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Illustrations:

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THE MORPHOMETRIC STUDY OF THE EFFECTS OF BISPEROXOVANADIUM (BPV(PHEN)) ON NEONATAL DRG NEURONS IN CULTURE

Natasa Todorovic[1], Gordana Stojadinovic[1], Kamal AlJamal[2], Miroslav Zivic[2]

[1]Institute for Biological Research "Sinisa Stankovic", University of Belgrade, Institute of National Importance for the Republic of Serbia, Bulevar Despota Stefana 142, 11000 Belgrade, Serbia
e-mail: nara@ibiss.bg.ac.rs
[2] Faculty of Biology, University of Belgrade, Studentski trg 16, 11158 Belgrade, Serbia
e-mail: ggrbic@ibiss.bg.ac.rs, kamalaljamal59@gmail.com, mzivic@bio.bg.ac.rs,

Abstract

Unlike the neurons in the CNS, the peripheral neurons have certain intrinsic regenerative capacity. After injury, peripheral neurons can switch to a cellular "state for growth", with the expression profiles similar to early developmental stages.

We looked at the changes of morphometric parameters induced in young peripheral neurons with treatments that in adult neurons have growth-stimulatory effect. The experimental treatments compared to control were: BpV (phen), an inhibitor of PTEN; and bFGF, basic fibroblast growth factor. The neurite growth was measured on cultured dissociated dorsal root ganglia neonatal neurons fixed 24h after treatment and immunostained with anti-neurofilament H (NF-H) phosphorylated antibody. FIJI Simple Neurite Tracer was used for morphometry of individual neurons. 24h post treatment, compared to control, total neurite length, length of primary and length of terminal branches, were increased by bFGF but not by BpV treatment. In all measured parameters related to the degree of branching, BpV-treated neurons had small dispersion of values and small mean values, reminiscent of literature data stating that BpV treated neurons are elongated and less branched. However, the BpV did not have a positive influence on neurite elongation, as was reported on adult neurons. In contrast, bFGF stimulated elongation of young neurons in the manner similar to the effects described on the adult neurons.

Key words: Elongation, Simple Neurite Tracer, Branching

1. Introduction

The obstacles to successful neurite regeneration are intrinsic to the very nature of the nervous system after the development is completed. Namely, mechanisms ensuring the stability needed for proper functioning of the nervous system are regeneration-inhibitive [1]. Peripheral nerves are usually considered to have a full outgrowth potential, but detailed studies suggested that their regeneration depends on the presence of the intact nerve encapsulating sheet, intrinsic neuronal and glial state and the presence of neuromodulators, among other factors [2]. We present the series of neuromorphometric measurements on the very young neurons, in the simple *in vitro* system for peripheral neurons: the dissociated cell culture from rat dorsal root ganglia (DRG). The animal age, postnatal day 2, was chosen as a time point of completed development, but still physiologically close to the growth state. The tested substances are well established to have stimulatory effects on the growth of adult neurons [3,4]

2. Materials and Methods

Neonatal (P2) rat dorsal root ganglia (DRG) dissociated cultures were plated on poly-D-Lysine-laminin coated glass cover slips. Thirty min afterwards, media with appropriate treatment was added to final concentrations: BpV $_{(phen)}$ (in further text: BpV) an inhibitor of PTEN (Phosphatase and TENsin homolog) [5], (80 nM) and bFGF (basic Fibroblast Growth Factor) (50 µM). The concentration for BpV was selected to be twice EC50 for PTEN, in order to insure efficient inhibition. NGF was supplemented to all groups. Axon growth was measured on cell cultures fixed 24h after treatment and immune-stained with anti-neurofilament H (NF-H) phosphorylated antibody (only neurons were labeled).

Images were acquired on fluorescent microscope; FIJI SNT plugin [6] was used for morphometry of individual neurons. The tracing and quantification was done only on the cells that were clearly separated. The final number of acceptable tracings for each group was: 13 (control); 8 (bFGF); 8 (BpV). ANOVA (with and without transformations) with post hoc testing, comparing to control group was used. The data is graphically presented as min-max with medians unless specified otherwise, and stated in the text as mean±SE.

3. Results and Discussion

There was the substantial variability in the neurite growth potential between individual neurons in dissociated culture, as expected.

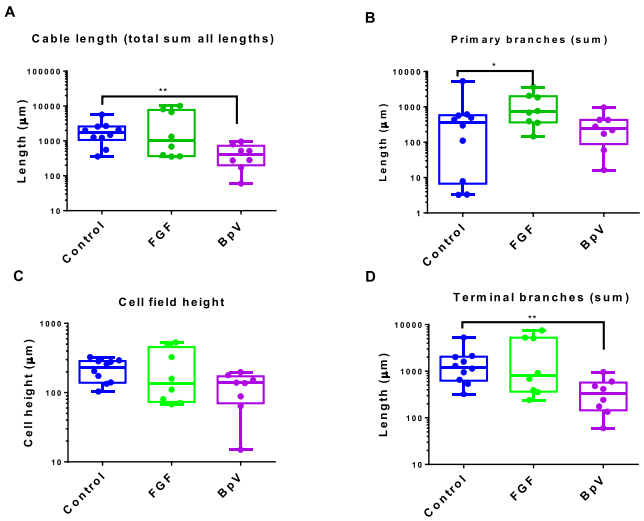

Fig. 1. The main length parameters, obtained from the analysis of traced images of neurons belonging to the control, bFGF-treated and BpV-treated group. A. Summed total lengths of the neurites in the cells. B. Lengths of primary branches (the processes originating in the soma). C. The longer dimensions of the cell rectangle field. D. Lengths of terminal branches (the processes ending with neurite tip). Min to max showing all points box plots with medians.

Figure 1A shows the total length of all neurites (cable length), for each cell in the groups examined. Group values in µm (mean ± SE) were: 1995 ± 505 (control); 3718 ± 1395 (bFGF); 465 ± 146 (BpV, p = 0.0012). Similar to the total cable length, , the length of primary branches in µm (Fig. 1B) was larger (p= 0.01) in bFGF group (1394 ± 454), compared to control (211 ± 767), while BpV group was not (353 ± 131). Length of the terminal branches (Fig 2D) followed the same pattern.

In contrast, BpV had the negative effect on the cell field height parameter (Fig. 1C). To summarize, data so far presented suggest that BpV is not ineffective on the young neurons: it acts in the direction of reduction of the area occupied by the cell. That could be the consequence of elongation inhibition or alternatively, could be caused by a decreased branching.

The graphs representing the Numbers of branches (NoB) and branch points (NoBP) for each group are shown in Figure 2. In the bottom panels, representing the distributions of values, in bFGF group histogram, portion of neurons with larger NoBP can be seen, not present in the controls. BpV-treated neurons had small NoBP (mean ± SE = 1.7 ± 0.6) compared to controls (13 ± 4), while bFGF-treated

ones had similar NoBP mean as the control group (15 ± 6) (Fig.2B).

In Fig.2A, the trend of opposing effects induced by BpV and bFGF is more dinstinct. The average NoB (mean ± SE) were: 20 ± 5 (control group), 37 ± 14 (bFGF group), 7 ± 2 (BpV group). The distribution of values (bottom panel) for the NoB in bFGF group is spread towards the increased numbers compared to control group ; The BpV group values are more uniform and tend to be smaller.

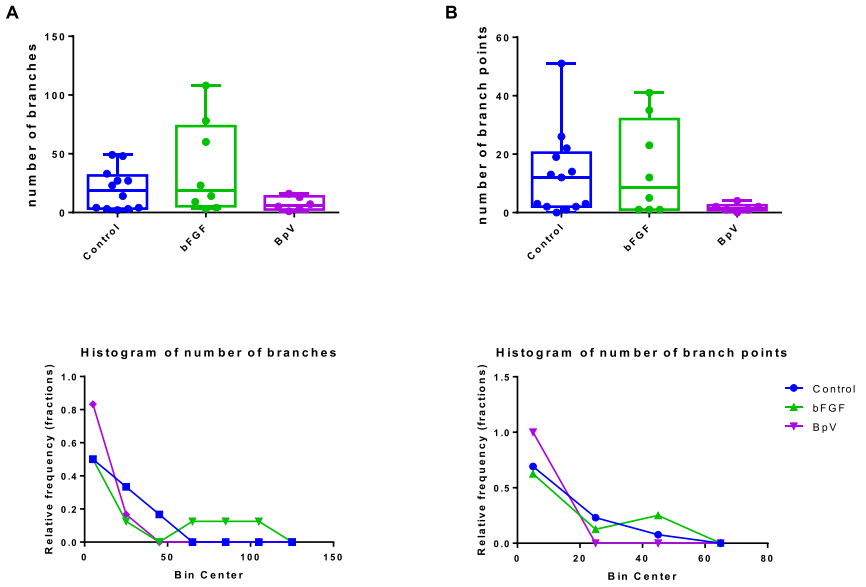

Fig. 2. The parameters of branching, number of branches (NoB) and number of branch points (NoBP), obtained from the analysis of traced images of neurons belonging to the control, bFGF-treated and BpV-treated group. A. The number of branches (top panel, min to max showing all points box plots with medians) and the distribution of the obtained values (bottom). B. The number of branch points (top) and the distribution of number of branch points values (bottom). Note the presence of large branch/branch point numbers almost exclusively in bFGF-treated group, and reduced spread in BpV-treated group.

The negative effect BpV has on branching is reminiscent of the effects described in the literature [7], with the important distinction that PTEN inhibition in adult neurons always induces growth stimulation in addition to a reduction of branching [3].

4. Conclusions

The neonatal DRG neurons in culture were growing less, when treated with BpV, PTEN inhibitor. This finding warrants for further investigation, in the context of contemporary attempts in a direction of clinical application of BpV in stroke and other neuronal injuries. The possible negative BpV effects on newly born neurons could have the profound consequences on memory and other neurogenesis-dependent functions.

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