Functional Changes in Potassium Conductances of the Human Neuroblastoma Cell Line SH-SY5Y During In Vitro Differentiation

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Tosetti, Patrizia, Vanni Taglietti, and Mauro Toselli. Functional changes in potassium conductances of the human neuroblastoma cell line SH-SY5Y during in vitro differentiation. J. Neurophysiol. 79: 648–658, 1998. The electrophysiological properties of voltage-dependent outward currents were investigated under voltage-clamp conditions in the human neuroblastoma cell line SH-SY5Y before and after in vitro differentiation with retinoic acid, by using the whole cell variant of the patch-clamp technique. Voltage steps to depolarizing potentials from a holding level of −90 mV elicited, in both undifferentiated and differentiated cells, outward potassium currents that were blocked by tetraethylammonium, but were unaffected by 4-aminopyridine, cadmium, and by shifts of the holding potentials to −40 mV. These currents activated rapidly and inactivated slowly in a voltage-dependent manner. In undifferentiated cells the threshold for current activation was about −30 mV, with a steady-state half activation potential of 19.5 mV. Maximum conductance was 4.3 nS and mean conductance density was 0.34 nS/cm². Steady-state half inactivation potential was −13.8 mV and 10% of the current was resistant to inactivation. Both activation and inactivation kinetics were voltage dependent. In differentiated cells the threshold for current activation was about −20 mV, with a half potential for steady-state activation of 37.0 mV. Maximum conductance was 15.2 nS and mean conductance density was 0.78 nS/cm². Steady-state half inactivation potential was −9.7 mV and 37% of the current was resistant to inactivation. Both activation and inactivation kinetics were voltage dependent. This diversity in potassium channel properties observed between undifferentiated and differentiated cells was related to differences in cell excitability. Under current-clamp conditions, the action potential repolarization rate in differentiated cells was about threefold faster than that of the abortive action potentials elicitable in undifferentiated cells. Furthermore, during prolonged stimulation, trains of spikes could be generated in some differentiated cells but not in undifferentiated cells.

INTRODUCTION

Delayed rectifier potassium channels are responsible for the repolarizing current that brings membrane potential back to its resting value after the depolarizing phase of action potential. Modifications of the electrophysiological parameters of this class of channels, because of gene mutations for example, might be implicated in some human diseases like ataxia and myokymia (Browne et al. 1994) and cardiac arrhythmia (Long QT Syndrome) (Curran et al. 1995; Sanguinetti et al. 1995).

The gating properties of delayed rectifier potassium channels have been extensively investigated in in vitro preparations from mouse, rat, and fly nervous system. Various voltage-gated potassium channels were cloned and characterized from different tissues of human origin. An insulinoma potassium channel (hPCN1) was expressed and studied in Xenopus oocytes (Philipson et al. 1991). A Shaker potassium channel, classified as Kv1.5, was cloned from human heart and transfected in a mouse cell line (Snyders et al. 1993; Tamkun et al. 1991). The gene for h-DRK1, the human homologous of rat DRK1 (Freh et al. 1989) and mouse mShab (Pak et al. 1991), was recently cloned from a human genomic DNA library (Albrecht et al. 1993) and its electrophysiological properties were investigated after cDNA injection in Xenopus oocytes (Benndorf et al. 1994). Because it is possible that host cells used for transfection confer to expressed exogenous channels properties that are not present in native channels (see also Shi et al. 1994), it is of interest to examine the properties of channels in their tissue of origin.

A quantitative study of potassium channels in native tissues however, is sometimes complicated by the presence of multiple overlapping ionic currents.

The human neuroblastoma cell line SH-SY5Y, established by repetitive subcloning of the SK-N-SH cell line (Biedler et al. 1978), exhibits the morphological and biochemical features of cells derived from the neural crest, like sympathetic neurons (Barnes et al. 1981; Ross et al. 1981). These cells can be induced to acquire a neuronal phenotype by prolonged treatment, with the differentiating agent retinoic acid (Pålman et al. 1984). Because of their capacity to differentiate in mature ganglion-like cells and to their cellular homogeneity, SH-SY5Y cells are a highly suitable model for studying the role ion channels play in the excitability of cells of human origin. The use of this cell line would also permit to combine molecular biological and electrophysiological approaches.

It was previously shown that in SH-SY5Y cells depolarizing voltage steps initially evoke a fast activating and full inactivating inward current, followed by an outward current component (Brown et al. 1994; Johansson 1994; Toselli et al. 1996). The inward current, identified as a tetrodixin (TTX)-sensitive Na⁺ conductance, was studied in detail, and changes in its gating properties during differentiation were related to the ability of SH-SY5Y differentiated cells to generate overshooting action potentials (Toselli et al. 1996).

The aim of the present study was to characterize and compare the nature, gating properties, and electrophysiological role of the native voltage-dependent outward current in both undifferentiated and differentiated SH-SY5Y cells. Channels expressed in the two types of cells were found to differ in their electrophysiological parameters, which might be related to the observed distinct ability of the two cell types to generate regular action potentials.
METHODS

Cell culture

SH-SY5Y cells were grown as monolayer in RPMI 1640 medium (GIBCO) supplemented with 10% heat inactivated fetal calf serum, 100 µg/ml streptomycin and 100 IU/ml penicillin. The medium was replaced three times a week. Cells were cultured in 75 ml plastic flasks in a 5% CO₂ atmosphere at 37°C; when confluent they were split in 35-mm plastic Petri dishes to be used for electrophysiological experiments. Differentiation of SH-SY5Y cells was achieved by treatment with 10 µM retinoic acid (RA) (Pålman et al. 1984). Differentiated cells were used after 14–18 days of RA treatment to obtain a high percentage of cells that showed clear morphological differentiation (Pålman et al. 1984). Undifferentiated cells were used 2–6 days after plating and no RA was added.

Solutions

During voltage- and current-clamp recording the cells were bathed in 125 mM NaCl, 4 mM KCl, 2 mM CaCl₂, 1.2 mM MgSO₄, 10 mM glucose, 0.3 µM TTX, 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)/NaOH (pH 7.4). The patch pipette contained 140 mM KCl, 4 mM NaCl, 0.02 mM CaCl₂, 0.8 mM ethylene glycol-bis(β-aminoethyl ether)-N,N',N″,N‴-tetraacetic acid (EGTA), 2 mM MgCl₂, 4 mM Mg-ATP, 10 mM HEPES/KOH (pH 7.4). Contamination by voltage-gated calcium currents was negligible in most undifferentiated cells but sizeable in most differentiated cells; 200 µM CdCl₂ was therefore added to the bath to suppress calcium currents when present. Chemicals were purchased from Sigma Chemical (St. Louis, MO). Changes of extracellular solutions were obtained by a fast multibarrel delivery system positioned close to the cell tested.

Electrophysiology

Electrophysiological recordings were carried out by using the whole cell patch clamp configuration (Hamill et al. 1981). Experiments were performed at room temperature (21 ± 2°C).

Stimulation, acquisition, data analysis, and curve fitting were performed with pCLAMP software (Axon Instruments, Burlingame, CA) and the packages ASYSTANT (Macmillan Software, New York) and ORIGIN (Microcal Software, Northampton, MA). Linear components of leak and capacitive currents were first reduced by analog circuitry and then almost completely cancelled with the P/N method. Patch pipettes had a resistance of 3–6 MΩ and occasionally, especially in current clamp experiments, their tips were coated with Sylgard to reduce capacitance.

Experiments were performed on small cells with short processes to guarantee sufficient voltage and space clamp control. Cells were discarded if current tracings showed signs of notchlike discontinuities. A sampling interval of 25 µs/point and series resistance compensation of 60–70% were applied when tail currents were studied. Currents were filtered at 3 kHz.

RESULTS

Under voltage-clamp conditions and using the solutions described in METHODS, a series of depolarizing voltage steps from −90 mV to potentials between −30 and +80 mV elicited an outward current in both undifferentiated and differentiated SH-SY5Y cells, as shown, respectively, in Fig. 1, A and B. This current was observed in ~93% of the clamped cells (n = 293). In undifferentiated cells, currents activated at about −30 mV, with sigmoidal time course and showed a slow but sizeable inactivation, particularly evident at potentials positive to +30 mV. In differentiated cells, the outward conductance activated at voltages positive to −20 mV and its time course of activation was similar to that observed in undifferentiated cells but its inactivation was less evident.

In Figure 1C the current-voltage relations are shown. They were obtained by averaging current density, obtained dividing peak current amplitude by cell area, measured at each
fig. 2. Pharmacology of outward currents in SH-SY5Y cells. A: block of outward current by extracellular tetraethylammonium (TEA, 20 mM) in an undifferentiated cell. B: effect of extracellular 4-aminopyridine (4-AP, 1 mM) on outward current of an undifferentiated cell. C: effect of change of holding potential from −90 to −40 mV on outward current of an undifferentiated cell. D: action of cadmium (200 μM) on outward current of an undifferentiated cell. Test potential was +50 mV for all cells. A, B, and D: outward currents recorded in control saline before and after drug application are labeled Contr and Wash, respectively.

A test potential in 11 undifferentiated (▲) and 13 differentiated (■) cells. In both types of cells the outward current displayed inward rectification—more pronounced in undifferentiated cells—at voltages positive to +60 mV.

Currents elicited from both undifferentiated and differentiated cells were suppressed by replacing K+ with 140 mM Cs+ in the patch pipette (n > 60) (see also Toselli et al. 1996), or by adding 20 mM tetraethylammonium (TEA) to the extracellular solution (Fig. 2A). On the other hand, focal perfusion with 5 mM 4-aminopyridine (4-AP) or lowering the holding potential from −90 to −40 mV had little or no effect on these currents, as shown in the sample tracings of Fig. 2B and C, respectively. The peak current measured at +50 mV was 98 ± 4% of control after application of 4-AP (n = 6) and it was 91 ± 7% of control after shifting from a Vh of −90 mV to a Vh of −40 mV (n = 6). Furthermore, addition to the extracellular solution of 200 μM Cd2+, which blocks completely voltage-dependent calcium channels in SH-SY5Y cells (Toselli et al. 1991), affected neither the shape nor the amplitude of the outward current, as shown in Fig. 2D (100 ± 3% of control at +50 mV, n = 4).

The time course and voltage-dependence of activation, the absence of a sizeable decrease in current amplitude at a given test potential after changing the holding potential from −90 to −40 mV, and the pharmacological evidences previously described strongly suggest that the outward current elicitable in both undifferentiated and differentiated SH-SY5Y cells is classifiable as a delayed rectifier potassium current (I钾) and also exclude contamination from fast inactivating (Ia) or calcium-activated potassium conductances.

**Conductance and steady-state activation**

The voltage dependence of I钾 steady-state activation was studied using the following protocol: a test potential of amplitude variable from −30 to +80 mV was applied and lasted the appropriate time for the current to reach its maximum; then the test pulse was followed by a repolarizing pulse to −50 mV. An example of currents elicited using this protocol is shown in Fig. 3A.

Tail currents, generated by relaxation of I钾 on return from different test potentials to −50 mV, could be fitted by a single exponential function. The amplitude of I钾 at the beginning of the repolarizing step was determined by extrapolating the current tail at time 0 of repolarization and correcting for current inactivation at the time of test pulse offset, following the equation h(t) = h_inf - (h_inf - h_o) exp(-t/t_h) (see Steady-state inactivation and Kinetics of inactivation). I钾 was converted to the conductance value (g钾) with the following equation

\[
g_{K} = I_{K}(V_{m} - E_{K})
\]

where \(V_{m}\) is the repolarization potential and \(E_{K}\) is the potassium equilibrium potential (−89.6 mV in the ionic conditions used for this study).
The instantaneous current-voltage (I-V) relation was determined by using the protocol illustrated in Fig. 4A and measuring the tail current amplitudes at 0.3 ms after the repolarization onset. The instantaneous I-V relation was linear between -60 and 0 mV in both differentiated and undifferentiated cells (not shown) and the use of the above equation was therefore justified. The reversal potential evaluated from the instantaneous I-V relation was \(-77 \pm 2\) mV for differentiated cells \((n = 7)\) and \(-76 \pm 3\) mV for undifferentiated cells \((n = 12)\); both potentials are \(\sim 10\) mV more depolarized to the theoretical value given by Nernst equation; this discrepancy could be because of an intracellular K\(^+\) concentration lower than expected.

For each cell tested, the maximal value of conductance \((g_{\text{max}})\) was evaluated by interpolating the sets of conductance values with the Boltzmann type equation \(g_K = g_{\text{max}}/\{1 + \exp[(V_m - V_{1/2})/k]\}\). The mean \(g_{\text{max}}\) values obtained were \(4.3 \pm 3.2\) nS \((0.34 \pm 0.21\) mS/cm\(^2\), obtained by averaging single densities) for undifferentiated cells \((n = 7)\) and \(15.2 \pm 8.1\) nS \((0.78 \pm 0.50\) mS/cm\(^2\) for differentiated cells \((n = 13)\), the differences being statistically significant (Student’s t-test, unpaired, confidence level 0.05). The \(g_{\text{max}}\) value for each cell was used to calculate the normalized conductance \((g_{\text{norm}})\), and the mean \(g_{\text{norm}}\) values from differentiated and undifferentiated cells are plotted versus membrane potential in Fig. 3B. When sets of \(g_{\text{norm}}\) values measured for each cell were fitted individually to a Boltzmann function, the mean parameters of the function were as follows: for undifferentiated cells, \(V_{1/2} = 22.1 \pm 1.2\) and \(k = -14.1 \pm 1.0\); for differentiated cells \(V_{1/2} = 37.0 \pm 4.4\) and \(k = -14.4 \pm 1.5\). The 15-mV difference in \(V_{1/2}\) between undifferentiated and differentiated cells was statistically significant \((t\text{-test, confidence level 0.05})\).

According to the Hodgkin and Huxley model (Hodgkin and Huxley 1952) the conductance \(g_K\) could be described by the equation

\[
g_K = g_{\text{max}}(n_{\text{act}})^n\]

where \(n_{\text{act}}\) is the steady-state value of the activation parameter \(n\). In both undifferentiated and differentiated cells the activating phase of the potassium current was best fitted by a single exponential raised to the third power \((x = 3)\) and therefore the steady state activation parameter resulted equal to \(n_{\text{act}} = (g_K/g_{\text{max}})^1/3\).

Mean \(n_{\text{act}}\) values for undifferentiated and differentiated cells are drawn versus membrane potential in Fig. 3C.
the $n_{\text{inf}}$ curve was best fitted by a Boltzmann equation and the average half activation potentials resulted to be $-4.6 \pm 7.2$ mV (undifferentiated cells, $n = 7$) and $11.3 \pm 6.5$ mV (differentiated cells, $n = 13$), while the average slope factors were $-17.3 \pm 2.3$ mV and $-20.6 \pm 2.4$ mV, respectively.

Kinetics of activation and deactivation

To investigate the activation kinetics of potassium currents in the voltage range between $-90$ and $+80$ mV, the values of the time constant of activation ($\tau_n$) were estimated by using two separate methods.

The first method measured $\tau_n$ values in the voltage range between $-70$ and $+20$ mV from relaxation of $I_k$ during repolarization to variable potentials after a 6 ms depolarizing step to $+50$ mV. The pulse protocol and tail currents obtained at five distinct repolarizing potentials are illustrated in Fig. 4A. $\tau_n$ values were then obtained by fitting potassium tail currents (nonlinear least squares curve fit) with a Hodgkin and Huxley type equation (Hodgkin and Huxley 1952)

$$I_{\text{tail}} = A[n_{\text{inf}} - (n_{\text{inf}} - n_0) \exp(-t/\tau_n)]^3 \times [h_{\text{inf}} - (h_{\text{inf}} - h_0) \exp(-t/\tau_h)]$$

where $A = G_{\text{max}}(V - V_k)$, $n_0$, and $h_0$ are the $n$ and $h$ values determined at the end of the depolarizing pulse and $n_{\text{inf}}$, $h_{\text{inf}}$, and $\tau_h$ are the activation and inactivation parameters at the repolarization potential. $n$, $h$, and $\tau_n$ parameters were fixed when experimentally available, otherwise they were free to vary between reasonable upper and lower bounds while fitting tail currents.

In the voltage range examined, the time constant of deactivation was voltage dependent for both undifferentiated and differentiated cells: $\tau_n$ increased with voltage and peaked at $-20$ mV for both cell types [$\tau_n = 8.2 \pm 3.5$ ms ($n = 8$) and $6.2 \pm 2.6$ ms ($n = 6$), respectively].

In the voltage range between $-30$ and $+50$ mV for undifferentiated cells and between $-20$ and $+50$ mV for differentiated cells $\tau_n$ was calculated directly from the activation phase of $I_k$. To this end, current tracings were fitted by a function of the type used for calculation of $\tau_n$ from tail currents. Similar values of $\tau_n$ were also obtained by fitting tracings with an exponential function raised to the third power

$$I_k = I_{\text{max}}[1 - \exp(-t/\tau_n)]^3$$

(see tracings in Fig. 4B), suggesting that, in the voltage and time range examined, the contribution of inactivation parameters to calculation of $\tau_n$ was negligible. Between $-20$ and $+50$ mV, $\tau_n$ decreased monotonically in both undifferentiated and differentiated cells. $\tau_n$ values measured at $-60$, $-40$, $-20$, $0$, and $+20$ mV were respectively $3.52$, $4.71$, $6.43$, $3.50$, and $2.18$ ms. B: onset of potassium currents in an undifferentiated cell on depolarization at different test potentials, fitted with a power function of third order; pulse protocol is indicated over current traces. Tail currents were fitted by nonlinear least-squares curve fit with Hodgkin-Huxley type equation:

$$I_{\text{tail}} = A[n_{\text{inf}} - (n_{\text{inf}} - n_0) \exp(-t/\tau_n)]\times [h_{\text{inf}} - (h_{\text{inf}} - h_0) \exp(-t/\tau_h)].$$

$\tau_n$ values measured at $-60$, $-40$, $-20$, $0$, and $+20$ mV were respectively $4.83$, $4.73$, $2.75$, $6.43$, $3.50$, and $2.18$ ms. B: onset of potassium currents in an undifferentiated cell on depolarization at different test potentials, fitted with a power function of third order; pulse protocol is indicated over current traces. A: time course of deactivation of K currents at different repolarization potentials in an undifferentiated cell. Membrane repolarization at potentials indicated followed a step depolarization to $+50$ mV of $3.5$ ms duration from a holding potential of $-90$ mV; pulse protocol is indicated over current traces. A period of $300$ $\mu$s was blanked after termination of test pulses. Tail currents were fitted by nonlinear least-squares curve fit with Hodgkin-Huxley type equation:

$$I_{\text{tail}} = A[n_{\text{inf}} - (n_{\text{inf}} - n_0) \exp(-t/\tau_n)]\times [h_{\text{inf}} - (h_{\text{inf}} - h_0) \exp(-t/\tau_h)].$$

$\tau_n$ values measured at $-60$, $-40$, $-20$, $0$, and $+20$ mV were respectively $3.52$, $4.71$, $6.43$, $3.50$, and $2.18$ ms.

FIG. 4. Activation kinetics of K currents. A: time course of deactivation of K currents at different repolarization potentials in an undifferentiated cell. Membrane repolarization at potentials indicated followed a step depolarization to $+50$ mV of $3.5$ ms duration from a holding potential of $-90$ mV; pulse protocol is indicated over current traces. A period of $300$ $\mu$s was blanked after termination of test pulses. Tail currents were fitted by nonlinear least-squares curve fit with Hodgkin-Huxley type equation:

$$I_{\text{tail}} = A[n_{\text{inf}} - (n_{\text{inf}} - n_0) \exp(-t/\tau_n)]\times [h_{\text{inf}} - (h_{\text{inf}} - h_0) \exp(-t/\tau_h)].$$

$\tau_n$ values measured at $-60$, $-40$, $-20$, $0$, and $+20$ mV were respectively $3.52$, $4.71$, $6.43$, $3.50$, and $2.18$ ms.


Potassium Currents in Neuroblastoma Cells

Steady-state inactivation

The voltage dependence of $I_K$ steady-state inactivation ($h_{\infty}$) was studied by measuring the peak potassium current elicited at $+50$ mV after a conditioning potential of varying amplitude and 660 ms duration; this protocol allowed the inactivation variable to reach its steady-state value at any investigated potential. $h_{\infty}$ was determined by normalizing...
the peak current to the maximum current evoked with a conditioning hyperpolarizing step to $-90 \text{ mV}$. The voltage protocol and two families of current tracings obtained from an undifferentiated and a differentiated cell are shown in Fig. 5A.

In Fig. 5B, average steady-state inactivation values for both undifferentiated and differentiated SH-SY5Y cells are plotted versus conditioning potential. Inactivation had a threshold of about $-30 \text{ mV}$ in both undifferentiated and differentiated cells; in neither of the two types of cells $I_K$ however reached full inactivation, even at very positive potentials: at $+60 \text{ mV}$, $I_K$ was $11 \pm 4\%$ of control in undifferentiated cells ($n = 12$) and $40 \pm 15\%$ of control in differentiated cells ($n = 16$), the difference being statistically significant ($t$-test, confidence level 0.05). Both steady-state inactivation curves showed a sigmoidal dependence on voltage that fitted well the Boltzmann type function

$$h_{inf} = S + (1 - S)/\{1 + \exp[(V - V_{1/2})/k]\} \quad (4)$$

where $V_{1/2}$ is the voltage at which the steady-state variable $h_{inf}$

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**Fig. 6.** Time course of potassium current inactivation at potentials indicated in an undifferentiated cell (A) and in a differentiated cell (B). Continuous lines superimposed to current tracings represent best fit obtained from least-squares nonlinear regression analysis to a single exponential function. $\tau_1$ is 106, 72, and 50 ms, respectively, at 0, +20, and +40 mV for undifferentiated cell and 415, 200, and 168 ms in differentiated cell.
is half-maximal, $k$ is a slope factor and $S$ is a dimensionless variable that represents the noninactivating portion of the current. Averaging the values obtained from single cell fits, the mean parameters resulted to be as follows: for undifferentiated cells $V_{1/2} = -13.8 \pm 6.7$, $k = 9.6 \pm 3.5$ and $S = 0.086 \pm 0.042$ ($n = 12$); for differentiated cells, $V_{1/2} = -9.7 \pm 5.8$, $k = 13.2 \pm 5.2$ and $S = 0.365 \pm 0.151$ ($n = 16$).

**Kinetics of inactivation**

At voltages positive to $-20$ mV the time constant of inactivation ($\tau_h$) was evaluated by fitting a single exponential to the falling phase of the current trace elicited at each test potential with a 660 ms step, as shown in the sample tracings of Fig. 6. The values of $\tau_h$ obtained with this method decreased monotonically with increasing voltages for both undifferentiated and differentiated cells. In a few differentiated cells the time course of inactivation displayed a biexponential decay, and a $\tau_h$ at least 10 times slower was also detectable (not shown). Because this slow $\tau_h$ was observed only occasionally, it was not taken into further consideration.

The time course of the recovery from inactivation of $I_K$ was evaluated in the voltage range between $-100$ and $-40$ mV. Sample tracings of $I_K$ recorded on step depolarizations at voltages positive to $+50$ mV after conditioning pulses of variable duration to hyperpolarizing potentials are shown in Fig. 7, A and B. Peak potassium currents obtained from these sample tracings were plotted as a function of the duration of the conditioning

![Image](http://www.jn.physiology.org/content/vol142/11/655/f7.large.jpg)

**FIG. 7.** Voltage dependence of potassium current inactivation kinetics. A and B: time course of recovery from inactivation of K channels at repolarization potentials of $-100$ and $-40$ mV for an undifferentiated cell (A) and at repolarization potential of $-60$ mV for a differentiated cell (B). Currents were measured on depolarization to $+50$ mV after a prepulse of variable amplitude ($V_c$) and duration ($\Delta t$), as shown by pulse protocol over current tracings. C: onset of recovery from inactivation obtained from current tracings shown in A and B as indicated: peak potassium currents at $+50$ mV were plotted vs. prepulse duration. Continuous lines represent best fit obtained from least-squares nonlinear regression analysis to a single exponential function for recovery at $-100$ mV ($\tau = 44$ ms), $-40$ mV ($\tau = 230$ ms), and $-60$ mV ($\tau = 30$ ms). D: time constants of inactivation ($\tau_h$) vs. membrane potential. $\tau_h$ values for potentials negative to $-40$ mV were measured as indicated in C ($n = 6$ for undifferentiated cells and $n = 7$ for differentiated cells); $\tau_h$ values positive to $-20$ mV were derived from decay of K current ($n = 11$ for undifferentiated cells and $n = 12$ for differentiated cells). Bars indicate means $\pm$ SD. (---): least-squares best fit with equations: $\tau_h = 45.5 + 10100/[61.1 \cdot \exp(V - 0.033) + 0.063 \cdot \exp(-0.146 \cdot V)]$ for undifferentiated cells and $\tau_h = 20.0 + 8943/[30.5 \cdot \exp(V - 0.029) + 0.091 \cdot \exp(-0.177 \cdot V)]$ for differentiated cells.
pulse (Fig. 7C). In both undifferentiated and differentiated cells, recovery from inactivation had a single exponential time course at all voltages tested. The averaged values of the time constants of inactivation and recovery from inactivation expressed as a function of membrane potential are shown in Fig. 7D.

**Electrophysiological role of the outward conductance**

It is known that, in SH-SY5Y cells, voltage-gated Na$^+$ conductances are present and are involved in the generation of overshooting action potentials in differentiated cells (Johansson 1994; Toselli et al. 1996) or abortive action potentials in undifferentiated cells (Toselli et al. 1996). Hereby we investigated whether or not the potassium conductance previously described is also involved in the excitability of these cells. Indeed, in the few cells expressing a negligible K current, the shape of the spikes or spikelets elicited under current clamp conditions was dramatically altered, because of the lack of the repolarizing phase. In cells where both the Na and the K conductance were available, the same effect could be reversibly obtained by adding 20 mM TEA to the extracellular solution (Fig. 8A). This clearly demonstrates that the outward current is the one responsible for the repolarizing phase of spikes and abortive action potentials in differentiated and undifferentiated cells respectively.

In a previous work we have shown that the rate of rise of action potentials increases during cell differentiation, as a consequence of changes in sodium channel gating (Toselli et al. 1996). Here the decaying phase of action potentials was investigated. In undifferentiated cells the average slope of spikelet decay (dV/dt) was $-4.3 \pm 1.7$ mV/ms ($n = 24$), while in differentiated cells the slope of action potential decay was $-11.5 \pm 4.7$ mV/ms ($n = 28$) (Fig. 8B). Cell capacity ($C_m$), measured in the same two groups of cells, was $12.5 \pm 4.3$ pF and $21.3 \pm 7.3$ pF in undifferentiated and differentiated cells, respectively. Because the rate of repolarization was about three times greater and $C_m$ nearly doubled in differentiated cells, we can conclude that the repolarizing capacitive current ($I_c = -C_m \cdot dV/dt$) was more than threefold faster in cells treated with retinoic acid. Finally, whereas multiple firing or even multiple spikelet generation was never observed in any undifferentiated cell in response to prolonged stimulations, trains of action potentials could be elicited in a fraction (5/7) of differentiated cells (Fig. 8C).

**DISCUSSION**

The present results show that the outward current measurable from the human neuroblastoma cell line SH-SY5Y is carried by potassium ions, on account of its reversal potential and of its block by external TEA and intracellular cesium ions.

In both differentiated and undifferentiated SH-SY5Y cells, this conductance had features apparently similar to the features of a “delayed rectifier” potassium conductance, according to its threshold and time course of activation and...
to its pharmacological properties. The potassium current was almost insensitive to 4-AP and cadmium and its amplitude did not change significantly on shifting the holding potential shift from −90 to −40 mV, indicating no sizeable contamination from A-type or calcium-dependent potassium conductances. A detailed analysis of kinetic properties and voltage dependence of activation and inactivation showed however differences between the currents measured in undifferentiated and differentiated cells.

Comparison of potassium-current properties in undifferentiated and differentiated cells

In undifferentiated cells, the potassium current was relatively small in amplitude; the current amplitude increases significantly in differentiated cells: maximum conductance changes from 4.3 to 15.2 nS. Because of a gradual increase in cell surface during differentiation, current density increases only about a factor of two (from 0.34 to 0.78 mS/cm²) but the difference is still statistically significant. These values are similar to those measured in guinea pig hippocampal neurons (Sah et al. 1988), undifferentiated IMR-32 neuroblastoma cells (Ginsborg et al. 1991), and rat suprachiasmatic nucleus neurons (Bouskila and Dudek 1995) but are more than 10-fold smaller than those measured in N1E-115 differentiated neuroblastoma cells (Moelenaar and Spector 1978) and rat sympathetic neurons (Belluzzi and Sacchi 1988). This fact could partly reflect a relatively low channel density even in differentiated cells. An increased expression of the related gene might be responsible of the higher K⁺ conductance after differentiation.

At both stages of differentiation the time course of activation was sigmoidal and best described by a power function of the third order. In differentiated cells however, we measured a shift of the potential for half-maximal activation of ~15 mV toward more positive potentials, compared with that of undifferentiated cells (see Fig. 3, B and C).

Kinetics of activation and deactivation looked similar in both differentiated and undifferentiated cells. In both cases the relation between time constant of activation and voltage was bell shaped with a maximum at about −20 mV. No significant differences between the two sets of values were measured in the two types of cells.

Potassium currents displayed a relatively slow but sizeable voltage- and time-dependent inactivation in both undifferentiated and differentiated cells. This has been considered to be a true gating property of the channels and not an artifact caused by K⁺ accumulation; although a small reduction in E_K cannot be excluded on prolonged depolarization, such effect cannot account for the magnitude of the outward current decline and for its voltage and time dependence.

Our results show that both steady-state properties and kinetics of potassium current inactivation changed during differentiation. For voltages positive to −30 mV the amount of steady-state current inactivation was progressively greater in undifferentiated cells than in differentiated cells; for instance at +60 mV steady-state inactivation was almost complete in undifferentiated cells, while it reached <70% in differentiated cells (see Fig. 5B).

In both types of cells, current inactivation was a relatively slow process when compared with activation: the smallest values of τ_h were measured between −100 and −60 mV (~20 ms in undifferentiated cells and 50 ms in differentiated cells) and the highest values at about −20 mV (333 and 517 ms in undifferentiated and differentiated cells, respectively).

The time constants of inactivation (τ_h) displayed a bell shaped voltage dependence in both differentiated and undifferentiated cells; τ_h curve for undifferentiated cells, however, reached a maximum at about −30 mV, whereas for differentiated cells the curve was shifted by ~10 mV toward more positive potentials, with a maximum at about −20 mV.

In a recent characterization of potassium currents in differentiated SH-SY5Y cells, Johansson et al. (1996) reported values for steady-state activation and inactivation much closer to those that we measured in undifferentiated cells. Furthermore, they reported a great variability from cell to cell concerning the time course of inactivation. These discrepancies are probably the result of the much longer period of differentiation of the cells tested in this study. With respect to this, it is noteworthy to observe that expression of functional voltage-gated Ca channels becomes also maximal and some gating properties of TTX-sensitive sodium channels change significantly after treatment of SH-SY5Y cells with RA for 15 days or longer (Toselli et al. 1991, 1996).

In conclusion, the outward conductances expressed in differentiated and undifferentiated cells display several distinct biophysical properties. Most of the features that characterize the K⁺ conductance of differentiated cells, rather than those observed in undifferentiated cells, are similar, at least qualitatively, to those displayed by several native potassium channels in mature mammalian neurons (Belluzzi and Sacchi 1988; Bouskila and Dudek 1995; Sah et al. 1988).

Channel properties alterations during differentiation could be the result of expression of different channel subunits (Swanson et al. 1990). Alternatively, different posttranslational processes could also contribute to generate potassium channels with distinct properties. For instance, Covarrubias et al. (1994) found that protein kinase C (PKC) specifically eliminates rapid inactivation of the cloned hKv3.4 K⁺ channel and that mutating one serine to aspartic acid in the N-terminal domain mimics the action of PKC. Concerning activation, it was shown that deletions at the C-terminus resulted in a shift of the voltage dependence of activation of drk1 channels (Van Dongen et al. 1990).

Electrophysiological implications

The change of the electrophysiological properties of the potassium conductance during in vitro differentiation of SH-SY5Y cells is not a surprising result, because a similar event also occurs to the TTX sensitive Na⁺ conductance present in the same cell line. Furthermore it was shown that alterations in sodium channel gating properties during differentiation are paralleled by the ability of these cells to generate overshooting action potentials (Toselli et al. 1996). Concerning the potassium conductance studied here, the marked prolongation of the action potential during focal application of TEA (see Fig. 8A) indicates that this current is responsible for the falling phase of action potential. Furthermore the different features acquired by the potassium conductance during prolonged treatment with retinoic acid might contribute, together with the changes in sodium channel gating, to
modify the excitability properties of these cells. This hypothes-
sis is corroborated by the following observations: 1) during
differentiation, potassium channel activation is shifted by
~20 mV toward positive potentials; this could contribute to
speed up the depolarizing phase of action potential and delay
repolarization; 2) in differentiated cells, the extent of steady-
state inactivation at positive potentials is much lower, current
inactivation is slower and recovery from inactivation is faster
than in undifferentiated cells; 3) a sizeable increase in the
outward current occurs during differentiation. These observa-
tions suggest that in differentiated cells repolarization could be
faster and more complete after an action potential.
Indeed, in current-clamp experiments, we have measured in
differentiated cells an action potential repolarization rate
about threefold faster than that measured in the abortive
action potentials observed in undifferentiated cells. A faster
and more pronounced repolarization might also contribute
to generate trains of spikes during prolonged stimulation.
Indeed this firing pattern was observed in some differentiated
but not in undifferentiated cells. These simple consid-
erations can give a qualitative explanation of the change of
the studied potassium conductances to changes in excit-
ability during differentiation in SH-SY5Y cells.

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