Differentiated neuroblastoma F-11 cells as an alternative in-vitro model to dorsal root ganglion neurons

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Abstract

We induced differentiation in F-11 cells to verify if they could show similarities with sensory neurons, in order to develop an alternative to animal models for research studies in the biomedical field.

Introduction

Dorsal Root Ganglion (DRG) neurons have been used for years to study the mechanisms underlying the somatosensory pathways and noxious stimuli. However, these cultures are often expensive, difficult to set up and subject to ethical issues. For these reasons, we tried to develop an alternative to animal models by using F-11 cell line, a hybridoma derived from embryonic rat DRG neurons and mouse neuroblastoma,[1] whose cryopreservation and thawing are simple and economic. F-11 cells could be differentiated into functional neurons by their maintenance on biocompatible substrates,[2] but their properties as sensory neurons remain so far unknown. Thus, in this work we differentiated them in serum-deprived medium in order to verify if they could develop properties of mature neurons and acquire functional similarities with DRG neurons.

Materials and Methods

Neuronal differentiation was induced by incubating F-11 cells in serum-deprived medium (1% FBS) for 10-14 days in culture. An electrophysiological investigation by using the patch-clamp technique in the whole-cell configuration was performed for studying the generation of electrical activity and the expression of voltage-dependent Na^+, Ca^{2+} and K^+ channels. Capsaicin, substance P, neurotransmitters (Acetylcholine and Glutamate) and acidic solutions were used to verify the expression of ion channels typical of sensory neurons. Data were presented as mean ± s.e.m. Mean comparisons between the properties of differentiated and undifferentiated cells (10% serum) were obtained by using the unpaired *t*-test or the non-parametric Mann–Whitney test. The number of responsive cells in the two conditions was compared by using the χ^2 test. The significance level was set for $p<0,05$.

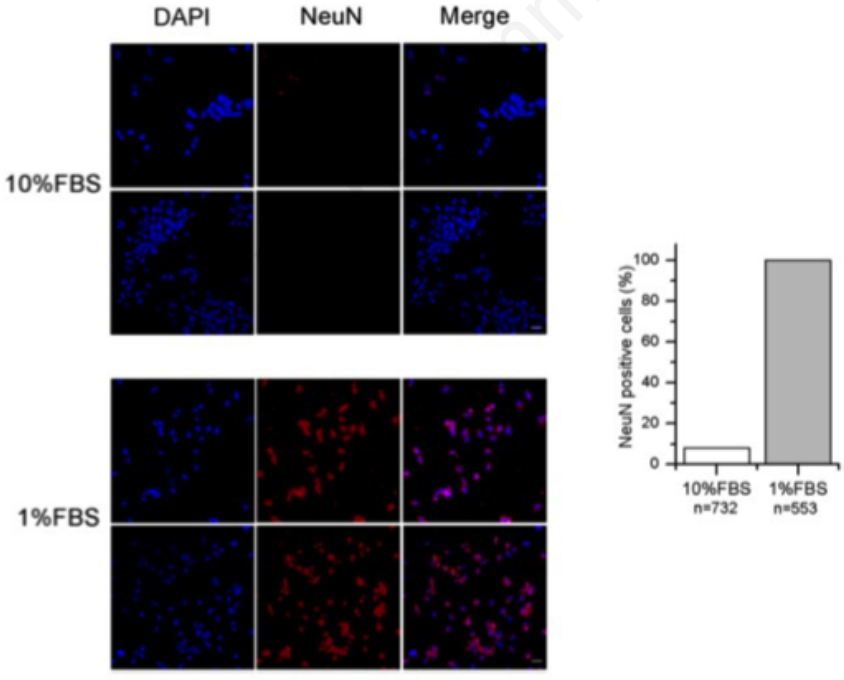

Figure 1. Differentiated F-11 cells express the neuronal nuclear antigen NeuN. The panels illustrate NeuN staining in red, DAPI in blue and the color overlay (merged) in F-11 cells maintained in 10% FBS (control) and 1% FBS (differentiated cells), respectively. Quantification of NeuN positive cells (histograms) in 10 different fields confirmed no or minor expression of this nuclear marker in 10% FBS compared to 1% FBS cultures.

Results

After 12–14 days in 1% FBS medium, F-11 cells showed properties of mature neurons: they stained positively for the neuronal nuclear protein NeuN (Figure 1) and

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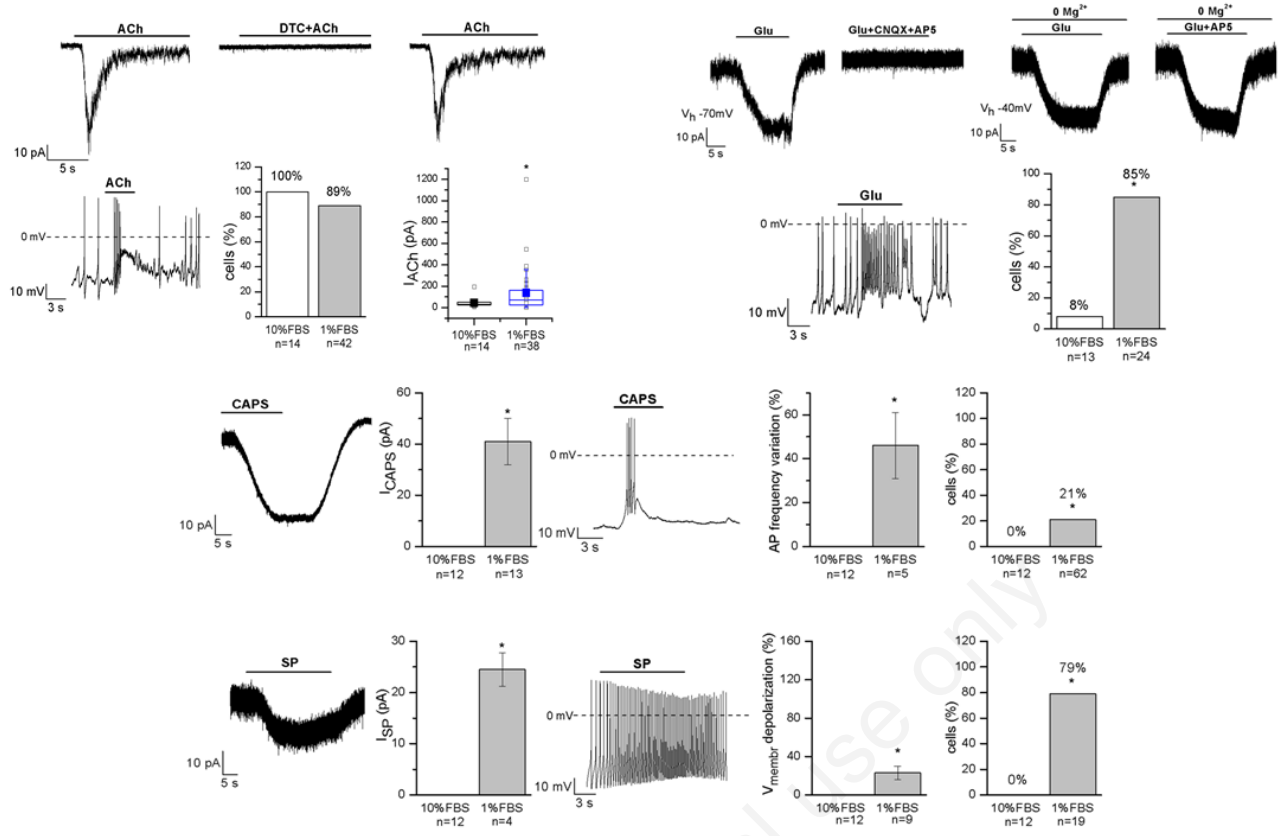

Figure 2. Differentiated F-11 cells display responses to Acetylcholine (ACh), Glutamate (Glu), Capsaicin and Substance P. ACh was effective on 100% of undifferentiated and on 89% of differentiated cells and its action was mediated by nAChRs, as demonstrated by d-tubocurarine (DTC) block. The percentage of Glu-responsive cells was significantly higher in differentiated than in undifferentiated cells. Glu-evoked effects were principally mediated by non-NMDA receptors, since AP5 administration in Mg_{2+}-free extracellular solution and at –40 mV did not affect them. Capsaicin (CAPS) and Substance P (SP) evoked responses in differentiated cells but had no effect on undifferentiated cells.

about 50% of the culture was characterized by neuronal networks of cells exhibiting typical neuronal morphology. Moreover, differentiated F-11 cells showed Na^+ currents consistent with those exhibited by primary DRG neurons. Responses to acetylcholine (ACh) were recorded in 89% of differentiated cells. Approximately the same percentage (70-80%) of rat DRG primary neurons was referred by literature to express functional nicotinic ACh receptors (nAChRs). Glutamate (Glu) was effective on non-NMDA receptors as in embryonic DRG neurons (Figure 2). Moreover, pH 5 induced desensitizing currents through proton-activated channels also expressed in DRG neurons. Responses to capsaicin and substance P were also recorded in 21% and 79% of differentiated cells respectively.

Discussion and Conclusions

Dissociated DRG neurons represent the ideal model for investigating sensory neurons, but their availability is limited by several issues. Therefore, the use of immortalized cell lines is considered a valid alternative to animal models. We demonstrated that differentiated F-11 cells represent a more accessible, simple and less expensive model compared to DRG neurons and, since they express some ion channels and receptors that are also expressed in sensory neurons, they might be employed for studying mechanisms involved in the detection and transmission of noxious stimuli and sensory inflammatory pain.[3]

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