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THREE TYPES OF VOLTAGE-DEPENDENT CALCIUM CURRENTS IN CULTURED HUMAN NEUROBLASTOMA CELLS

MASAO KITO[1], MITSUO MAEHARA[2], and KAZUYOSHI WATANABE[3]

[1] *Department of Pediatrics, Chubu Rosai Hospital, Nagoya,*
[2] *Department of Pediatrics, Chubu National Hospital, Obu, and*
[3] *Department of Pediatrics, Nagoya University School of Medicine, Nagoya, Japan*

ABSTRACT

The voltage-dependent calcium current (I_{Ca}) in cultured human neuroblastoma cells (NB-I) was studied by whole-cell recording. The low-threshold current (I_l), the high-threshold, fast inactivating current ($I_{h.f.}$), and the high-threshold, slow inactivating current ($I_{h.s.}$) were identified. I_l was blocked by Ni^{2+}. $I_{h.f.}$ was blocked by ω-conotoxin GVIA. $I_{h.s.}$ was blocked by nifedipine, and enhanced by Bay K 8644. These characteristics indicate that I_l, $I_{h.f.}$ and $I_{h.s.}$ are consistent with the T-, N- and L-type I_{Ca}, respectively.

Key words: Calcium channel current, Whole cell recording, Human neuroblastoma NB-I

INTRODUCTION

Voltage-dependent Ca^{2+} channels play important roles in the regulation of many cellular functions.[1)] Recently, in addition to T- (low-threshold, transient) and L- (high-threshold, long-lasting) types of Ca^{2+} channels, a third type of Ca^{2+} channel (N-type; neither T nor L) has been shown in the cultured dorsal root ganglion neurons of the chick and mouse.[2,3)] Carbone et al. reported T-, N- and L-type Ca^{2+} channels in human neuroblastoma IMR32 cells.[4)] In the present study we describe three types of I_{Ca} in the neuroblastoma cells of human origin named NB-I.[5)]

MATERIALS AND METHODS

The human neuroblastoma cell line (NB-I) established by Miyake et al. was used.[5)] Cells were cultured in RPMI 1640 medium, pH 7.4, supplemented with 10% fetal calf serum at a temperature of 37°C. NB-I cells were replaced on a small glass-covered culture dish and incubated for two to seven days before use.

A whole-cell recording of the patch-clamp techniques was applied to record I_{Ca} of the neuroblastoma cell under voltage-clamp conditions.[6)] The cut-off frequency of the recording system was 700 Hz. The recording chamber with a bath volume of 0.2 ml in which NB-I cells were mounted was perfused with a gravity-fed perfusion system at a rate of 2 ml/min. I_{Ca} were evoked by applying step depolarizations of a 400-ms duration from −100 mV to +80 mV at 10 mV steps from the holding potential. The holding potentials were set at −80 mV for I_l and $I_{h.f.}$ and at −30 mV for $I_{h.s.}$. I_{Ca} were separated by sensitivity to the holding potential. Experiments were conducted at room temperature (22 to 25°C).

Correspondence: Dr. M. Kito, Department of Pediatrics, Chubu Rosai Hospital, Koumei 1-10-6, Minato-ku, Nagoya City, 455 Japan. Telephone (052) 652-5511 Fax (052) 653-3533

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The normal external solution contained (mM): NaCl (36.7), $BaCl_2$ (51.2), $MgCl_2$ (1.18), glucose (11.8), HEPES-Na (10.0), and tetraethylammonium-Cl (TEA) (23.6) at pH 7.4. The patch pipette was filled with a solution containing (mM): Cs-Aspartate (106.2), CsCl (23.6), $MgCl_2$ (4.95), ATP-Na_2 (4.95), EGTA (9.9), HEPES-Na (4.95), and $CaCl_2$ (1.26) at pH 7.0. The resistance of the patch pipette was between 3 and 5 MΩ in the normal external solution. The voltage-dependent Na^+ current was differentiated from I_{Ca} by its time constant. Tetrodotoxin (TTX) 3μM did not affect any of the I_{Ca} in NB-I cells (not shown). The voltage-dependent K^+ current was blocked by the use of Cs^+ as a dominant cation in the patch pipette solution and by the addition of TEA to the external solution. Ba^{2+} was used in the normal external solution because it is more permeant to the Ca^{2+} channel and also easier to analyze the amplitude with when compared with Ca^{2+}.[6)]

The susceptibility to various Ca^{2+} channel blockers varies with the type of Ca^{2+} channel. We studied the blocking effects of some inorganic blockers (Ni^{2+}, Cd^{2+} and La^{3+}), dihydropyridine Ca^{2+} channel blocker (nifedipine) and ω-CgTX[7)] on different types of I_{Ca} in NB-I cells.

The membrane currents were recorded by using a pre-amplifier of CEZ 2100 (Nihon Kohden, Tokyo, Japan). Data were analyzed by using the PCLAMP ver 5.51 (Axopatch, USA). The numerical values were expressed as mean values ± S.E.M.

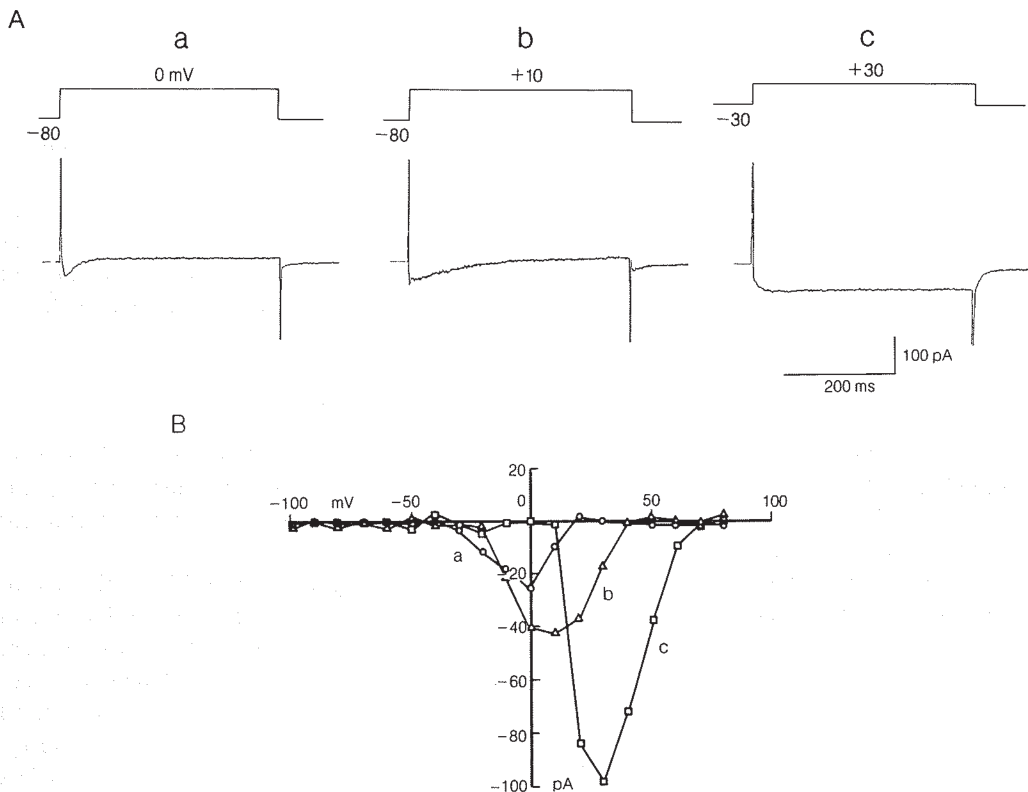

Fig. 1. The voltage-dependent Ca^{2+} currents in human neuroblastoma (NB-I) cell line. (A) The typical inward currents evoked by applying a step-depolarization from a holding potential of −80 mV to the test potentials indicated to record $I_{l.}$(a) and $I_{h.f.}$(b) and −30 mV for $I_{h.s.}$(c). (B) The typical current-voltage relationships of $I_{l.}$(a), $I_{h.f.}$(b) and $I_{h.s.}$(c). Data were obtained from different cells.

RESULTS

Fig. 1 shows three types of I_{Ca} recorded in NB-I cells. Fig. 1-A shows the time course of I_{Ca} evoked by a depolarizing stimulation of 400-ms duration. Fig. 1-B shows the current-voltage relationship (I-V curve). Il was activated by a depolarizing potential more positive than −50 mV, and was rapidly inactivated during the depolarizing test potentials with a time constant of 22.5 ± 5.7 ms (n = 4) at a test potential of −10 mV (Fig. 1-A-a and Fig. 1-B-a). $I_{h.f.}$ was activated at a relatively large depolarization potential more positive than −20 mV, and decayed with a

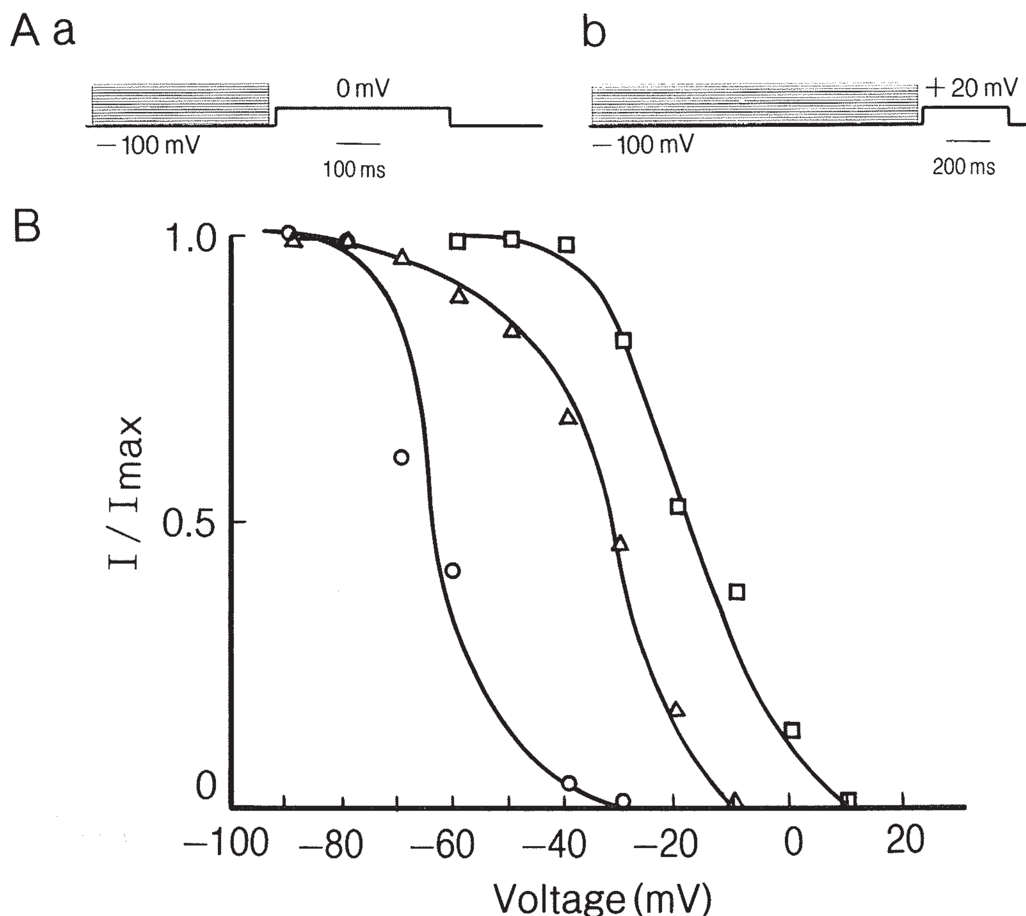

Fig. 2. The voltage dependency of inactivation of $I_{l.}$, $I_{h.f.}$, and $I_{h.s.}$
The Ca^{2+} currents for $I_{l.}$ were evoked by applying a step-depolarization from a holding potential of −100 mV to the test potential of 0 mV. The test pulses were preceded by a 400-ms prepulse between −100 mV and +60 mV at 10 mV steps. The Ca^{2+} currents for $I_{h.s.}$ and $I_{h.s.}$ were evoked by applying a step-depolarization from a holding potential of −100 mV to the test potential of +20 mV. The test pulses were preceded by a 1500-ms prepulse between −100 mV and +60 mV at 10 mV steps. ○: The peak amplitudes of the inward currents plotted against the voltage levels during the prepulse. Data points were fitted with a smooth curve derived from the Boltzmann equation. $I/Imax = [1+\exp(V-V_{1/2})/k]^{-1}$ where $V_{1/2} = -64$ mV and k = 4.1 mV. △: $I/Imax = [1+\exp(V-V_{1/2})/k]^{-1}$ where $V_{1/2} = -32$ mV and k = 10.9 mV. □: $I/Imax = [1+\exp(V-V_{1/2})/k]^{-1}$ where $V_{1/2} = -18$ mV and k = 8.3 mV. $V_{1/2}$: mid-point, k: slope parameter

time constant of 120 ± 8.8 ms (n = 5) at a test potential of 20 mV (Fig. 1-A-b and Fig. 1-B-b). $I_{h.s.}$ was activated at depolarization potentials (V_t more positive than 0 mV) and showed little inactivation during a 400-ms depolarization (Fig. 1-A-c and Fig. 1-B-c).

Fig. 2 shows the voltage dependency of inactivation of $I_{l.}$, $I_{h.f.}$ and $I_{h.s.}$. $I_{l.}$ was strongly inactivated between −80 mV and −40 mV, and completely inactivated at −30 mV prepulse. The data points for $I_{l.}$ were fitted with a continuous smooth curve derived from the Boltzmann equation with a mid-point of −64 mV and a slope parameter of 4.1 mV (n = 5). The data points for $I_{h.f.}$ were fitted with a smooth curve derived from the Boltzmann equation with a mid-point of −32 mV and a slope parameter of 10.9 mV (n = 7). The data points for $I_{h.s.}$ were also fitted by the Boltzmann equation with a mid-point of −18 mV and a slope parameter of 8.3 mV (n = 4).

In Table 1, the electrophysiological and pharmacological properties of the three types of I_{Ca} ($I_{l.}$, $I_{h.f.}$ and $I_{h.s.}$) recorded in NB-I cells are summarized. Relative conductances were measured when recordings were made with 10 mM-external Ca^{2+} instead of 50 mM-external Ba^{2+}. Extracellular application of 100μM Ni^{2+} inhibited $I_{l.}$ by 82.6% (n = 8). On the other hand, 100μM Cd^{2+} inhibited $I_{h.f.}$ and $I_{h.s.}$ by 90.5% (n = 3) and 97.0% (n = 3), respectively. La^{3+} at 10μM inhibited $I_{h.s.}$ by 95.8% (n = 3). Nifedipine at 10μM inhibited $I_{h.s.}$ by 90.1% (n = 3). ω-CgTX at 5μM inhibited $I_{h.f.}$ by 66.6% (n = 4). Bay K 8644 10μM, a L-type Ca^{2+} channel agonist,[8] enhanced $I_{h.s.}$ by 32.4% (n = 9) when compared with the control state.

$I_{l.}$, $I_{h.f.}$, $I_{h.s.}$, $I_{l.} + I_{h.f.}$, $I_{l.} + I_{h.s.}$, $I_{h.f.} + I_{h.s.}$, and $I_{l.} + I_{h.f.} + I_{h.s.}$ were detected in 23.5%, 2.5%, 21.0%, 0%, 39.5%, 3.7%, and 9.9% of the NB-I cells (n = 81) examined, respectively. Mean amplitudes of $I_{l.}$, $I_{h.f.}$, and $I_{h.s.}$ were 25.8 ± 8.7 pA, 34.4 ± 7.7 pA, and 45.2 ± 18.3 pA, respectively. Two cells (2.5%) had only $I_{h.f.}$. The time course of $I_{h.f.}$ and the current-voltage

Table 1. The Electrophysiological and Pharmacological Properties of the Three Types of Ca^{2+} Currents in NB-I Cells. Each value represents the mean values ± S.E.M.

	$I_{l.}$	$I_{h.f.}$	$I_{h.s.}$
Acitivation range (for 50mM Ba^{2+})	> −50mV	> −20mV	> 0 mV
Inactivation rate (τ : ms) (50mM Ba^{2+})	22.5±5.7 (−10mV)	120.4±8.8 (20mV)	>400
Relative conductances (Ca^{2+}/Ba^{2+})	1.02	0.68	0.17
Ni^{2+} (100μM) inhibition	82.6±15.3 %	38.7±18.8 %	69.6±6.8 %
Cd^{2+} (100μM) inhibition	11.4±3.2 %	90.5±0.5 %	97.0±0.4 %
La^{3+} (10μM) inhibition	24.3±5.5 %	66.7±16.7 %	95.8±0.9 %
Nifedipine (10μM) inhibition	20.6±0.6 %	40.0±9.6 %	90.1±5.9 %
ω-CgTX(5μM) inhibition	12.9±7.6 %	66.6±12.2 %	26.9±8.0 %
Bay K 8644 (10μM) enhancement.	−5.8±18.6 %	−2.6±11.6 %	32.4±27.2 %

relationship of $I_{h.f.}$ in Fig. 1 and the voltage dependency of inactivation of $I_{h.f.}$ in Fig. 2 were obtained from these two cells.

DISCUSSION

Recent reports have demonstrated that there are at least three types of Ca^{2+} channels in various neuronal cells such as sensory neurons,[2,3] and cultured rat hippocampal neurons.[9] Although the presence of three types of Ca^{2+} channels has been demonstrated in other human neuroblastoma IMR32 cells,[4] there is no other report of three types of Ca^{2+} channels in human neuroblastoma cells. In the cultured NB-I cells, we found three types of I_{Ca}, and named them, $I_{l.}$(low-threshold current), $I_{h.f.}$(high-threshold, fast inactivating current) and $I_{h.s.}$(high-threshold, slow inactivating current). Ni^{2+} was more effective in blocking $I_{l.}$ than $I_{h.}$. On the other hand, Cd^{2+} was more effective in blocking $I_{h.}$ than $I_{l.}$ Nifedipine was more effective in blocking $I_{h.s.}$ than $I_{1.}$ and $I_{h.f.}$. ω-CgTX at 5μM inhibited $I_{h.f.}$ more than $I_{l.}$ and $I_{h.s.}$. Bay K 8644 enhanced $I_{h.s.}$, whereas it did not enhance $I_{l.}$ and $I_{h.f.}$. I_{l}, $I_{h.f.}$ and $I_{h.s.}$ seem to be consistent with the T-, N- and L-type I_{Ca}, respectively.[2,3]

In conclusion, three types of Ca^{2+} channels reported in the several neuronal cells, i.e., T-, N- and L-type Ca^{2+} channels, were also found in NB-I cells. The neuroblastoma cell line has the advantages of easy maintenance and acquisition from the primary culture or the cytological isolation of the neurons.

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REFERENCES

1) Miller, R.L.: Multiple calcium channels and neuronal function. *Science*, 235, 46−52 (1987).
2) Fox, A.P., Nowycky, M.C., and Tsien, R.W.: Kinetic and pharmacological properties distinguishing three types of calcium currents in chick sensory neurons. *J. Physiol.* (Lond), 394, 149−172 (1987).
3) Kostyuk, P.G., Shuba, Y.M., and Savchenko, A.N.: Three types of calcium channels in the membrane of mouse sensory neurons. *Pflugers. Arch.*, 411, 661−669 (1988).
4) Carbone, E., Sher, E., and Clementi, F.: Ca currents in human neuroblastoma IMR32 cells: Kinetics, permeability and pharmacology. *Pflugers. Arch.*, 416, 170−179 (1990).
5) Miyake, S., Shimo, Y., and Kitamura, T.: Characteristics of continuous and functional cell line NB-1, derived from a human neuroblastoma. *Autonomic Nervous System*, 10, 115−120 (1973).
6) Hamil, O.P., Marty, A., Neher, E., Sackmann, B., and Sigworth, F.J.: Improved patch clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflugers. Arch.*, 391, 85−100 (1981).
7) Kasai, H., Aosaki, T., and Fukuda, J.: Presynaptic Ca-antagonist ω-conotoxin irreversibly blocks N-type Ca-channels in chick sensory neurons. *Neurosci. Res.*, 4, 228−235 (1987).
8) Nowycky, M.C., Fox, A.P., and Tsien, R.W.: Long-opening mode of gating of neuronal calcium channels and its promotion by the dihydropyridine calcium agonist Bay K 8644. *Proc. Natl. Acad. Sci. U.S.A.*, 82, 2178−2182 (1985).
9) Ozawa, S., Tsuzuki, K., Iino, M., Ogura, A., and Kudo, Y.: Three types of voltage-dependent calcium current in cultured rat hippocampal neurons. *Brain Res.*, 495, 329−336 (1989).