XLVIII CONGRESSO DELLA SOCIETA' ITALIANA DI NEUROLOGIA
Napoli, 14 - 17 Ottobre 2017

Treating a human neuroectodermal cell line with retinoic acid induces the appearance of neuron-like voltage-gated ionic currents

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Introduction

TB cells are a human neuroectodermal cell line able to morphologically differentiate towards a neuronal-like phenotype if cultured in presence of retinoic acid (RA) 10 µM. These cells were isolated from the cerebrospinal fluid (CSF) specimen of a patient with clinical diagnosis of primary leptomeningeal melanomatosis and characterized by immunological and ultrastructural analysis [1]. The main aim of this work is to study the electrophysiological properties of TB cells by whole cell patch-clamp technique to try to understand if, together with morphological and biochemical modifications, the cells also undergo functional changes after RA-treatment.

Methods

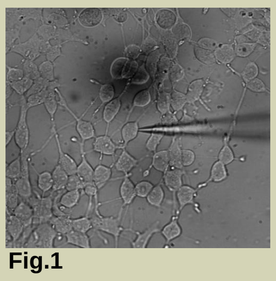

Fig.1

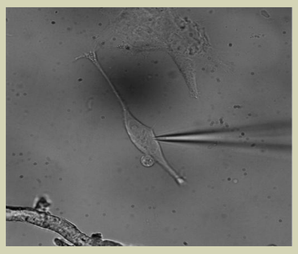

Fig.2

TB cells plated at high (Fig.1) and low (Fig.2) density on plastic coverslips, as appeared at 40 X water immersion objective of an up-right microscope, were prepared as reported previously[1]. Briefly, cells were seeded in 6-wells plates using DMEM medium supplemented with 15% FBS and 1% pen/strep. After 24 hours, cells were treated with 10 µM RA to induce the differentiation. TB cells without RA were also prepared (control). Whole cell patch clamp recordings were performed by means of glass capillaries filled with intracellular solution. Electrophysiological recordings were performed after 7 days of RA treatment by submerging them in extracellular solution whose composition was similar to artificial cerebrospinal fluid (aCSF). To avoid cell detachment, aCSF flow rate was kept very low (< 1 mL/min).
To record sodium currents aCSF composition was (in mM): 126 NaCl, 2.5 KCl, 1.2 MgCl2, 1.2 NaH2PO4, 2.4 CaCl2, 10 glucose, 10 HEPES, 290 mOs/L; intracellular solution contained (in mM): Cs-Methansulphonate 100, TEACl 18, EGTA 2, HEPES 10, Mg2ATP 4, Na3GTP 0.3, Phosphocreatine 10; for Ca2+ currents, extracellular solution contained (in mM): TEACl 100, HEPES 10, CaCl2 4, MgCl2 3, 4-amynopyridine 5, D-Glucose 25; or alternatively: extracellular solution KCl 5.4, NaCl 138, $CaCl_2$ 2, $MgCl_2$1, HEPES 10, D-Glucose 10 with intracellular solution KCl 130, NaCl 5, $CaCl_2$ 1, $MgCl_2$ 2, MgATP 2, $GTPNa_2$ 0.5, EGTA 10, HEPES 10, D-Glucose 10.
Solutions containing only Ca^{2+} : (Medium) CsMethanesulfonate 143.4; CaGluconate 2, MgGluconate 1, HEPES 10, D-Glucose 10; (intracellular solution) CsMethanesulfonate 136, CaGluconate 1, MgGluconate 2, MgATP 2, EGTA 10, HEPES 10. Solutions containing only Cl^- and Ca^{2+} : (Medium) CsCl 143.4, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, D-Glucose 10; (intracellular solution) CsCl 136, $CaCl_2$ 1, $MgCl_2$ 2, MgATP 2, EGTA 10, HEPES 10.

Results and Discussion

Treating TB cells with RA upregulates a fast-inactivating inward current mediated by Na^+ ions.

Upon depolarizing voltage steps, untreated TB cells displayed pronounced outward currents that were strongly reduced when a cesium-based pipette solution was used (Fig. 4). Under this experimental condition, a small inward-going fast-inactivating current at 4,63 ± 0,343 ms (N=14) from the beginning of the depolarizing step was unmasked (Fig. 5, black line).

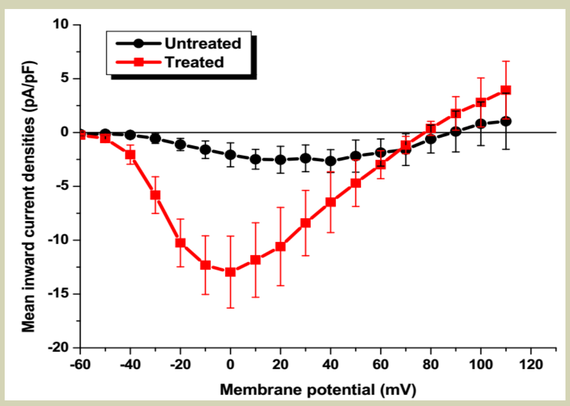

Fig.3

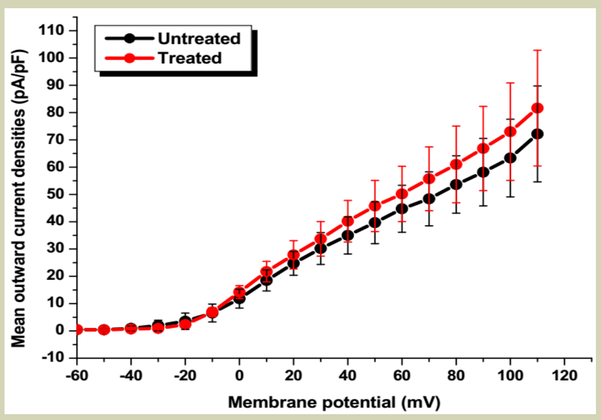

Fig.4

A 7-days RA treatment caused a marked increase in the amplitude of the fast-inactivating inward current, started to activate at -35 mV and reached maximal peak between -5 and 0 mV (Fig.5). The reversal potential of this current (fig. 3) is compatible with Na^+ permeability. Although the fast kinetics of activation and inactivation of this current together with their I-V relationship (Fig.3) resembles that of neuronal voltage-dependent Na^+ currents, we tested if these currents were inhibited by TTX and if they were carried by Na^+.

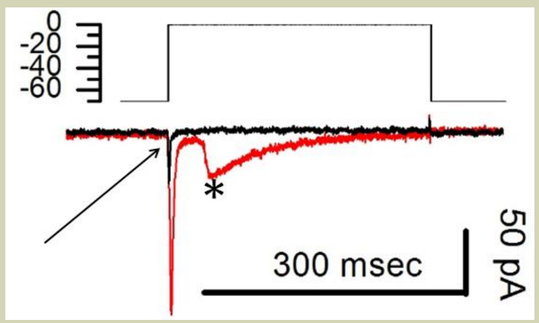

Fig.5

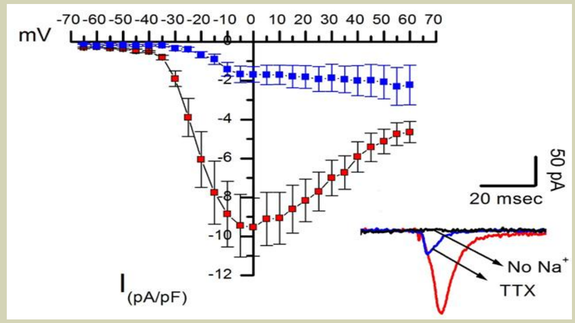

Fig.6

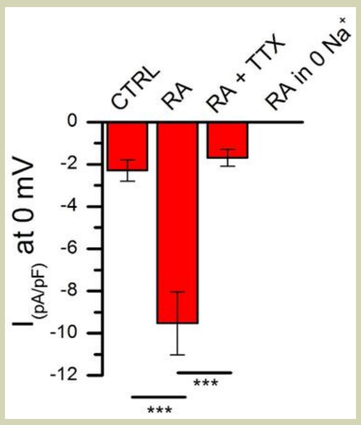

Fig.7

Fig. 6 shows the I-V relationship of fast-activating inward currents in RA-TB cells before (red squares) and during (blu squares) application of TTX (1 µM). Currents were strongly reduced by TTX at all steps of depolarization, while completely abolished by removal of extracellular sodium. While the current-to-voltage relationship (I-V) in CTRL-TB cells (Fig. 3 and 4, N=14) and in RA-TB cells (Fig. 3 and 4, N=38) was similar for both inward and outward components, RA treatment didn't enhance outward currents (Fig.4). Example traces are shown in the inset (the black trace was recorded in the Na^+ -free aCSF, the blu trace in presence of TTX). Bar plot in Fig. 7 shows mean values of fast Na^+ current amplitudes in CTRL-TB cells (N=14), RA-TB cells (N=38), RA-TB cells in the presence of TTX (N=8), and in RA-TB cells in the absence of Na^+ ions in the aCSF (N=21).
These experiments confirmed that RA upregulated a neuron-like TTX-sensitive Na^+ current.

Note that in almost 50% of our experiments, RA treatment induced the expression of a second fast-activating inward component with a time peak at 15 ms (Fig. 8). Although the ultimate ionic nature of this second component is not known, experiments performed with solutions alternatively containing only Ca2+ or Cl- and Ca2+ showed that it is not chloride dependent (Fig. 9), but very likely to be Na+ dependent as suggested by its IV relationship (data not shown).

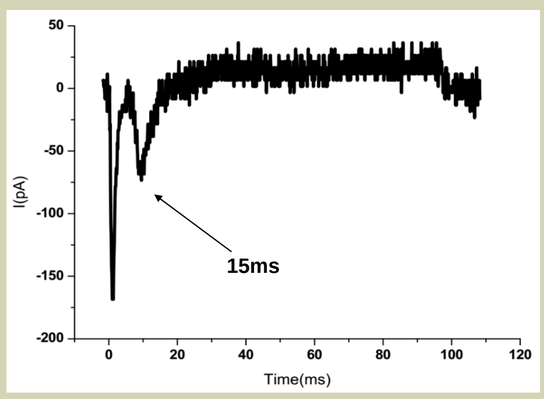

Fig.8

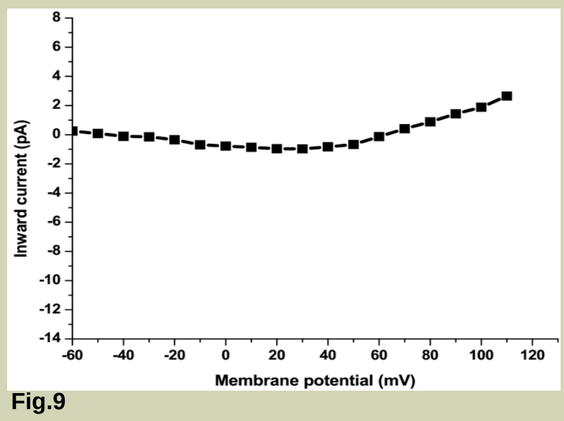

Fig.9

RA induces the expression of a slow-inactivating inward current mediated by Ca^{2+} ions in TB cells

A depolarizing voltage step from -70 mV to 0 mV holding potential applied to RA-TB cells in Na^+ -free aCSF elicited the appearance of a late-onset third inward peak (Fig.5, * on red trace) at 21,81 ± 3,288 ms (N=7) after the onset of the depolarizing step, confirming that this was not carried by Na^+. Instead, this current was completely blocked by Cd^{2+} (300µM; Fig.10 orange trace), a broad spectrum blocker of Ca^{2+} currents. In some RA-TB cells, in the presence of Cd^{2+} an outward current was unmasked. Fig. 11 shows the I-V relationship of the Ca^{2+} current recorded from RA-TB-cells in Na^+-free aCSF (N=21).
Fig. 9 shows the IV relationship obtained by subtracting the current traces obtained with the solutions (medium and extracellular) containing only Ca^{2+} ions from traces obtained with solutions containing both Cl^- and Ca^{2+} ions.

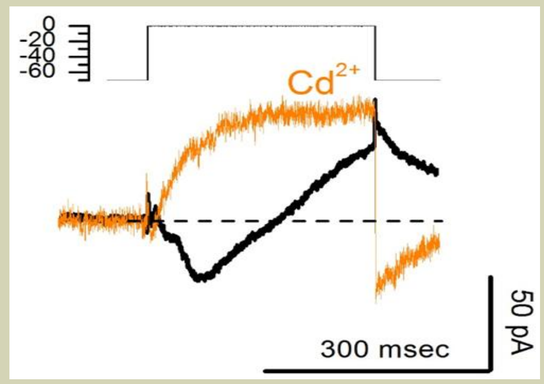

Fig.10

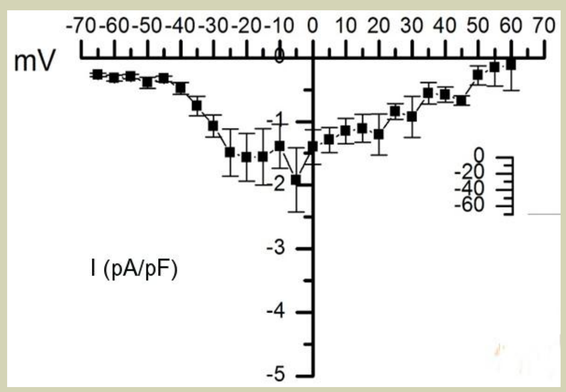

Fig.11

In conclusion, RA-treated TB cells show several neuron-like ionic currents when stimulated by depolarizing voltage protocols. A first appearing fast-inactivating current, which is clearly Na^+ dependent as shown by TTX experiments (see Fig. 6 and 7), is strongly potentiated by RA treatment. A second fast component emerged in almost 50% of our experiments (Fig. 8). Finally, RA caused the appearance of a third slow-onset inward current mediated by Ca^{2+} ions, as confirmed by Cd^{2+}-induced inhibition. Despite RA-treated TB cells did not produce typical action potentials upon electrical stimulation, such treatment potentiated and induced the expression of neuron-like Na^+ and Ca^{2+} currents, respectively.

Conclusion

When treated with retinoic acid, TB cells undergo morphological and biochemical changes [1] while acquiring functional properties [2].
A 7-days RA treatment dramatically increases the amplitude of the fast inward current respect to non-treated TB cells and it induces the expression of two other inward currents, one of which has a definite slow onset and inactivation. While the fast component is due to sodium entry, the slow components is due to calcium. No effect of RA is noted on the outward current. We can conclude that RA primarily modulates expression or functionality of sodium and calcium currents in TB cells.

References

[1] Sorrentino G., Monsurrò MR., Pettinato G., Vanni R., Zuddas A., Di Porzio U., Bonavita V. (1999). Establishment and characterization of a human neuroectodermal cell line (TB) from a cerebrospinal fluid specimen. Brain Res. 827(1-2):205-9.
[2] Arianna Polverino, P. Sorrentino, G. Sorrentino, V. Di Maio, S. Santillo Electrophysiological characterization of a novel human neuroectodermal cell line, SIN-2016 47° Congresso Nazionale della Società Italiana di Neurologia Venezia 22 - 25 ottobre 2016.