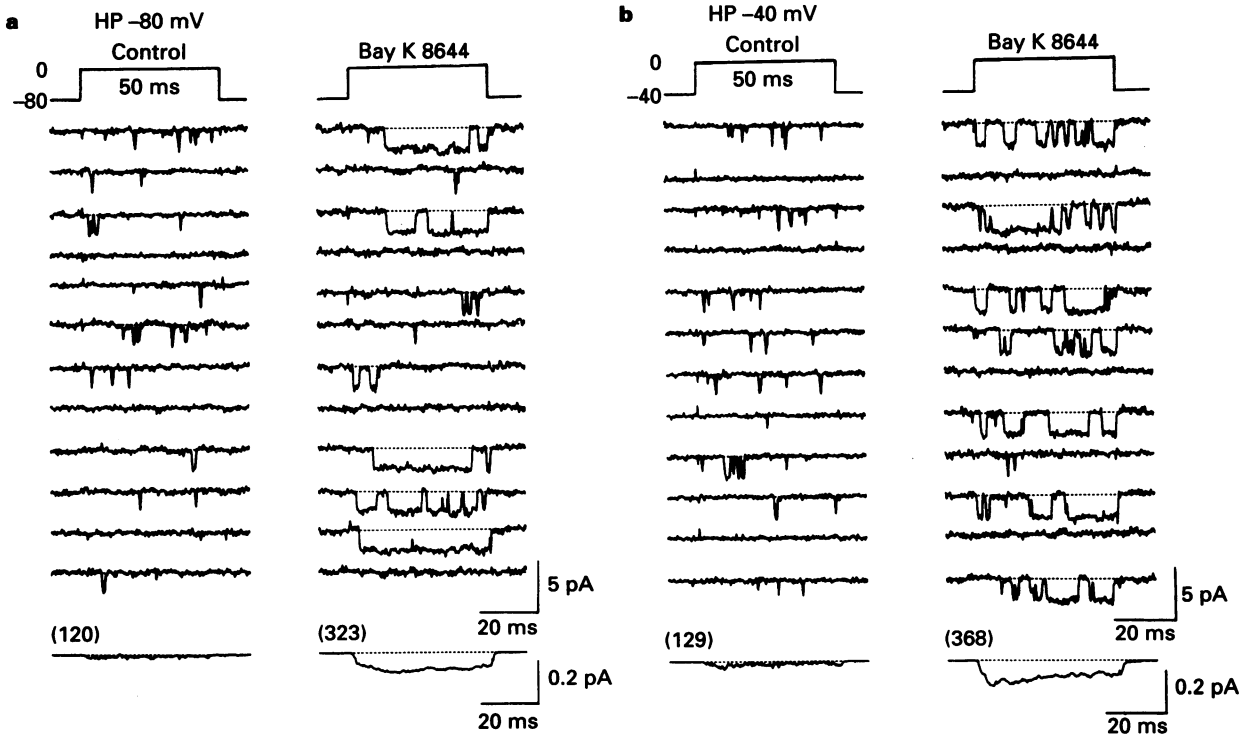

Figure 2 Effects of Bay K 8644 on the single Ca^{2+} channel currents. HPs were −80 mV (a) and −40 mV (b). Ca^{2+} channel activity was elicited by depolarizing pulses to 0 mV for a duration of 50 ms repetitively at 0.5 Hz, in a control depolarizing solution (left) and in the presence Bay K 8644 (1 μM) (right). The bottom traces represent averaged currents.

Effect of DHPs on open time distribution of Ca^{2+} channel

Figure 4 shows a representative example of the effect of YC-170 on the open time distribution of Ca^{2+} channels. Table 1 shows summarized data from several experiments with YC-170. In the control condition, the open time distributions were fitted by a single exponential curve at HPs of −80 mV or −40 mV. Treatment of cardiomyocytes with YC-170 resulted in the appearance of a second exponential component having a slower time constant in the open time distribution. The time constant of the first component was increased at HP of −80 mV, but not at HP of −40 mV. The time constant of the second component was slower at HP of −80 mV than at HP of −40 mV.

Bay K 8644 and CGP 28392 also produced the second slower component in the open time distribution (Table 1). However, the slower time constants are larger at HP of −40 mV than at HP of −80 mV in the presence of Bay K 8644 and CGP 28392. Therefore, only YC-170 showed a voltage-dependent attenuation of prolongation of the open times.

Effect of DHPs on closed time distribution of Ca^{2+} channel

Figure 5 shows representative histograms of the closed time distribution of the Ca^{2+} channel in the absence and presence of YC-170. In the control condition, the distributions were fitted by two exponential curves at either HP. Time constants of the fast components were not changed by application of YC-170. However, YC-170 decreased the time constant of the slow component from 11.8 ms to 4.9 ms at HP of −80 mV, but slightly increased it at HP of −40 mV (from 7.7 ms to 9.3 ms). Similar results are expressed in the summarized data in Table 1.

Effects of the other DHPs on time constants of the closed time distribution are also shown in Table 1. Bay K 8644 shortened the slow time constant of the closed time distribution at HP of −80 mV. At HP of −40 mV, Bay K 8644 also shortened the slow time constant, but not significantly. CGP 28392 rather prolonged the slow time constant of the closed time distribution at −40 mV, while it shortened the slow time constant at HP of −80 mV.

Discussion

In the present study, a voltage-dependent Ca^{2+}-agonist effect of YC-170 was observed in single channel recording, as suggested in our previous report (Nakaya *et al.*, 1986). In open-time kinetics of Ca^{2+} channels, YC-170 produced a second slow component of open time distribution at both HPs, indicating that YC-170 prolonged open times. Therefore, the YC-170-induced prolongation of open times cannot explain the voltage-dependent effect of YC-170 on NP_o, because YC-170 prolonged open times at HP of −40 mV, at which YC-170 decreased NP_o. CGP 28392 produced a relatively weak voltage-dependent effect on NP_o. However, CGP 28392 produced a second slow component of open time kinetics at both HPs, and the time constant at HP of −40 mV was larger than that at HP of −80 mV. Therefore, these data for YC-170 and CGP 28392 indicate that the voltage-dependence of the Ca^{2+}-agonist effect cannot be fully explained by the Ca^{2+}-agonist-induced prolongation of the open times.

Effects of YC-170 on closed-time kinetics appeared to produce a voltage-dependent Ca^{2+} agonist effect, because the time constant of the slow component of closed-time distribution was reduced at HP of −80 mV, but increased at HP of −40 mV. Bay K 8644 reduced the time constant of the slow component at HP of −80 mV. At HP of −40 mV, Bay K 8644 also reduced the slow time constant, but not significantly. CGP 28392 increased the time constant of the slow component at HP of −40 mV but reduced that at HP of −80 mV. Effects of Bay K 8644 and CGP 28392 on closed time kinetics corresponded to a voltage-related change in their Ca^{2+} agonist effects.

All these data concerning single-channel kinetics demon-

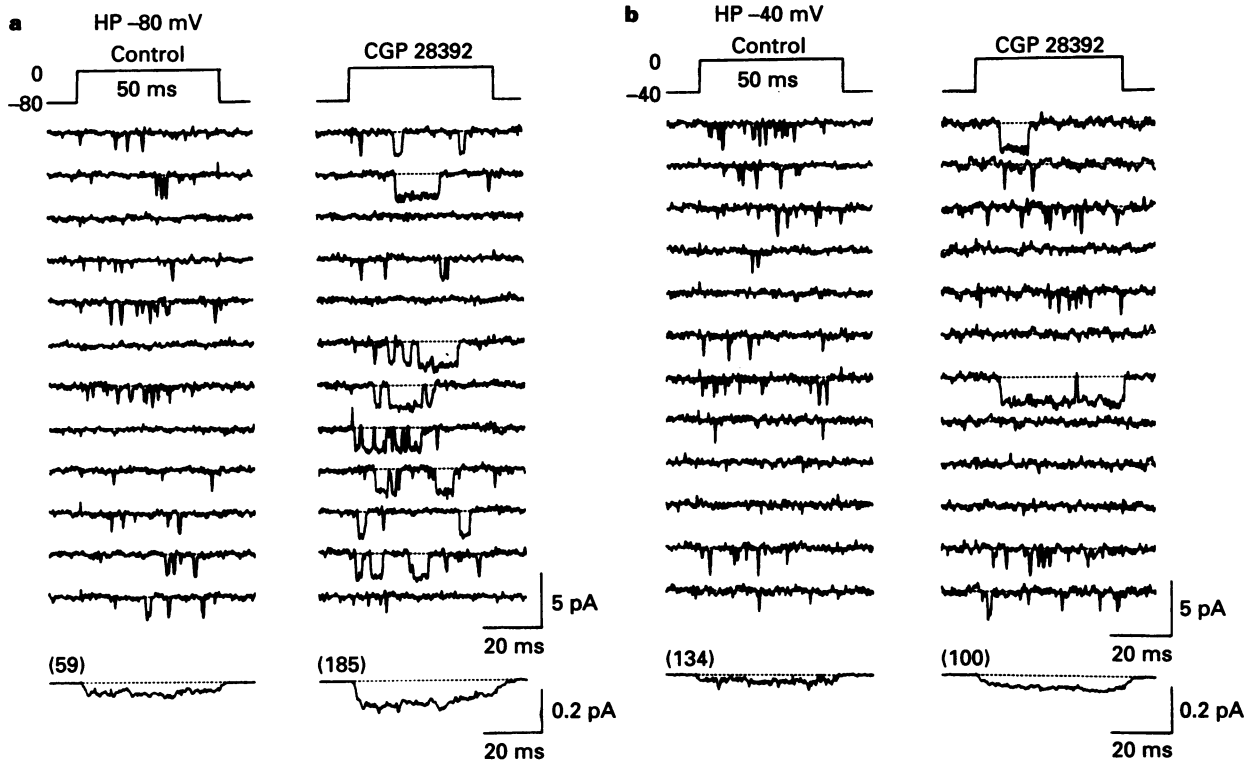

Figure 3 Effects of CGP 28392 on single Ca^{2+} channel currents. HPs were −80 mV (a) and −40 mV (b). Ca^{2+} channel activity was elicited by depolarizing pulses to 0 mV with a duration of 50 ms repetitively at 0.5 Hz, in a control depolarizing solution (left) and in the presence CGP 28392 (10 μM) (right), respectively. The bottom traces are averaged currents.

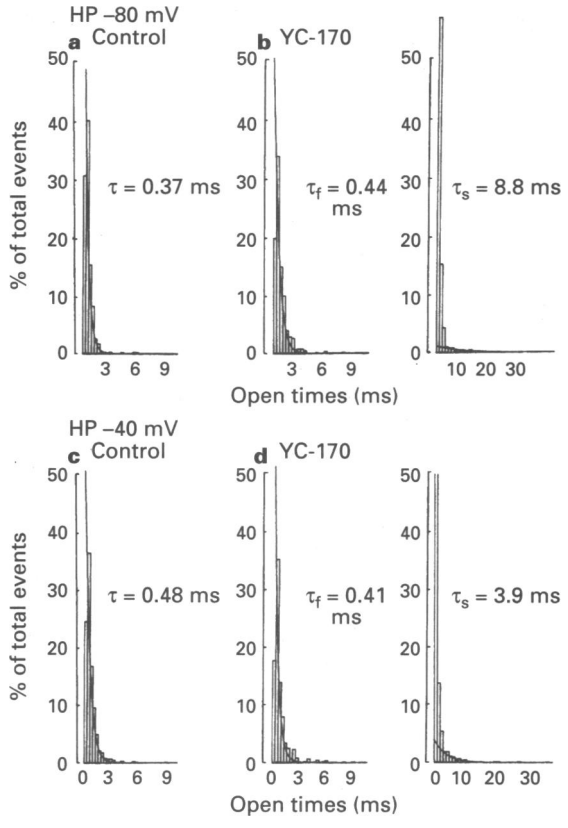

Figure 4 Upper panels: Open-time histograms of Ca^{2+} channels at HP of −80 mV under control conditions (a) and in the presence of YC-170 (10 μM) (b) from the best four patches. The data from four patches were pooled to construct the histogram for each value. The histograms were fitted by one or two exponential curve(s) by means of least square analysis. Under the control conditions, the histogram was fitted by single exponential curve and the time constant (τ) is given. In the presence of YC-170, the histogram was fitted by two exponentials, and the time constant of the fast component (τ_f) and that of the slow component (τ_s) are given. Lower panels: Open-time histograms of Ca^{2+} channels at HP of −40 mV under control conditions (c) and in the presence of YC-170 (10 μM) (d) from the best three patches.

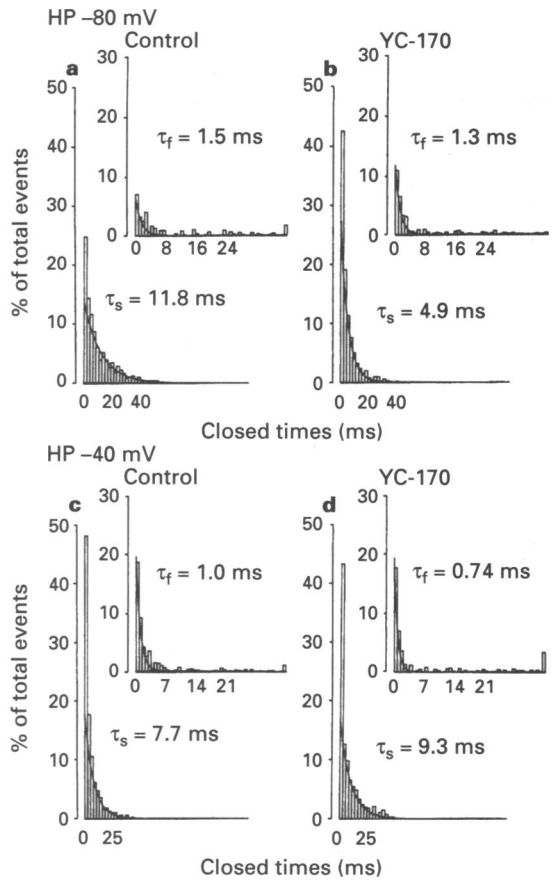

Figure 5 Upper panels: Closed-time histograms of Ca^{2+} channels at HP of −80 mV under control conditions (a) and in the presence of YC-170 (10 μM) (b) from the best four patches. The histograms were fitted with two exponential curves and the time constant of the fast component (τ_f), and that of the slow component (τ_s) are given. Lower panels: Closed-time histograms of Ca^{2+} channels at HP of −40 mV under control conditions (c) and in the presence of YC-170 (10 μM) from the best three patches (d).

strated that the voltage-dependent decrease (or reverse) of the Ca^{2+} agonist action of YC-170 and CGP 28392 may be mainly attributed to a voltage-dependent effect on closed times by the two DHPs. However, the mechanisms of these drug effects are still complicated. At HP of −80 mV, at which they increased NP_o, they shortened the slow time constant of closed time kinetics in addition to prolonging open times. Therefore, YC-170 and CGP 28392 possess two different mechanisms for the Ca^{2+} agonist effect.

Kass (1987) showed that the Ca^{2+} channel agonist effects of DHPs were attenuated at less negative holding potentials in a whole cell voltage-clamp study. At the single channel level, Hess *et al.* (1984) proposed the modal hypothesis in which the

Table 1 Effects of YC-170 (10 μM), Bay K 8644 (1 μM) and CGP 28392 (10 μM) on time constants of open-closed kinetics of Ca^{2+} channels

	Time constant for open times (ms)				*Time constant for closed times* (ms)			
	HP=−80 mV		*HP=−40 mV*		*HP=−80 mV*		*HP=−40 mV*	
	fast	*slow*	*fast*	*slow*	*fast*	*slow*	*fast*	*slow*
Control	0.36±0.03	–	0.47±0.07	–	3.0±0.8	16.3±1.1	1.5±0.4	8.9±0.7
YC-170 (10 μM)	0.62±0.05*	6.6±1.4	0.50±0.08	4.2±0.5	2.0±0.5	8.5±2.6*	2.1±0.6	13.3±0.5*
(*n*)	(4)		(5)		(4)		(5)	
Control	0.48±0.13	–	0.54±0.01	–	2.1±0.6	14.4±0.4	2.2±0.3	14.2±1.1
Bay K 8644 (1 μM)	0.64±0.13	5.8±2.3	1.07±0.03*	9.7±1.3	1.5±0.2	8.2±1.6*	2.1±0.4	10.7±0.7
(*n*)	(3)		(3)		(3)		(3)	
Control	0.50±0.03	–	0.52±0.07	–	1.7±0.1	12.0±2.5	1.9±0.4	9.1±1.1
CGP 28392 (10 μM)	0.68±0.15	6.3±1.3	0.76±0.15	9.4±2.2	1.9±0.4	9.6±2.2*	2.7±0.4	15.3±1.2*
(*n*)	(5)		(5)		(5)		(5)	

The slow time constants in control were absent because open time distributions in control were fitted by one exponential curve. Mean±s.e. *$P<0.05$ vs. control.

DHP Ca^{2+} agonists enhance the Ca^{2+} channel activity through prolongation of the open time of channels, but they did not show the relationship between the prolongation of open time and the level of the membrane potentials. On the other hand, Sanguinetti *et al.* (1986) showed that Bay K 8644 shifted the activation and inactivation curves of the Ca^{2+} current to negative potentials, and proposed a 'C-O-I' sequential model which provides a reasonable background for explaining how these shifts produced the prolongation of open time and the voltage-dependent attenuation of its agonist effect. In either hypothesis, it is speculated that the prolongation of open time might be attenuated at less negative potentials, since the probability of inactivated state (I) increases with less negative potentials. However, the present results showed that the three DHPs prolonged the open times of Ca^{2+} channel even at HP of -40 mV, at which YC-170 decreased NP_o. Therefore, another mechanism may contribute to the voltage-dependent attenuation of their agonist action.

Brown *et al.* (1984) showed that CGP 28392 and Bay K 8644 enhanced reopening of the L-type Ca^{2+} channels and the enhanced reopening resulted in their agonist effects. Brown *et al.* (1986) also showed that nitrendipine, a DHP Ca^{2+} antagonist, enhanced reopening of the Ca^{2+} channel, in addition to prolongation of openings. They postulated two affinity sites of the Ca^{2+} channel to DHP; a site mediating a stimulatory effect due to prolonged openings, and another site mediating stimulatory or inhibitory effects depending upon the membrane potentials. Tohse *et al.* (1991) reported that Bay K 8644 exerted its agonist action on the L-type Ca^{2+} channels of chick embryonic cells without the prolongation of the mean open time. We concluded that Bay K 8644 increased recruitment of functional channels because the L-type Ca^{2+} channels in chick embryonic cells have the characteristic property of exhibiting spontaneous long-lasting opening (Tohse & Sperelakis, 1990; Tohse *et al.*, 1992). In the other words, these reports indicate in common that the DHP agonists may increase the number of opening events per unitary time (a frequency of opening). The increase in the frequency of opening is independent of the prolongation of the mean open time, and seems likely to be a candidate mechanism for the voltage-dependence of the agonist effect. In the present study, we showed that the reduction of closed times by YC-170 was reversed with depolarization of HP, and related to their agonist action. The reduction of closed times means an enhancement of reopenings of channels.

It was reported that the stereoisomers of some DHP derivatives including Bay K 8644, have opposing actions (agonist and antagonist actions). YC-170 and CGP 28392 used in this study were also racemic mixtures, so it was possible that the voltage-dependence of the agonist action might be caused by the differential effects of the stereoisomers. However, it seems that the voltage-dependence of the agonist action of DHP agonists cannot be explained only by the differential effects of stereoisomers, because the pure agonist enantiomers of 202-791 and Bay K 8644 were reported to exert voltage-dependent effects on Ca^{2+} channels (Kokubun *et al.*, 1986; Sanguinetti *et al.*, 1986).

In the present study, YC-170 enhanced the activities of the Ca^{2+} channels of rabbit ventricular myocytes at HP of -80 mV while it did not enhance those at HP of -40 mV. In our previous report (Nakaya *et al.*, 1986), YC-170 did not increase the force of contraction in normally-polarized guinea-pig papillary muscles. However, YC-170 markedly increased the force of the contraction in normally-polarized rabbit papillary muscles ($n=2$, unpublished observation). Therefore, there may be species differences between rabbit and guinea-pig in the agonist effect produced by YC-170.

In conclusion, it is suggested that two mechanisms may be involved in the agonist actions of YC-170. One is an increase in the mean open time of Ca^{2+} channels and the other is an increase in frequency of openings of Ca^{2+} channels. The voltage-dependence of agonist effects of DHP Ca^{2+} channel agonists may be mainly due to the voltage-dependence of the latter mechanism. YC-170, having steeper voltage-dependence of its enhancement of channel reopening would be a good candidate for the management of AMI.

The authors wish to thank Ms Y. Yonezawa and Ms R. Yamazaki for their excellent secretarial assistant.

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(Received July 29, 1994
Revised April 28, 1995
Accepted June 16, 1995)