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Effects of dimethylsulfoxide on membrane currents of neuroblastoma × glioma hybrid cell

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Giga-ohm seal whole cell recording technique was used to examine ionic currents changes induced by dimethylsulfoxide (DMSO) in neuroblastoma × glioma hybrid NG 108-15 cells. DMSO (0.5–1%) reversible blocks sodium, potassium and calcium currents and shifts by about 6 mV the sodium inactivation curve towards more negative voltages.

Dimethylsulfoxide (DMSO) is known to have multiple biological actions, including induction of Friend leukemic and neuroblastoma cell differentiation [1,2] and analgesia [3]. Effects of DMSO on membrane phospholipids have been reported [4]. While one might suggest that the modifications in the electrical excitability of cells are related to these effects, the mechanism of action of DMSO on nerves remains unknown [3,5]. In order to further investigate the mode of action of DMSO on cell membrane, we analysed its effects on ionic currents in neuroblastoma × glioma hybrid NG 108-15 cells. We show that 0.5–1% DMSO (v/v) reversibly blocks sodium, potassium and calcium currents and shifts the sodium inactivation curve towards more negative voltages. The findings are discussed in relation to effects of anesthetics on nerve membrane and to the ability of DMSO to induce cell differentiation.

The present experiments were carried out on voltage clamped neuroblastoma × glioma hybrid NG 108-15 cells. The cells were grown in DMEM culture medium containing 5% fetal calf serum and HAT (hypoxanthine 100 μM, aminopterin 0.4 μM, thymidine 16 μM) as previously described [12]. To induce morphological differentiation, 2% DMSO and/or 0.5 to 1 mM dibutyryl-cAMP were added to the culture medium for 48 to 72 h, after which the DMSO-containing medium was removed and replaced by DMEM with the following additives: HAT, 1% fetal calf serum, 1% Ultroser G (IBF) and 0.5 mM dibutyryl-cAMP. This medium was renewed every 2 days. The voltage clamp was based on the succion pipette, giga-ohm seal whole cell recording technique [6]. The standard external solution had the following composition (mM): 140 NaCl; 5 KCl; 1 $CaCl_2$; 2 $MgCl_2$; 10 Hepes; 1 glucose (pH 7.3). Stimuli were applied, from a holding potential of −80 mV, at a frequency of 1 Hz when sodium current (I_{Na}) and potassium (I_K) were recorded. A lower frequency (0.25 Hz) was used in calcium current (IC_a) studies. The series resistance was compensated. The composition of the medium contained in the pipette was (mM): 120 CsF, 40 CsCl for I_{Na} recordings; 120 KF, 40 KCl for I_K recordings and 140 CsCl, 1 $MgCl_2$, 3 EGTA, 40 Hepes, 11 glucose (pH 7.3) for I_{Ca} recordings. 0.3 μM TTX were present in the external solution both when

Abbreviations: TEA, tetraethylammonium; TTX, tetrodotoxin.

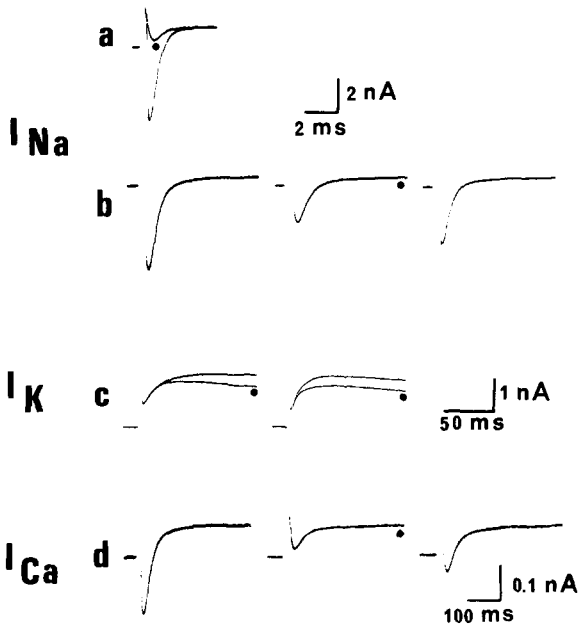

Fig. 1. Reversible block of membrane currents by DMSO. Filled circles indicate current traces in the presence of DMSO. I_{Na} was recorded during depolarizations to 0 mV preceded by 50 ms hyperpolarizations to −120 mV. (a) Superimposed Na^+ current traces recorded under control conditions and in the presence of 1% DMSO. (b) Traces of Na^+ current recorded in the presence of 0.5% albumin; under control conditions (left), after 3 min superfusion with a solution containing 1% DMSO (middle) and after 10 min wash with DMSO-free solution (right). The transient downward current could be fully blocked by TTX (0.3 μM). (c) I_K was recorded during depolarizations to +35 mV. Each trace corresponds to an average of six recordings. Superposition of current traces recorded under control conditions and after 3 min superfusion with a solution containing 1% DMSO (left). Superposition of current traces recorded in the presence of 1% DMSO and after 4 min wash with DMSO-free solution containing 0.5% albumin (right). The delayed upward current could be fully blocked by 20 mM TEA. (d) I_{Ca} was recorded during depolarizations to −10 mV. Averaged traces under control conditions (left), 3 min after addition of 0.5% DMSO and after 30 min wash with DMSO-free solution. The transient inward current could be fully blocked by 1 mM Co^{2+}.

potassium and calcium currents were studied. Furthermore, for I_{Ca} recordings, 20 mM TEA and 18 mM $CaCl_2$ were added to the external solution. The temperature was 18–20°C.

Fig. 1 presents the effects of 0.5 and 1% DMSO on sodium, potassium and calcium currents. In the presence of 1% DMSO, I_{Na} was almost completely blocked within 2 min (Fig. 1a) and this effect was poorly reversible even after a 20 min wash with DMSO-free solution. Reversibility of I_{Na} blockade was accelerated by washing with an external DMSO-free solution containing 0.5% albumin (w/w), which by itself had no effect on I_{Na}. In the presence of albumin (0.5%), 1% DMSO reduced I_{Na} to about 50% of the control value within 1 min, and about 75% reversibility occurred after 10 min of wash with DMSO-free solution containing albumin (Fig. 1b). Five cells were tested in this manner and gave similar results. The effects of DMSO (0.5% without albumin) on time and voltage parameters of sodium conductance were analysed, both under control conditions and in the presence of DMSO. In two independent experiments, these parameters were measured in the same cell, the results of one of these experiments are shown in Fig. 2. In several other experiments, a shift in Na activation and inactivation curves was observed after a control period of superfusion, before DMSO addition. In this case DMSO effects were not analysed. Such a modification in Na activation and inactivation curves has been previously described [7]. In order to

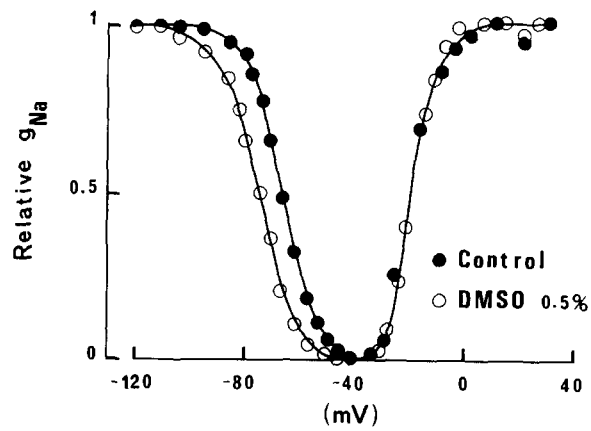

Fig. 2. Effect of DMSO (0.5%) on the voltage dependence of Na conductance activation and inactivation. Normalized values for inactivation (left curves) were calculated as the ratio of I_{Na} at −10 mV following a 50 ms prepulse at each membrane potential to that following a 50 ms prepulse at −120 mV. The curves were drawn according to the equation:

$$h_\infty = 1/\left[1+\exp\frac{(V-V_h)}{k_h}\right] \quad (1)$$

with $k_h = 7$ mV and $V_h = -67$ mV and −75 mV in control and DMSO-treated cells, respectively.

TABLE I

SODIUM CURRENT PARAMETERS

V_h and k_h, characterizing the steady-state inactivation defined by Eqn. 1 (legend to Fig. 2), correspond to the voltage required to reach half-maximal inactivation and the steepnes of the inactivation curve, respectively.

		Control ($n = 8$)	0.5% DMSO ($n = 7$)
Maximum g_{Na}	(nS)	123.4 ±25.5	87.1 ±17.0
$V(g_{Na} = 0.5)$	(mV)	−19.0 ± 3.1	−18.9 ± 1.5
$V(h_\infty = 0.5)$	(mV)	−68.0 ± 1.2	−73.7 ± 1.2
k_h	(mV)	8.1 ± 0.6	7.4 ± 0.4
Time to peak (at 0 mV)	(ms)	0.88± 0.10	0.89±0.13
τ_h (at 0 mV)	(ms)	0.65± 0.04	0.53± 0.04

avoid this, for each culture tested, Na conductance parameters were analysed, first in several cells without DMSO, then in several other cells after DMSO addition to the external solution. Since no significant differences were observed between the values of the individual parameters tested, under each of these experimental conditions, the results for each experimental group were pooled and are summarized in Table I. In addition to a decrease in maximum conductance, DMSO induced a shift of about 6 mV towards more negative voltages of the steady-state inactivation curve and a decrease by about 20% in the inactivation time constant (measured at 0 mV) (Table I and Fig. 2). The other parameters of Na conductance (shape and position of the activation-voltage curve, slope of the inactivation-voltage curve, time to peak of I_{Na}) remained unchanged. None of these DMSO effects were frequency dependent.

Potassium current (I_K) was decreased by DMSO (1% without albumin) and the block was reversed within about 5 min by washing with DMSO-free solution containing 0.5% albumin (Fig. 1c). In the presence of DMSO, a fast inactivation-like decrease in I_K was observed. In two cells, I_K, measured after 150 ms depolarization, was decreased to 70% and 76% of control values, respectively. The decrease in I_K induced by DMSO was apparently voltage independent (Fig. 3).

DMSO also caused a decrease in calcium current (I_{Ca}) and the block was reversed by removing DMSO from the external solution (Fig. 1d). In two cells, 0.5% DMSO decreased I_{Ca} to 23% and

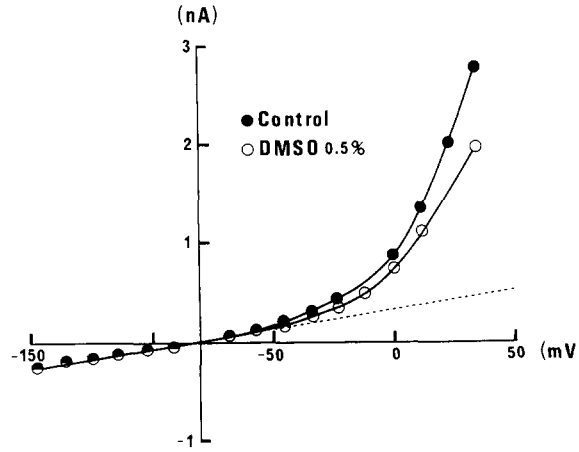

Fig. 3. Potassium current voltage curves in the absence and in the presence of DMSO. The current was measured at the end of 150 ms pulses at indicated membrane potentials. The dashed line is the extrapolation of leakage current recorded during hyperpolarizations.

to 28% of control values. The amplitude of calcium current reported here was somewhat smaller than that reported by Bodewei et al. [8]. This could be attributed to variability in the cell line properties. Because of the relatively small amplitude of the calcium current (observed in five cultures), the effect of DMSO on calcium conductance parameters was difficult to assess.

Two major conclusions can be drawn from the present studies of ionic conductances in neuroblastoma × glioma hybrid cells: (1) DMSO reversibly decreases the membrane ionic currents and (2) it shifts the steady-state Na inactivation curve towards more negative voltages. In some respects, the electrophysiological effects of DMSO on neuroblastoma cells resemble those of local anesthetics and hydrocarbons on nerve axons [10,11]. These effects may in part explain the analgesic properties of DMSO [3]. However, it must be noted that 1% DMSO does not alter membrane currents of the node of Ranvier (Benoit, E., personal communication) and 6% DMSO decreases conduction velocity of frog sciatic nerve only to a slight extend [5]. This variation of DMSO-induced effects might reflect differences in membrane properties between various nerve cells types or between normal and malignant cells. The inhibitory effects of DMSO on membrane currents in neuroblastoma cells, observed in this study

after a short term treatment, were somewhat unexpected since an increase in electrical excitability [2] and an increase in the abundance of voltage-sensitive Na channels, assessed by scorpion toxin binding, have been previously reported [12], for neuroblastoma cells, the differentiation of which was induced by DMSO. However, it must be pointed out that in these cells the maximum rate of rise of the action potential was only slightly enhanced or even decreased [2], in spite of an increase in resting membrane potential [2] and a likely enhancement of Na^+-channel density [12]. This observation is in agreement with our results demonstrating that DMSO blocks ionic currents. A question deserving attention is whether the effects of DMSO on membrane properties (shown here as an alteration in membrane currents) are related to the ability of this compound to inhibit the proliferation of various cell types and to induce a phenotypic and electrical differentiation of malignant cells [2,4,13]. It is known that a modification of the intracellular sodium concentration interferes with the cellular mitogenic activity [14,15]. By decreasing Na influx DMSO might cause a reduction in intracellular sodium concentration and consequently induce a decrease in the rate of cell division. Three other facts have to be taken into account: (i) It has been reported that volatile anesthetics alter the membrane and/or cytosolic calcium concentration [16,17]; (ii) in various preparations, the intracellular free-calcium level plays an important role in mitogenic activity [18–20]; (iii) in muscle cells, cytosolic calcium is known to exert a negative feedback on biosynthesis of TTX-sensitive Na^+ channels [21,22]. As the present results show that the effects of DMSO on membrane currents are similar to those of certain lipophilic local and general anesthetics [10,11], one might suggest that changes in intracellular free-calcium and/or sodium are involved in the DMSO-induced blockade of mitogenic activity and in the cell differentiation. The DMSO-induced increase in electrical excitibility and the abundance of Na channels in differentiated neuroblastoma cells might be relevant to a decrease in the intracellular free calcium level.

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