Roles of $\alpha_1$ and $\alpha_1/\beta$ Subunits Derived from Cardiac L-Type Ca$^{2+}$ Channels on Voltage-Dependent Facilitation Mechanisms

Tae-Seong LEE, Katsushige ONO, Tetsuo HADAMA*, Yuzo UCHIDA†, and Makoto ARITA

Departments of Physiology, * Cardiovascular Surgery, and † Surgery, Oita Medical University, Hasama, Oita, 879-5593 Japan

Abstract: Strong depolarization pulses facilitate L-type Ca$^{2+}$ channels in various cell types including cardiac myocytes. The mechanisms underlying prepulse facilitation are controversial with respect to the requirements for channel subunits, cAMP-dependent protein kinase, and additional anchor proteins. The properties of voltage-dependent facilitation of the L-type Ca$^{2+}$ channel was studied in recombinant cardiac $\alpha_1$ subunits with or without cardiac $\beta$ subunit, expressed in Chinese hamster fibroblast cells. The magnitude of voltage-dependent $I_{Ba}$ facilitation in the $\alpha_1$ subunit channel is dependent on the duration of the prepulse as well as on the interval duration between prepulse and test pulse. The characteristics of this facilitation were not affected by coexpression of the $\beta$ subunit. These results indicate that cardiac $\alpha_1$ subunits exhibit voltage-dependent facilitation because of their own intrinsic structure, independent of any other accessory subunit or additional regulatory proteins, and that cardiac $\beta$ subunits have no essential regulatory role at the onset or continuance of the voltage-dependent facilitation. [Japanese Journal of Physiology, 51, 337–344, 2001]

Key words: calcium channel, facilitation, $\alpha_1$ subunit, $\beta$ subunit, Chinese hamster fibroblast (CHW) cells.

The high-threshold (L-type) voltage-dependent cardiac calcium (Ca$^{2+}$) channel plays a crucial role in excitation–contraction coupling in cardiac and other muscles. The cardiac Ca$^{2+}$ channel comprises four subunits: $\alpha_1$, $\beta$, and $\alpha_2/\delta$. The pore-forming $\alpha_1$ subunit involves the most important functional elements of the L-type Ca$^{2+}$ channel, including a selectivity filter, a voltage sensor, a motif for Ca$^{2+}$-dependent inactivation, and a binding site for dihydropyridines (DHPs) [1–3]. The functional role of the $\alpha_2/\delta$ subunit is less clear; some investigators [4, 5] have reported that it modifies the $\alpha_1$ subunit function; thus the activation times of the barium current could be reduced 50%. Others claim that the $\alpha_2/\delta$ subunit plays a role in the acceleration of current activation and inactivation [6]. The cardiac $\alpha_1$ subunit current, generated by interactions with or without specific $\beta$ subunits, has been studied with a variety of expression systems. A homologous coexpression of the cardiac $\alpha_1$ subunit ($\alpha_{1c}$) with the cardiac $\beta$ subunit ($\beta_{2a}$) increased both the amplitude of the Ba$^{2+}$ current and the rate of channel activation [7, 8]. A coexpression of the cardiac $\alpha_1$ subunit ($\alpha_{1c}$) with the skeletal $\beta$ subunit ($\beta_1$) similarly increased the density of the L-type Ca$^{2+}$ current [4, 5] and increased the rate of inactivation [4, 9]. Moreover, Ca$^{2+}$ currents of cardiac $\alpha_1+\alpha_2$ subunit combinations were increased in the presence of the brain/cardiac $\beta$ subunit ($\beta_3$) [8]. These findings suggest that although their structures are different, $\beta_1$, $\beta_2$, and $\beta_3$ subunits play similar regulatory roles in the kinetic modulation of channel openings by interacting with the $\alpha_1$ subunit.
The activity of L-type Ca\(^{2+}\) channels can be influenced by strong predepolarization. This interesting and unique property of Ca\(^{2+}\) channel behavior is known as facilitation. Although there is general agreement about prepulse-induced changes in gating kinetics as a phenomenon underlying the increase of whole-cell current, the responsible molecular mechanisms are a matter of controversy. The \(\alpha_1\) subunit exhibits a primary function common to that of the native L-type Ca\(^{2+}\) channel, but it remains uncertain whether the \(\alpha_1\) subunit alone can produce voltage-dependent facilitation of the current in L-type Ca\(^{2+}\) channels. Furthermore, much less is known of the function of the \(\beta\) subunit on facilitation mechanisms. In an early study of the rat cardiac \(\alpha_{1C}\) subunit, it was concluded that the \(\alpha_{1C}\) subunit was unable to produce voltage-dependent facilitation, regardless of the presence or absence of the cardiac \(\beta\) subunit, but the other isoforms (\(\beta_1\), \(\beta_3\), \(\beta_4\)) were all capable of producing facilitation when combined with the \(\alpha_{1C}\) subunit [10]. Other investigations concluded that cardiac \(\alpha_{1C}\) subunits exhibit voltage-dependent facilitation only when coexpressed with auxiliary \(\beta\) subunits (\(\beta_1\) or \(\beta_2\)) [11, 12]. Thus the obvious question of importance is whether the \(\alpha_1\) subunit alone is able to produce voltage-dependent facilitation, or whether the \(\beta\) subunit, which is coexpressed with \(\alpha_1\), affects the evolution of the facilitation. We therefore performed experiments by using Chinese hamster fibroblast (CHW) cells permanently transfected with cDNA of the cardiac \(\alpha_1\) subunit alone or of the \(\alpha_1\) subunit together with the cardiac \(\beta_2\) (\(\beta_{2a}\)) subunit.

**METHODS**

**Cell transfection and cell culture.** Chinese hamster fibroblast (CHW 1102) cells were maintained in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, and 100 \(\mu\)g/ml streptomycin in an atmosphere of 95% O\(_2\) plus 5% CO\(_2\) at 37°C. CHW cells were stably transfected either with class 1C-a (rabbit cardiac) \(\alpha_1\) subunit alone (CHW \(\alpha_1\)) or with class 1C-a \(\alpha_1\) subunit and \(\beta\) type 2 (rabbit cardiac \(\beta_{2a}\)) subunit (CHW \(\alpha_1/\beta\)) of the L-type calcium channel. The construction of plasmids carrying the cDNAs encoding the cardiac \(\alpha_1\) and cardiac \(\beta\) subunits have been described previously [13]. The cDNA encoding the rabbit cardiac L-type Ca\(^{2+}\) channel \(\alpha_1\) subunit [5], which was inserted into the mammalian expression vector, pKHN, and the rabbit cardiac L-type Ca\(^{2+}\) channel \(\beta_{2a}\) subunit cDNA [14], which was subcloned into p912023(B), were kindly provided by Dr. L. Birnbaumer. CHW cells were cotransfected with 20 \(\mu\)g of \(\alpha_{10}/pKNH\) and 20 \(\mu\)g of \(\beta_{2a}/p912023(B)\) by using the calcium phosphate precipitation method. Transfected cells were selected because of their resistance to DMEM supplemented with 10% dialyzed FCS and 300 \(\mu\)g/ml G418 (a neomycin analogue).

**Electrophysiology.** CHW cells were seeded onto glass-bottom dishes and incubated in culture medium for 3 to 48 h. Whole-cell patch configuration was used to record the macroscopic current from transfected cells (EPC-8, HEKA Elektronik, Lambrecht, Germany). Patch pipettes with resistances ranging from 1.5 to 4 MΩ were used for recording. Series resistance was compensated electrically, as much as possible, without oscillation (60 to 75%). Capacitive artifacts were minimized by using built-in circuitry of the amplifier. The remaining transients and linear leakage currents were eliminated by using p/4 subtraction (Pulse/Pulsefit, HEKA Elektronik). In all protocols, the holding potential was clamped at −80 mV. To plot the current–voltage relationships, transfected CHW cells were stimulated by 500 ms of square pulses from −60 to +80 mV (increment=10 mV) with 5 s of sweep interval. To investigate the relationship between the prepulse potential and facilitation, 400 ms of prepulses from −80 to +150 mV (increment=10 mV) and 500 ms of a test pulse at +30 mV with 10 ms of interval were applied with 5 s of sweep interval. To investigate the relationship between prepulse duration and facilitation, variable durations (from 0 to 500 ms) of prepulses at +100 mV were applied 10 ms before 500 ms of the test pulse at +30 mV with 5 s of sweep interval. To investigate the facilitation, depending on duration of the interval between the prepulse and the test pulse, variable interval durations (from 5 to 1,000 ms) were inserted between pulses with 5 s of sweep interval. The amplifier output was filtered at 5 kHz and digitally sampled at 10 kHz by using an ITC-16 interface (Instrutech Corp., Greatneck, NY, USA), and stored on a 90-MHz Pentium–based computer under control of a data acquisition program (Pulse/Pulsefit, HEKA Elektronik). The data are expressed as mean±standard deviation (SD) and were analyzed by using paired and unpaired t-tests, or by analysis of variance with a nesting design, when appropriate. Statistical significance was defined by \(p<0.05\). All experiments were conducted at room temperature (20 to 23°C).

**Solutions.** The recording chamber was filled with a solution of the following components (mM): BaCl\(_2\) (40), MgCl\(_2\) (1.0), tetraethylammonium chloride (TEA-Cl) (80), 4-aminopyridine (4-AP) (5.0), glucose (10), HEPES (10), and 4,4′-diisothiocyanato-
stilbene-2,2’-disulfonic acid (DIDS) (0.1). (The pH was adjusted to 7.3 with 0.1 N TEA-OH.) The patch-clamp electrode contained (mM): CsCl (130), MgCl2 (2.0), ATP-Mg (2.0), GTP-Na2 (0.5), EGTA (5.0), and HEPES (5.0). (The pH was adjusted to 7.3 with 0.1 N TEA-OH.) TEA-Cl and DIDS were added to block any existing endogenous K+ and Cl− channels. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

RESULTS

Transfection of CHW cells with cDNA encoding either the α1 or α1 plus β subunits of the cardiac Ca2+ channel has led to the stable expression of functional L-type Ca2+ channels through more than 40 passages. Cell capacitance was 14.5±2.1 pF for cells expressing the α1 subunit (n=12), and it was 15.9±3.4 pF for cells expressing both the α1 and β subunits (n=16), values that are statistically identical. Throughout the observation period for up to 40 passages, cell capacitance was unchanged (data not shown). Functional cardiac L-type Ca2+ channels were reconstituted in CHW cells by use of the calcium phosphate precipitation method. Because the existence of any intrinsic β subunits in CHW cells interferes with current evaluations in this heterologous expression system, the expression of detectable mRNAs for α1 and α1/β subunits was confirmed in advance by Northern analysis in our stable cell lines (α1 cell, α1/β cell) by collaborators in a previous study [13, 15], and the lack of any detectable mRNA for these subunits in untransfected CHW cells has also been confirmed [13]. During a typical test pulse from a holding potential (VH) of −80 mV to a test potential (VT) of +30 mV in a recording solution containing 40 mM Ba2+, the Ba2+ current (Iba) in the α1 subunit channel displayed very little decay phase or inactivation (Fig. 1A). The coexpression of the β subunit with α1, however, consistently enhanced the expressed current and caused a shift of the current–voltage (I–V) curve in the hyperpolarized direction by 20 mV (Fig. 1B). The maximum peak current was 123±27 pA in the α1 subunit current (α1 current) and was 357±67 pA in the α1/β subunit current (α1/β current). This result implies a modulation by and a synergy of the β subunit with the pore-forming α1 subunit of the L-type Ca2+ channel, which is consistent with most documented functions of the β subunit in the L-type Ca2+ channel.

The application of a positive prepulse of +150 mV

Fig. 1. A: Typical whole-cell current records in Chinese hamster fibroblast (CHW) cells expressing cardiac α1 and α1/β L-type Ca2+ channels in the presence of 40 mM Ba2+ during a test pulse to +30 mV from a holding potential of −80 mV. B: Current–voltage relationships with 40 mM Ba2+ are shown by peak current mean±SD from CHW cells expressing α1 subunit alone (α1 current: ○) and α1/β subunits (α1/β current: ●). The maximum peak current was 123±27 pA (8.5±1.9 pA/pF: n=11) at +30 mV in the α1 current and 357±67 pA (22.4±4.2 pA/pF: n=8) at +10 mV in the α1/β current. C: Prepulse-dependent facilitation of α1 and α1/β channel currents. Each panel of the figure shows responses to a test pulse at +30 mV with (●) or without (○) a prepulse at +150 mV, as shown in the pulse protocol (upper).
for a 400 ms duration revealed that the $I_{Ba}$ amplitude during a subsequent test pulse was increased in the $\alpha_1$ current as well as in the $\alpha_1/\beta$ current (Fig. 1C). The amplitude of the facilitation current was strongly voltage-dependent with 400 ms prepulses from $+40$ to $+150$ mV (data not shown). The prepulse-dependent facilitation of $I_{Ba}$ demonstrated in $\alpha_1$ and $\alpha_1/\beta$ cells was found to be similar to that for facilitated native cardiac L-type channels [16]. Although the prepulse-dependent facilitation of $I_{Ba}$ both in $\alpha_1$ and $\alpha_1/\beta$ channels is apparent, it is unclear whether the increase in the peak inward current following conditioning depolarizations can be modified by the duration of the prepulse of $+150$ mV. Because a prepulse of highly depolarized potentials could inactivate the Ca$^{2+}$ channel at the same time, interactions of channel facilitation and inactivation might be affected by depolarization durations.

To determine the time course of development and decline of current facilitation, the durations of 100 mV prepulses are varied from 0 ms (no pulse) to 500 ms either in the $\alpha_1$ or the $\alpha_1/\beta$ channel (Fig. 2). The interstimulus interval was maintained at 5 s to allow complete recovery from potentiation and/or inactivation. The increase in peak inward current following prepulses of increasing duration was limited up to approximately 300 ms duration, usually either in the $\alpha_1$ or $\alpha_1/\beta$ channel (Fig. 2, C, D). This is probably affected by the slow inactivation of both the $\alpha_1$ and $\alpha_1/\beta$ channels. Nevertheless, $I_{Ba}$ was not greatly diminished by depolarized prepulses up to 400 ms either in the $\alpha_1$ or $\alpha_1/\beta$ channel. With longer (>400 ms) prepulse duration, $I_{Ba}$ in $\alpha_1/\beta$ channel became smaller in size than that without the prepulse, though a difference between $\alpha_1$ and $\alpha_1/\beta$ currents was not elucidated (Fig. 2F).

The reversal of facilitation was studied by a change of repolarization intervals at $-80$ mV (Fig. 3). The extent of facilitation declined with the length of the interval between the prepulse and the test pulse. Prepulses from $-80$ to $+100$ mV, followed by an interval of 10 ms at $-80$ mV, facilitated $I_{Ba}$ by 1.5 to 2.0 times in most cases, either in the $\alpha_1$ or $\alpha_1/\beta$ channel, and the

![Fig. 2. Dependence of $I_{Ba}$ facilitation on prepulse duration of $\alpha_1$ and $\alpha_1/\beta$ currents. A, B: Original demonstration of enhancement of $I_{Ba}$ by various prepulse durations (from nil to 500 ms) in $\alpha_1$ current (A) and $\alpha_1/\beta$ current (B). The left upper trace shows the command potential. C, D: Current traces by test pulses at $+30$ mV following prepulse, which were obtained from A and B with different time and current scales. Note that the $\alpha_1$ current (C) and the $\alpha_1/\beta$ current (D) are shown by different current scales. E: Current ($I_{Ba}$)-prepulse duration relation of $\alpha_1$ (○) and $\alpha_1/\beta$ current (●) by number of cells indicated, which was obtained by the protocol shown in the upper inset. Values are expressed as mean±SD. Error bars were placed only at 0 and 500 ms of prepulse duration for clarity. *: p<0.05. F: Normalized $I_{Ba}$-prepulse duration relation of $\alpha_1$ (○) and $\alpha_1/\beta$ current (●) from plots in E. #: p=0.25.](image-url)
facilitation was maximum at this interval duration. The facilitation current was decreased when the interval duration was prolonged (Fig. 3E). The prepulses of +100 mV for 400 ms did not augment $I_{Ba}$ in most cases when the interval duration was 200 ms or longer, regardless of the presence or absence of the $\beta$ subunit (Fig. 3F). The reduction of the facilitated $I_{Ba}$, depending on interval duration, had a pair of time courses: fast and slow. The fast-declining phases were well fitted by a single exponential curve either in the $\alpha_1$ or $\alpha_1/\beta$ channel, reflecting a possible waning phase of facilitation. The waning phase of the facilitation of $\alpha_1/\beta$ current ($\tau=11.7$ ms) was marginally faster than that of $\alpha_1$ current ($\tau=15.0$ ms). The other slow time course, however, continued for 1,000 ms in interval duration (data not shown). The amplitudes of $I_{Ba}$ when followed by an interval of more than 100 ms were smaller than those without the prepulse, indicating that this slow phase may include reduction of the current because of the incomplete recovery from channel inactivation.

DISCUSSION

By the use of the cDNA of the DHP-sensitive L-type Ca$^{2+}$ channel $\alpha_1$ subunit derived from rabbit cardiac muscle, the present study demonstrated for the first time that the $\alpha_1$ subunit channel, which was expressed in CHW cells, exhibited voltage-dependent facilitation independently of the presence of the $\beta$ subunit, and cardiac $\beta$ subunits have no essential regulatory role in this mechanism. Even though the cardiac L-type Ca$^{2+}$ channel is composed of the heterotrameric subunits of $\alpha_1$, $\beta$, and $\alpha_2/\delta$, the $\alpha_1$ subunit is sufficient for the generation of voltage-dependent facilitation of the current because of its own molecular conformation that responds to changes in the transmembrane depolarizing potentials.

The activity of the L-type Ca$^{2+}$ channels can be potentiated by high-voltage pulse(s) or frequent stimulations, known as “facilitation” [16, 17]. Facilitation has been considered to be a form of positive feedback and to represent an apparently unique property of Ca$^{2+}$
channels. Facilitation induced by strong depolarizations has been described not only in the cardiac L-type Ca\(^{2+}\) channel, but also in various other L-type Ca\(^{2+}\) channel types [18–20]. Multiple molecular mechanisms have been suggested to underlie facilitation of L-type Ca\(^{2+}\) channels. In cardiac myocytes, a Ca\(^{2+}\)-dependent facilitation and a voltage-dependent (Ca\(^{2+}\)-independent) facilitation of L-type Ca\(^{2+}\) channels have been described [16, 21–23]. Previous investigations using cardiac myocytes have suggested that this Ca\(^{2+}\)-dependent facilitation is due to the activation of Ca\(^{2+}\)-calmodulin protein kinase II, which phosphorylates the Ca\(^{2+}\) channels and leads to an increase in Ca\(^{2+}\) current [24–26]. Alternatively, the voltage-dependent (Ca\(^{2+}\)-independent) facilitation of the L-type Ca\(^{2+}\) channel in cardiac myocytes may be due to a voltage-dependent conformational switch of the channel protein, leading to altered gating [16]. The mechanism underlying prepulse-induced facilitation, however, is controversial with respect to the requirements of channel subunits; it has been suggested that multiple molecular mechanisms underlie the facilitation of L-type Ca\(^{2+}\) channels. Previous studies using heterologous expression systems have provided additional or even controversial information on voltage-dependent facilitation of the L-type Ca\(^{2+}\) channel in regard to the role of the β subunit. Two previous studies expressing rabbit α\(_{1C}\) subunit coexpressed with β\(_{2a}\) or β\(_{3}\) subunit failed to detect voltage-dependent facilitation [27, 28]. A recent study, however, reported that the cardiac Ca\(^{2+}\) channel α\(_{1C}\) subunit exhibited voltage-dependent facilitation only when coexpressed with β\(_{1a}\) or β\(_{3a}\) subunit [12], which is in sharp contrast to this study. The apparent difference between these studies and the present one may be attributed to the methodological difference between the studies, and differences in α\(_{1C}\) isoforms. Kamp et al. [12] used a rabbit cardiac α\(_{1C}\) isoform that has alternative splicing in domain IV S3, whereas the full structure of the rabbit α\(_{1C}\) isoform was used in the present study.

Alternatively, and even more important, differences in experimental protocol can modify facilitation results, especially in evaluating the role of the β subunit. The functional role of the Ca\(^{2+}\) channel β subunit, coexpressed with the α\(_{1}\) subunit, has been studied with regard to current amplitude, rate of activation, rate of inactivation, number of DHP binding sites, and even the “maturation” of channels [7, 27]. In the present study, a coexpression of the β subunit did not affect the basic characteristics of the facilitated current: The dependence of the prepulse durations and time-dependent abolition of the facilitation after prepulse were nearly identical with or without the β subunit. The increase in the net inward current in the presence of the β subunit was probably due to the increased number of channels expressed and to modified activation/inactivation kinetics, which have been studied extensively in previous studies [1, 4–6]. Because the coexistence of the β subunit did not alter the fundamental mechanisms of facilitation of the Ca\(^{2+}\) current, it is assumed that the α\(_{1}\) subunit has a key molecular structure that produces voltage-dependent facilitation, and the β subunit adds no functional role to this mechanism. In assessing the role of the β subunit on facilitation, it is especially important to exclude any additional modulatory function of the β subunit on the α\(_{1}\) subunit current. We carefully chose the test potential of +30 mV to minimize the effect of the shift of the activation curve in the presence of the β subunit. The chosen test potential was in the voltage range where the maximum chord conductance should be observed both in the α\(_{1}\) and α\(_{1}/β\) channel currents (Fig. 1B). Consequently, improper protocols for the voltage-dependent facilitation in α\(_{1}\) and α\(_{1}/β\) channels might exaggerate the function of the β subunit on the channel activation and may mask the intrinsic mechanism of the α\(_{1}\) subunit on facilitation, notably at the hyperpolarized potentials for the test pulse. We chose the prepulse potential of +100 mV for the studies of prepulse duration (Fig. 2), and of interval duration (Fig. 3) on the changes of the facilitation current. As a result of our own experiments, we found that \(I_{Ba}\) of the α\(_{1}\) or α\(_{1}/β\) channel at +30 mV after the prepulse of +100 mV was smaller than that without prepulse (data not shown). Therefore the demonstration of the nonuniform changes in the test pulse current as a function of the prepulse potentials would provide evidence that facilitation modulation of the Ca\(^{2+}\) channel would have appeared at even more hyperpolarized potentials, instead of only at highly depolarized potentials that displayed apparent current augmentation, which apparently interacted with changes resulting from steady-state inactivation. Namely, the relationship between prepulse potential and facilitation current examined by standard two-pulse protocols, similar to the examination of the relationship between membrane potential and steady-state inactivation, reveals the U-shaped relationship, which has been reported for the relationship between the prepulse voltage and the \(I_{Ca}\) inactivation in cardiac myocytes [23, 29, 30]. These mechanisms can be described by a combined regulation of the Ca\(^{2+}\)-dependent potentiation and the voltage-dependent (Ca\(^{2+}\)-independent) facilitation [12, 17, 23]. Because we used Ba\(^{2+}\) as the charge carrier throughout the experiments, we observed only a voltage-dependent facilitation of the ex-
pressed channels in this study, instead of a Ca$^{2+}$-dependent potentiation. An important conclusion is that facilitation of the L-type Ca$^{2+}$ channel current is purely voltage-dependent in guinea-pig ventricular cells [22] and in frog ventricular cells where facilitation can occur even when Ba$^{2+}$ replaces Ca$^{2+}$ as the charge carrier [31].

It is possible that evolution of the current facilitation is influenced by the cell’s metabolic state because the L-type Ca$^{2+}$ channel current is known to be affected by phosphorylation [32, 33]. The literature has some discrepancies with regard to the contribution of channel phosphorylation to voltage-dependent facilitation. Facilitation in guinea-pig ventricular cells [34] and recombinant α$\text{1}/\beta_{1,2a}$ channels [12] develops independently of channel phosphorylation. It has been suggested, however, in chromaffin cells that phosphorylation is the main mechanism underlying the prepulse-induced facilitation of the L-type Ca$^{2+}$ channel [18]. Previous investigations have demonstrated that protein kinase does not regulate L-type Ca$^{2+}$ channels expressed in mammalian cells unless additional regulatory proteins are present, such as AKAP79 [28, 35]. In the present study, we expressed α$\text{1}$ and α$\text{1}/\beta$ subunits in Chinese hamster fibroblast cells; very little AKAP was detected by immunofluorescent staining in these cell lines (personal communications). Furthermore, it has been suggested that basal phosphorylation activity or protein kinase activity was very small in native Chinese hamster fibroblast cells. Therefore the present study examined voltage-dependent facilitation under conditions where voltage-dependent phosphorylation by protein kinase might not occur. Both the cardiac α$\text{1}$ and β subunits contain multiple potential phosphorylation sites [33], although it is still not known which subunit(s) of the DHP-sensitive Ca$^{2+}$ channel have the greatest responsibility for regulating the channels mediated by protein phosphorylation. Since our present study did not include an examination of the effect of activation or inhibition of cAMP-dependent protein kinase, we could draw no conclusions regarding the contribution of phosphorylation to the appearance of voltage-dependent facilitation. Further studies are required to understand how each subunit (and its phosphorylation) may additionally modulate the mechanisms giving rise to facilitation in Ca$^{2+}$ channels. When these results are considered together, the facilitation currents of the α$\text{1}$ subunit channel in the present study are smaller than those in the wild-type L-type Ca$^{2+}$ channel in cardiac myocytes. Although our present study demonstrates that the α$\text{1}$ subunit alone can produce current facilitation after the depolarization pulse, the mechanism for the voltage-dependent facilitation may not be due solely to the α$\text{1}$ subunit. Other unknown factors or subunits, exclusive of the β subunit, could be candidates for this mechanism.

In conclusion, we clearly demonstrated that the cardiac α$\text{1}$ subunit of the DHP-sensitive Ca$^{2+}$ channel exhibits voltage-dependent facilitation, and that this characteristic is unaffected by the presence or absence of the cardiac β subunit. The underlying submolecular mechanism of the α$\text{1}$ subunit remains to be determined.

We sincerely thank Dr. Stuart Green for providing the stable cell lines expression rabbit cardiac L-type Ca$^{2+}$ channel α$\text{1C}$ subunit and α$\text{1C}/\beta_{2}$ subunits. This study was supported in part by the Japan Heart Foundation and an IBM Japan Research Grant (to K.O.), and by Grants-in-Aid for Scientific Research (Nos. 07807008, 09670049) from the Ministry of Education, Science, Sports and Culture of Japan (to K.O.).

REFERENCES

10. Cens T, Managoni ME, Richard S, Nargeot J, and Charnet P: Coexpression of the β2 subunit does not induce voltage-dependent facilitation of the class C L-type Ca$^{2+}$ channel.


31. Schouten VJA and Morad M: Regulation of Ca²⁺ current in frog ventricular myocytes by the holding potential, cAMP, and frequency. Pflügers Arch 415: 1–11, 1989


