The Gating and Conductance Properties of $\text{Ca}_v3.2$ Low-Voltage-Activated T-Type Calcium Channels

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Abstract: Calcium channels are essential for excitation-contraction coupling and pacemaker potentials in cardiac muscle cells. Whereas L-type Ca^{2+} channels have been extensively studied, T-type channels have been poorly characterized in cardiac myocytes. We describe here the functional properties of recombinant $\text{Ca}_v3.2$ T-type Ca^{2+} channels expressed in mammalian cell lines. The T-type Ca^{2+} current showed a rapid activation and an inactivation phase in response to depolarization, and it displayed a window current over the voltage range from -60 to -40 mV in 1 to 10 mM external Ca^{2+} . Barium (Ba^{2+}) and strontium (Sr^{2+}) permeate the channel with similar activation kinetics. On the other hand, monovalent cations, Li^+ and Na^+ , permeate the T-type Ca^{2+} channel more easily than the L-type Ca^{2+} channel. The permeability order of the $\text{Ca}_v3.2$ T-

type Ca^{2+} channel among monovalent and divalent cations was determined as $\text{Ba}^{2+} > \text{Mn}^{2+} > \text{Ca}^{2+} > \text{Sr}^{2+} > \text{Li}^+ > \text{Na}^+$ with the permeability order of 1.39:1.25:1.00:0.95:0.55:0.29. The ionic conductance sequence for cations relative to calcium was $\text{Sr}^{2+} > \text{Ba}^{2+} > \text{Ca}^{2+} > \text{Li}^+ > \text{Mn}^{2+} > \text{Na}^+$ with the conductance ratio of 1.39:1.21:1.00:0.40:0.23:0.11. The permeation profile of manganese (Mn^{2+}) is complex. Mn^{2+} permeates the Ca^{2+} channel with a permeability similar to Ca^{2+} or Ba^{2+} , but with a much smaller current density, resulting in a much smaller conductance. The properties relating to progression and recovery from inactivation in the $\text{Ca}_v3.2$ channel are substantially identical with either Ca^{2+} or Ba^{2+} as the charge carrier. [The Japanese Journal of Physiology 53: 165–172, 2003]

Key words: calcium channel, monovalent cations, divalent cations, permeability, conductance.

Among the several types of voltage-dependent Ca^{2+} channels identified in various excitable cells, only L-type and T-type Ca^{2+} channels are expressed in cardiac myocytes. The influx of Ca^{2+} ions through these voltage-gated Ca^{2+} channels during an action potential serves to trigger the Ca^{2+} transient and to regulate the intracellular calcium store in cardiac myocytes. In contrast to the L-type Ca^{2+} channel, specific blockers have not been available for the low-voltage-activated T-type Ca^{2+} channel. T-type Ca^{2+} channels can be activated by small depolarizations near the maximum diastolic potential or the resting membrane potential of myocytes and could help to generate pacemaker activities in nodal cells in the heart [1]. In sinoatrial and atrioventricular nodal cells, Ca^{2+} currents are thought to play an important role in the electrogenesis of im-

pulse generation [1]. In pathological conditions, such as cardiomyopathic myocytes and postinfarction myocytes, it is postulated that the excessive Ca^{2+} overload and the arrhythmias may be related to the abnormal expression of T-type Ca^{2+} channels in cardiac myocytes [2, 3].

Current traces of the T-type Ca^{2+} channel obtained by voltage-clamp methods in excitable cells inevitably contain other Ca^{2+} channel components to a certain degree. This is because the voltage range for the activation of the T-type Ca^{2+} channel and that for other Ca^{2+} channels widely overlap, which makes it difficult to identify the specific electrophysiological properties of the T-type Ca^{2+} channel. Several relatively specific antagonists for the L-type Ca^{2+} channel are helpful to distinguish the L-type current from other voltage-de-

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pendent Ca^{2+} channels. Indeed, dihydropyridine agonists and antagonists have helped to identify the L-type Ca^{2+} channel in cardiac myocytes and to demonstrate that it is encoded by $\alpha_1\text{C}$ ($\text{Ca}_v1.2$) cDNA [4]. Nevertheless, the precise description of T-type Ca^{2+} channel kinetics has not been achieved. The availability of recombinant T-type Ca^{2+} channels for expression in mammalian cell systems enables us to research their pharmacological and biophysical characteristics [5–7]. Genes encoding the T-type Ca^{2+} channel pore subunits have been identified and are designated $\alpha_1\text{G}$ ($\text{Ca}_v3.1$), $\alpha_1\text{H}$ ($\text{Ca}_v3.2$), and $\alpha_1\text{I}$ ($\text{Ca}_v3.3$) [5–7, 8–10]. Taking advantage of the ability to express a pure population of the recombinant T-type Ca^{2+} channels together with the use of voltage clamp protocols, we investigate activation and inactivation properties here, as well as the cationic permeability of the $\alpha_1\text{H}$ ($\text{Ca}_v3.2$) T-type Ca^{2+} channel.

MATERIALS AND METHODS

Expression of Ca^{2+} channel proteins and culture. The $\alpha_1\text{H}$ subunit derived from human heart, which forms cardiac T-type Ca^{2+} channels, was stably expressed in human embryonic kidney (HEK)-293 cells with no auxiliary subunits. A profile and a procedure for channel expression were described in detail in a previous report [5]. cDNA encoding the human cardiac $\alpha_1\text{H}$ subunit (*CACNA1H*) was inserted into the transfection vector pcDNA3, and HEK-293 cells were transfected with 2 μg of this vector by using the calcium phosphate precipitation method. Recombinant HEK-293 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% fetal calf serum, 100 U ml^{-1} penicillin, and 100 g ml^{-1} streptomycin, in an atmosphere of 95% O_2 plus 5% CO_2 at 37°C . This medium was supplemented with 300 g ml^{-1} G418 (neomycin analogue) for the selection of recombinant HEK-293 cells.

Electrophysiology. Recombinant cells were seeded onto glass-bottom dishes and incubated in a culture medium for 12–48 h before the electrophysiological measurements. Macroscopic Ca^{2+} channel currents were recorded in the whole-cell configuration by using an Axopatch 200A amplifier (Axon Instruments, Burlingame, CA, USA) at room temperature (22 – 25°C). Patch pipettes were pulled from 75 mm plain capillary tubes (Broomall, PA, USA) with a micropipette puller, Model P-97 (Sutter Instrument, Novato, CA, USA), and were fire-polished. The electrode had a resistance of 1.8 – $3.5\text{ M}\Omega$ when the pipette was filled with the pipette solution (see below). Series resistance was compensated electrically as much as possible

without oscillation (60–75%). Capacitance was canceled by the built-in circuitry of the amplifier. Current signals were usually filtered at 3.3 kHz and digitized at 10 kHz, and stored on a 90-MHz Intel Pentium-based computer using a Microsoft Windows 95 operating system under the control of a data acquisition program, pCLAMP 6.0 software, also from Axon Instruments. The statistical data are expressed as the mean $\pm$ standard error (SE).

Determination of permeability and conductance ratios. The ratio of the peak inward current in the presence of extracellular cation X to the peak inward current in the presence of Ca^{2+} was used to determine the relative conductance (G_X/G_{Ca}). The permeability ratio (P_X/P_{Ca}) for a given cation was calculated by using a modified Goldman-Hodgkin-Katz equation [11]:

$$E_X - E_{\text{Ca}} = RT/ZF \ln(P_X[X]_o/P_{\text{Ca}}[\text{Ca}]_o), \quad (1)$$

where E_X and E_{Ca} are the reversal potentials for the tested cation (X) and Ca^{2+} , respectively, Z is the valence of the tested cation, and R is the gas constant (in energy units), T , the absolute temperature, and F , Faraday's constant. The value of the factor RT/F in this equation is given as 25.69 mV at 25°C , or the experimental room temperature in this study. Permeability ratios can provide equivalent interpretations of ion selectivity [12]. Reversal potentials and slope conductance were calculated by fitting the current–voltage relationship to a Boltzmann distribution function:

$$I = G_{\text{max}}(V_m - V_{\text{rev}})/\{1 + \exp[(V_m - V_{0.5})/k]\}, \quad (2)$$

where I is the peak I_{CaT} at the given test potential V_m , V_{rev} is the reversal potential, G_{max} is the maximal slope conductance, $V_{0.5}$ is the half-point of the relationship, and k is the slope factor. Voltage-dependent inactivation was similarly determined with a Boltzmann equation:

$$I/I_{\text{max}} = 1/\{1 + \exp[(V_m - V_{0.5})/k]\}, \quad (3)$$

where V_m is the membrane potential, $V_{0.5}$ is the mid-potential, and k is the slope factor.

Solutions. The recording chamber was filled with a solution of the following components (mM): tetraethylammonium chloride (TEA-Cl) (120), 4-aminopyridine (4-AP) (5), glucose (10), HEPES (10) and 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS) (0.1), and a chlorine compound of a monovalent or divalent cation with the concentration shown in each experimental protocol. The pH was adjusted to 7.4 with 0.1 N TEA-OH. The patch-clamp electrode contained (mM): CsCl (130), MgCl_2 (2.0), ATP (2.0), GTP (0.5), EGTA (5.0), and HEPES (5.0). The pH

Fig. 1. Current-voltage (I - V) relationship for human $\text{Ca}_v3.2$ channels. Representative whole-cell Ca^{2+} current (A) and Ba^{2+} current (B) obtained from a transfected HEK-293 cell at 10 s intervals in response to 400-ms depolariza-

tion pulses of 5 mV steps from a holding potential of -100 mV. I - V relationships were obtained from representative current traces with 0.1 – 50 mM Ca^{2+} (A) or Ba^{2+} (B).

was adjusted to 7.2 with 0.1 N Cs-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

Current activation and inactivation kinetics

Current activation kinetics were studied by using a depolarization pulse of 400-ms duration ranging from -100 to $+90$ mV in 5 mV steps applied from a holding potential of -100 mV (Fig. 1) with Ca^{2+} or Ba^{2+} as the charge carrier. As shown in Fig. 1, the T-type Ca^{2+} current became detectable at about -60 mV, peaked near -25 mV, and reversed at a membrane potential close to $+30$ mV with either Ca^{2+} or Ba^{2+} at a concentration of 10 mM as the charge carrier. The peak inward currents doubled as $[\text{Ca}^{2+}]_o$ was raised from 1 to 10 mM. A similar increase in the maximum peak current was observed when the concentration of external Ba^{2+} was changed to 10 mM, from 1 mM. The current-voltage relationships with Ca^{2+} and Ba^{2+} as the charge carrier are shown in Fig. 1 and indicate no remarkable differences in the voltage dependency of activation of the Ca^{2+} current and the Ba^{2+} current in the $\text{Ca}_v3.2$ channel. Raising $[\text{Ca}^{2+}]_o$ or $[\text{Ba}^{2+}]_o$ shifted the threshold for electrical dependence of Ca^{2+} channel activation, resulting in shifts of the maximum peak

potentials and of the reversal potentials with either Ca^{2+} or Ba^{2+} almost as if an electrical bias had been added to the membrane potential.

Steady-state inactivation kinetics were studied with either Ca^{2+} or Ba^{2+} as the charge carrier. We measured the channel availability by applying a 1,000-ms prepulse of various potentials prior to a test pulse at 0 mV. Figure 2 shows the activation and steady-state inactivation curves with 1 or 10 mM Ca^{2+} or Ba^{2+} as the charge carrier, indicating that inactivation occurred around -80 mV with either 1 mM Ca^{2+} or Ba^{2+} . The averaged data points for activation curves could be fitted by a single Boltzmann equation with the parameter values $V_{0.5} = -46.6 \pm 0.1$ mV and $k = 3.9 \pm 0.1$ mV for 1 mM Ca^{2+} , and $V_{0.5} = -48.9 \pm 0.2$ mV and $k = 5.3 \pm 0.1$ mV for 1 mM Ba^{2+} . The averaged experimental points for steady-state inactivation curves were fitted by a Boltzmann equation with values of $V_{0.5} = -57.7 \pm 0.4$ mV and $k = -4.4 \pm 0.3$ mV for 1 mM Ca^{2+} , and $V_{0.5} = -64.1 \pm 0.1$ mV and $k = -4.7 \pm 0.1$ mV for 1 mM Ba^{2+} . Activation and steady-state inactivation curves overlap in the voltage range of -60 to -40 mV with 1 mM Ca^{2+} and -70 to -50 mV with 1 mM Ba^{2+} , where the channels were activated during the prepulse and not completely inactivated. The current at this voltage, referred to as a window current, is one of the properties characteristic of the T-type Ca^{2+} current. No significant difference between Ca current

Fig. 2. Voltage dependence and concentration dependence of steady-state inactivation and activation for Ca^{2+} and Ba^{2+} currents in the $\text{Ca}_v3.2$ channel. The activation parameter, or chord conductance (G), was calculated by dividing the peak current measured for each test potential by the driving force. Steady-state inactivation was assessed by measuring the channel availability or the proportional peak current at 0 mV after a 1-s long pre-pulse for various potentials. Smooth solid curves represent Boltzmann fits to the data, yielding half-maximal activation potentials of: 1 mM Ca^{2+} , $V_{0.5} = -46.6 \pm 0.1$ mV ($k = 3.9 \pm 0.1$ mV); 10 mM Ca^{2+} , $V_{0.5} = -40.9 \pm 0.1$ mV ($k = 3.9 \pm 0.1$ mV); 1 mM Ba^{2+} , $V_{0.5} = -48.9 \pm 0.2$ mV ($k = 5.3 \pm 0.1$ mV); 10 mM Ba^{2+} , $V_{0.5} = -40.2 \pm 0.1$ mV ($k = 4.7 \pm 0.1$ mV). Smooth dotted curves represent Boltzmann fits to the data, yielding half-maximal inactivation potentials of: 1 mM Ca^{2+} , $V_{0.5} = -57.7 \pm 0.4$ mV ($k = -4.4 \pm 0.3$ mV); 10 mM Ca^{2+} , $V_{0.5} = -53.6 \pm 0.3$ mV ($k = -5.2 \pm 0.2$ mV); 1 mM Ba^{2+} , $V_{0.5} = -64.1 \pm 0.1$ mV ($k = -4.7 \pm 0.1$ mV); 10 mM Ba^{2+} , $V_{0.5} = -58.7 \pm 0.2$ mV ($k = -5.0 \pm 0.1$ mV).

Fig. 3. Divalent and monovalent cation selectivity of $\text{Ca}_v3.2$. Representative divalent and monovalent cationic currents, generated by the $\text{Ca}_v3.2$ channel, were obtained by increasing the depolarization steps from -90 to $+50$ mV for the 400-ms test pulse starting from a holding potential of -100 mV. Subsequent current-voltage (I - V) relationships with Ca^{2+} , Ba^{2+} , Sr^{2+} , and other cations (Mn^{2+} , Na^+ , Li^+) as the charge carrier (10 mM) are shown in (B, C).

and Ba current in the shifts of steady-state inactivation and activation curves in the depolarizing direction by the increase of cation concentration from 1 to 10 mM was observed.

Divalent and monovalent cation permeation

We further studied other cationic permeation by replacement with strontium (Sr^{2+}), manganese (Mn^{2+}), sodium (Na^+), and Lithium (Li^+). Exposure of $\text{Ca}_v3.2$ to the Sr^{2+} solution resulted in inward currents comparable to the Ca^{2+} -mediated current. It is interesting that the replacement with Mn^{2+} resulted in inward currents with a shift of the current-voltage relation-

ship in the depolarizing direction (Fig. 3C). The replacement of Ca^{2+} in the external bath solution with Mg^{2+} resulted in barely detectable inward currents (data not shown). It is even more interesting that the exposure of the $\text{Ca}_v3.2$ channel to Na^+ or Li^+ solutions resulted in unexpectedly large inward currents comparable to those of Mn^{2+} as the permeant charge carrier, with a shift of the current-voltage relationship in the hyperpolarizing direction (Fig. 3C). Reversal potentials of the $\text{Ca}_v3.2$ channel by the use of these divalent and monovalent cations (10 mM) were accordingly measured: Ca^{2+} , 27.1 ± 0.9 mV ($n=4$); Ba^{2+} , 32.1 ± 1.8 mV ($n=6$); Sr^{2+} , 26.4 ± 0.4 mV

($n=5$); Mn^{2+} , 30.1 ± 2.6 mV ($n=5$); Li^+ , 4.3 ± 0.9 mV ($n=5$); Na^+ , -11.7 ± 1.6 mV ($n=5$). The permeability ratio ($P_{\text{Ba}}/P_{\text{Ca}}$), determined by the reversal potentials at 10 mM $[\text{Ca}^{2+}]_o$ and in Ca^{2+} -free solution according to Eq. 1 was 1.39 for Ba^{2+} (Table 1). This value is much larger than that found in the L-type Ca^{2+} channel for Ba^{2+} ($P_{\text{Ba}}/P_{\text{Ca}}$: 0.40) [13, 14]. Ionic permeability ratios for other cations were calculated. The $\text{Ca}_v3.2$ channel is more permeable only for Ba^{2+} and Mn^{2+} than for Ca^{2+} and other cations. The maximum slope conductance was calculated by fitting the current-voltage relationship to a Boltzmann distribution function as shown in Eq. 2 for each permeant cation (10 mM). The conductance ratio was determined by the ratio of the maximum slope conductance (G_{max}) in the presence of extracellularly applied tested cations (10 mM) to the maximum slope conductance in the presence of 10 mM Ca^{2+} (Table 1). Only Ba^{2+} and Sr^{2+} have higher channel conductance or current density through the $\text{Ca}_v3.2$ channel than Ca^{2+} does as the charge carrier. It is interesting that $\text{Ca}_v3.2$ is thus more permeable for Mn^{2+} than for Ca^{2+} , despite the smaller current density observed with Mn^{2+} as the charge carrier.

Fast and slow inactivation properties

The inactivation kinetics of the channel were studied by using a depolarization pulse of 400 ms from -100 to $+20$ mV in 10 mV steps driven from a holding potential of -100 mV. The recorded currents were well fitted by a single exponential function. The current decay was slow at near threshold voltage and was accelerated at positive potentials. Thus as shown in Fig. 4, the inactivation time constants (T_{au}) were strongly voltage-dependent in the range of -60 to -30 mV for Ca^{2+} , Ba^{2+} and Sr^{2+} . The voltage dependency of T_{au} was shifted to positive potentials with Mn^{2+} and to hyperpolarizing potentials with Na^+ as the charge carrier.

Native T-type Ca^{2+} channels have been distinguished from L-type Ca^{2+} channels by their rapid and nearly complete inactivation property. To corroborate this kinetic property, we have investigated the change of the peak current in the $\text{Ca}_v3.2$ channel in response to repetitive stimulation of 0.2, 1, and 5 Hz, using Ca^{2+} or Ba^{2+} as the charge carrier (Fig. 5). In the presence of 10 mM $[\text{Ca}^{2+}]_o$, the current decreased after several repetitive stimulations at 5 Hz and reached the steady-state level. The use-dependent re-

duction of the peak Ca^{2+} and Ba^{2+} currents. Changes in the peak current at 0 mV (pulse duration of 100 ms) from a holding potential of -100 mV at three different stimulation frequencies (0.2, 1, and 5 Hz) are plotted with 10 mM Ca^{2+} (A) or Ba^{2+} (B) as the charge carrier.

Table 1. Ionic permeability and conductance ratios of the $\alpha 1\text{H}$ channel.

	Cations (n)				
	Ba^{2+} (6)	Sr^{2+} (5)	Mn^{2+} (4)	Li^+ (4)	Na^+ (4)
P_x/P_{Ca}	1.39	0.95	1.21	0.42	0.23
G_x/G_{Ca}	1.21	1.39	0.23	0.39	0.11

The permeability ratios for cations (P_x/P_{Ca}) and conductance ratios (G_x/G_{Ca}) over Ca^{2+} were calculated from the equations shown in MATERIALS AND METHODS. n : number of experiments.

Fig. 4. Time constants for fast inactivation (current relation) in various cationic currents. Extracellular divalent and monovalent cation concentrations were 10 mM. The decay of the current was fitted by a single exponential. Inactivation time constants (τ) are plotted against the respective test potentials.

Fig. 5. Use-dependent reduction of the peak Ca^{2+} and Ba^{2+} currents. Changes in the peak current at 0 mV (pulse duration of 100 ms) from a holding potential of -100 mV at three different stimulation frequencies (0.2, 1, and 5 Hz) are plotted with 10 mM Ca^{2+} (A) or Ba^{2+} (B) as the charge carrier.

Fig. 6. Time course of recovery from inactivation with Ca^{2+} or Ba^{2+} as the charge carrier.

Ca^{2+} (A) or Ba^{2+} (B) currents were inactivated by a 500-ms pulse to 0 mV, after which the membrane potential was stepped to 0 mV for a 200-ms duration, following a period ranging from 1 to 3,000 ms at -100 mV. The inset shows the pulse protocol. Smooth curves were fitted to the data by using the sum of two exponentials, yielding fast (Ca^{2+} , 12.9 ms; Ba^{2+} , 23.7 ms) and slow (Ca^{2+} , 372.3 ms; Ba^{2+} , 487.6 ms) time constants for 10 mM Ca^{2+} or Ba^{2+} as the charge carrier.

duction of the current was not observed at the stimulation rate of 0.2 Hz and was $77 \pm 3\%$ at the rate of 1 Hz. The onset and the steady-state reduction of the Ba^{2+} current and the dependence of these events on the stimulation frequency were nearly identical to those with equimolar $[\text{Ca}^{2+}]_o$.

We then examined the time and cationic dependence of recovery from inactivation by using the protocol illustrated in Fig. 6. Recovery from inactivation was complete at -100 mV with either $[\text{Ca}^{2+}]_o$ or $[\text{Ba}^{2+}]_o$. It is striking to note that the time course was essentially identical with either cation, suggesting a Ca^{2+} -independent inactivation process, together with the data in Fig. 5.

DISCUSSION

The present study describes the biophysical characteristics of the human T-type Ca^{2+} channel, $\text{Ca}_v3.2$. The functional expression of the $\text{Ca}_v3.2$ ($\alpha 1H$) clone in HEK-293 cells produced currents with the essential kinetic properties of cardiac T-type Ca^{2+} currents. Specifically, the voltage dependence of activation ($V_{0.5}$: -46.6 mV, 1 mM Ca^{2+}) is clearly in the low-voltage-activated range, and inactivation ($V_{0.5}$: -57.7 mV, 1 mM Ca^{2+}) also occurs at more negative voltages than for the L-type Ca^{2+} channel. Inactivation is not only rapid, but also nearly complete.

General characteristics of the $\text{Ca}_v3.2$ channel. There is accumulated evidence that the current through the $\text{Ca}_v3.2$ channel reproduces in large part, but not exactly, the biophysical and pharmacological characteristics of currents through guinea-pig atrial T-type Ca^{2+} channels. Activation shows the same voltage dependence as the native channel in rabbit sinoatrial node cells, consistent with the low-voltage-activated T-type Ca^{2+} channel [1]. The inactivation of human $\text{Ca}_v3.2$ channels occurs at voltages that are

more positive by about 20 mV than for native rabbit T-type Ca^{2+} channels [1]. This could reflect a species difference [15], a splicing variant [7], or an accessory subunit that is missing from the $\text{Ca}_v3.2$ channel expressed in HEK-293 cells [16–19]. The general feature of the $\text{Ca}_v3.2$ channel inactivation has been previously explored with 10 mM Ba^{2+} as the charge carrier [5]. Our present study not only confirmed the feature of the T-type Ca^{2+} channel [5, 20], but also demonstrated the slow recovery from inactivation, which is referable to the use-dependent availability of the channel. The time course of recovery from inactivation could be fit as a biexponential. The fast and slow processes of recovery from inactivation in the $\text{Ca}_v3.2$ channel (Fig. 6) are relatively faster than those in the T-type Ca^{2+} channel in canine cardiac Purkinje cell [21], which may reflect different kinetics of the T-type Ca^{2+} channel in distinct isoforms and/or species [8].

Divalent and monovalent cation permeation. The most striking findings in this study are seen with the use of various cations as the charge carrier. Studies on permeating ion-dependent channel conductance through the native T-type Ca^{2+} channel have been poorly investigated because the native T-type Ca^{2+} channel current is more or less superimposed on the ubiquitous L-type Ca^{2+} channel current even with the antagonism of the L-type Ca^{2+} channel, particularly in the possible ranges for the reversal potential. This study took advantage of the ability to express a pure population of human recombinant T-type ($\text{Ca}_v3.2$) Ca^{2+} channels in HEK-293 cells. By measuring the precise reversal potentials of various cationic currents through the $\text{Ca}_v3.2$ channel, the ionic permeability ratios for T-type Ca^{2+} channels were obtained according to the Goldman-Hodgkin-Katz theory. The permeability ratio of the $\text{Ca}_v3.2$ channel for divalent and monovalent cations based on reversal potential measurements was ordered as fol-

lows: $\text{Ba}^{2+} > \text{Mn}^{2+} > \text{Ca}^{2+} > \text{Sr}^{2+} > \text{Li}^+ > \text{Na}^+$. L-type Ca^{2+} channels are nearly impermeable to Li^+ and Na^+ : Ionic permeability ratios, P_X/P_{Ca} , for the L-type Ca^{2+} channel were 0.002 for Li^+ and 0.001 for Na^+ [11]. The permeation of Li^+ and Na^+ through the $\text{Ca}_v3.2$ channel is therefore quite surprising (Fig. 3, Table 1). On the other hand, the conductance ratio of $\text{Ca}_v3.2$ channel was ordered as follows: $\text{Sr}^{2+} > \text{Ba}^{2+} > \text{Ca}^{2+} > \text{Li}^+ > \text{Mn}^{2+} > \text{Na}^+$. It is interesting that despite the higher permeability observed with Mn^{2+} as the charge carrier, the $\text{Ca}_v3.2$ channel has smaller current density for Mn^{2+} than for Ca^{2+} . A similar observation on Mn^{2+} permeability was reported in the epithelial Ca^{2+} channel cloned from rabbit kidney [19]. The finding that Mn^{2+} is highly permeable through the $\text{Ca}_v3.2$ channel with low conductance could be explained by the slow rate of dehydration [22]. It is postulated that the transport of Mn^{2+} through the channel is retarded because of the slow rate of dehydration within the channel pore. The observation that Ba^{2+} is highly permeable with a large conductance ratio through the $\text{Ca}_v3.2$ channel, together with a higher dehydration rate than Mn^{2+} , may partially support this speculation. Overall, it is intriguing that the $\text{Ca}_v3.2$ channel was relatively nonselective toward the monovalent and divalent cations tested, unlike the high selectivity of the L-type Ca^{2+} channel to Ca^{2+} and Ba^{2+} .

The most important differences in the permeant ion selectivity between the L-type Ca^{2+} channel and the $\text{Ca}_v3.2$ channel could be derived from the molecular differences in the pore-forming P loops in domains I–IV. The L-type Ca^{2+} channel shows a high selectivity for Ca^{2+} over more plentiful ions such as Na^+ or K^+ by virtue of its high-affinity binding of Ca^{2+} within the pore [23], and this binding involves four conserved glutamate residues [24] in equivalent positions in the putative pore-lining regions of domains I–IV in the L-type Ca^{2+} channel $\alpha 1C$ subunit. In contrast, all the low-voltage-activated Ca^{2+} channels, including $\text{Ca}_v3.2$, have aspartate instead of glutamate residues in the same P region of domains III and IV. This difference may be the main cause of the distinct ion selectivity in the $\text{Ca}_v3.2$ channel, which is newly revealed in this study. Furthermore, the $\text{Ca}_v3.2$ channel has a lysine residue (positively charged) instead of a phenylalanine residue (neutral) in the position next to the aspartate as part of a possible selection filter in the same P region of domain III. It is also speculated that molecular mutations not only in the ion-selective motifs in the P region of the Ca^{2+} channel, but also in their peripheral amino acids composition could have additional influences on their ion selectivity. A similar

speculation was indicated for the distinction selectivity in the mutant sodium channels [24]. Based on this information, another supposable explanation for the high permeability of Mn^{2+} could be offered by electrostatic interactions between the $\text{Ca}_v3.2$ channel protein and Mn^{2+} , though they are ignored in the Goldman-Hodgkin-Katz theory. Indeed, Eisenman describes conductance selectivity entirely as an electrostatic phenomenon, arising from a binding competition between the dehydration and attraction of ions to a channel pore (Eisenman's theory) [25, 26]. The $\text{Ca}_v3.2$ channel contains a narrow region with the glutamate and aspartate residues near the extracellular end that forms the selectivity filter and is responsible for many of its distinctive characteristics [5, 6, 18]. The narrow section of the channel prevents ions from passing one another, making this a single-file model. The highly charged glutamate and aspartate groups create a deep potential well that attracts surrounding cations [5, 27, 28]. Once an ion enters this region of the channel, the depth of this well makes it difficult for that ion to exit on its own [27, 28]. Thus in the $\text{Ca}_v3.2$ channel, it is plausible that Mn^{2+} conductance is by far the lowest among the divalent cations because of the preferred chemical interaction of Mn^{2+} , besides the difference in diffusion coefficients.

In conclusion, we analyzed the kinetic characterizations of the human $\text{Ca}_v3.2$ channel, a subclass of the low-voltage-activated Ca^{2+} channel, in terms of activation and inactivation properties as well as cation permeability, taking full advantage of the ability to express a pure population of recombinant T-type Ca^{2+} channels.

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