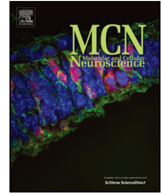
Molecular and Cellular Neuroscience 75 (2016) 50–62

Contents lists available at ScienceDirect

Molecular and Cellular Neuroscience

journal homepage: www.elsevier.com/locate/ymcne



An electrophysiological study on the effects of BDNF and FGF2 on voltage dependent Ca^{2+} currents in developing human striatal primordium

Roberta Squecco [a,*], Eglantina Idrizaj [a], Annamaria Morelli [b], Pasquale Gallina [c], Gabriella B. Vannelli [b], Fabio Francini [a]

[a] Department of Experimental and Clinical Medicine, Section of Physiological Sciences, University of Florence, viale Morgagni 63, 50134 Florence, Italy
[b] Department of Experimental and Clinical Medicine, Section of Anatomy and Histology, University of Florence, Largo Brambilla 3, 50134 Florence, Italy
[c] Department of Surgery and Translational Medicine, University of Florence, Largo Palagi 1, 50139 Florence, Italy

ARTICLE INFO

Article history:
Received 31 January 2016
Revised 24 May 2016
Accepted 27 June 2016
Available online 28 June 2016

Keywords:
Human striatal primordium
Neurotrophic factor BDNF
Neurotrophic factor FGF2
Electrophysiology

ABSTRACT

Over the past decades, studies in both Huntington's disease animal models and pilot clinical trials have demonstrated that replacement of degenerated striatum and repair of circuitries by grafting fetal striatal primordium is feasible, safe and may counteract disease progression. However, a better comprehension of striatal ontogenesis is required to assess the fetal graft regenerative potential. During neuronal development, neurotrophins exert pleiotropic actions in regulating cell fate and synaptic plasticity. In this regard, brain-derived neurotrophic factor (BDNF) and fibroblast growth factor 2 (FGF2) are crucially implicated in the control of fate choice of striatal progenitor cells. In this study, we intended to refine the functional features of human striatal precursor (HSP) cells isolated from ganglionic eminence of 9–12 week old human fetuses, by studying with electrophysiological methods the effect of BDNF and FGF2 on the membrane biophysical properties and the voltage-dependent Ca^{2+} currents. These features are particularly relevant to evaluate neuronal cell functioning and can be considered reliable markers of the developmental phenotype of human striatal primordium. Our results have demonstrated that BDNF and FGF2 induced membrane hyperpolarization, increased the membrane capacitance and reduced the resting total and specific conductance values, suggesting a more efficient control of resting ionic fluxes. Moreover, the treatment with both neurotrophins enhanced N-type Ca^{2+} current amplitude and reduced L- and T-type ones. Overall, our data indicate that BDNF and FGF2 may help HSP cells to attain a more functionally mature phenotype.

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1. Introduction

In the human brain, the corpus striatum is composed of two large subcortical nuclear masses, the caudate nucleus and the putamen, similar in their constituent neurons, intrinsic circuits, and neurophysiology. These nuclei are the main part of the basal ganglia and work with the cortex through a complex network to co-ordinate movements, cognition and emotion, thus controlling the execution of planned and motivated behaviors (Obeso et al., 2008). Selective neuronal loss of the striatum, accounts for most of the clinical features of Huntington's disease (HD), a genetic neurodegenerative disorder characterized by cognitive, motor and psychiatric impairments, which inexorably leads to death within 15–30 years (Novak and Tabrizi, 2010). No proven medical treatments are currently available to counteract the devastating course of the disease. Over the past decades, studies in both HD animal models (Peschanski et al., 1995; Armstrong et al., 2000) and pilot clinical trials (Bachoud-Lévi et al., 2006; Reuter et al., 2008; Gallina et al., 2010; Zuccato et al., 2010; Onorati et al., 2014; Paganini et al., 2014) have demonstrated that replacement of degenerated striatum and repair of circuitries by grafting fetal striatal primordium is feasible, safe and may counteract disease progression, thus prospecting an effective strategy to treat HD patients. The achievement of this practice depends on the ability of the grafted cells to proliferate, differentiate, and re-establish impaired circuitries (Tuszynski, 2007). However, several causes may hamper graft survival, such as hypoxia, mechanical

Abbreviations: BDNF, brain-derived neurotrophic factor; C_m, cell capacitance; FGF2, fibroblast growth factor 2; G_m, resting membrane conductance; G_m/C_m, specific conductance; G_{max}, maximal conductance for the activating current; HD, Huntington's disease; HP, holding potential; HSP, human striatal precursor; HVA, high voltage-activated Ca^{2+} currents; I_a, activating current; I_{Ca}, Ca^{2+} currents; $I_{Ca,max}/C_m$, current specific maximal size; I_m, steady-state membrane current; k_a, steepness factor for activation curve; k_h, steepness factor for inactivation curve; LVA, low voltage-activated Ca^{2+} currents; R_a, access resistance; R_m, membrane resistance; RMP, resting membrane potential; τ, time constant of the transient's decay; V_a, potential eliciting the half-maximal activation; V_h, potential eliciting the half-maximal inactivation; V_{rev}, apparent reversal potential.

* Corresponding author.
E-mail address: roberta.squecco@unifi.it (R. Squecco).

http://dx.doi.org/10.1016/j.mcn.2016.06.008
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injury, neurotrophic factors deficiency and free radical production (Cicchetti et al., 2009; Watmuff et al., 2012). *In vitro* modelling is thus necessary to help understand how primordium cells could face those acute and chronic stressors, but several challenges remain to be faced before using this therapy widely. Hopefully, optimization of transplantation protocols and improvement of functional outcome may be reached thanks to a better comprehension of striatal ontogenesis and fetal tissue potential.

During neuronal development, neurotrophic factors exert pleiotropic actions in regulating cell fate, synaptic plasticity, as well as circuitry formation and maintenance (Park and Poo, 2013). In this regard, brain-derived neurotrophic factor (BDNF) and fibroblast growth factor 2 (FGF2) have been crucially implicated in the control of proliferation and fate choice of striatal progenitor cells (Grothe and Timmer, 2007; Cohen-Cory et al., 2010). In previous studies, we demonstrated that human striatal precursor (HSP) cells isolated from 9 to 12-week-old human fetuses, possessed the machinery for long-term survival, proliferation and differentiation (Sarchielli et al., 2014), even under hypoxic conditions (Ambrosini et al., 2015). In addition, this HSP cell plasticity was maintained *in vitro* by the mentioned neurotrophins BDNF and FGF2 in a peculiar manner. Indeed, if on one hand these neurotrophins promoted an undifferentiated state of HSP cells inducing neurogenesis, migration and survival, on the other hand, they stimulated neuritogenesis and thereby maturation of the main component of HSP cell population (Sarchielli et al., 2014). Since HSP cells endogenously produce BDNF and FGF2, these neurotrophins could also play an important autocrine–paracrine role in determining progenitor cell biology during early striatum development. Based on this background (for a review see Zuccato et al., 2010) and on recently published research (Onorati et al., 2014), we here intended to focus the present electrophysiological study on the effect of BDNF and FGF2 treatment on the passive membrane properties and the different voltage-dependent Ca^{2+} currents present in neuronal cells from HSP, known to be good marker of the cell fate. In fact, the expression of the type of Ca^{2+} channels depends on the stage of differentiation, on the type of neuron considered and of transcripts expressed. Ca^{2+} currents are usually small or absent in mesenchymal (Benvenuti et al., 2006; Heubach et al., 2004; Li et al., 2005) or neuronal stem cell (Moe et al., 2005) but appear in neuronal precursors with a prevalent L-type current (D'Ascenzo, et al., 2006). Therefore, the early appearance of L-type Ca^{2+} current has a privileged role in the regulation of gene transcription. In contrast, N-, P/Q- and R-type Ca^{2+} channels are expressed later and are primarily responsible for initiation of synaptic transmission early in development and during maturation in most systems studied to date (Reid et al., 2003; Catterall and Swanson, 2015). Of note, the sensitivity to growth factors varies among the different types of Ca^{2+} currents and the different kinds of neurons: in fact, a specific growth factor can determine opposite effects such as enhancement or inhibition with different strength, time-dependence and may dramatically differ depending on how it is delivered (Ji et al., 2010). Based on this background and on recently published research (Onorati et al., 2014), we here intended to focus the present electrophysiological study on the effect of BDNF and FGF2 treatment on the passive membrane properties and the different voltage-dependent Ca^{2+} currents present in neuronal cells from HSP (n-HSP). To date, in fact, the effects of these growth factors on the electrical properties of the newly formed fetal human striatal precursors have been scarcely investigated. Actually, whether neuronal progenitor cells exhibit voltage- and ligand-gated currents, features characteristic of neurons, and whether these currents are differentially regulated by growth conditions is an important question that has already been addressed in previous studies in different cell models (Sah et al., 1997). Consequently, the present results are particularly relevant to show that neurotrophins treatment of human striatal precursors significantly enhances the acquisition of functional features, indicative of a mature neuronal phenotype.

2. Materials and methods

2.1. Cell cultures

The primary human striatal precursor (HSP) cells were isolated and propagated *in vitro* from human fetal striatal primordium of 9–12 week-old legally aborted fetuses, as described previously (Gallina et al., 2008, 2010; Sarchielli et al., 2014). The use of human fetal tissue for research purposes was approved by the National Ethics Committee and the Committee for investigation in Humans of the University of Florence (Protocol no 678304). HSP cells are a mixed population including neural stem cells, glial- and neuronal-committed progenitors and mature neurons with a striatal phenotype, with this latter component being highly expressed, as previously demonstrated by an extensive characterization (Sarchielli et al., 2014; Ambrosini et al., 2015). For electrophysiological experiments, 10^5 cells were seeded onto coverslips, serum starved for 24 h and then stimulated with 50 ng/ml BDNF or 50 ng/ml FGF2 alone or after 30 min of preincubation with 200 nM of their receptor inhibitors k252a and PD173074, respectively. After a 24 h incubation (37 °C), the stimulated or unstimulated cells were analyzed by electrophysiology. In a further set of experiments, we tested the effects of both growth factors given in combination for 24 h (50 ng/ml FGF2 + 50 ng/ml BDNF). Finally, to test a time-dependence of the response to growth factors, we performed electrophysiological records under acute treatment and at a later time point (48 h).

All the electrophysiological recordings were performed in selected cells with a marked spindle-shaped neuronal morphology (n-HSP). The statistical analysis included only selected n-HSP showing: 1) voltage-dependent L-type Ca^{2+} currents and a RMP more negative than −40 mV, since cells that did not show L-type Ca^{2+} currents and with RMP more positive to such a value are usually considered stem cells (Heubach et al., 2004; Li et al., 2005; Moe et al., 2005; Benvenuti et al., 2006; D'Ascenzo et al., 2006); 2) a membrane time constant >10 ms, since glial-like cells, although have generally a RMP close to −70 mV, have a time constant definitely <10 ms (Westerlund et al., 2003) suggesting a less wide cell surface. Accordingly, the reported data are related to the cells that satisfied these criteria, namely the 70% of the patched control cells, the 84% of BDNF- and 82% of FGF2-treated cells.

2.2. Solutions for electrophysiological experiments

Coverslips with the adherent n-HSP cells in control medium were first superfused at a rate of 1.8 ml min^{-1} with a physiological bath solution having the following composition (mM): 150 NaCl, 5 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 10 D-glucose and 10 HEPES (pH 7.4 with NaOH). To block the high voltage activated Na^+ channels sensitivity, we used TTX (1 μM). Ca^{2+} currents were recorded in a Na^+- and K^+-free solution, namely a TEA-Ca^{2+} bath solution, containing (mM): 10 $CaCl_2$, 145 TEABr and 10 HEPES. For the specific blockade of L-type Ca^{2+} channels, Nifedipine (Sigma) was diluted at 10^{-2} M in DMSO and stored at 4 °C; it was used at 3 μM. ω-Conotoxin-GVIA (ω-CTx-GVIA) and ω-agatoxin-IVA (ω-Aga-IVA (both from Alomone Labs, Jerusalem, Israel) were diluted in distilled water at 5×10^{-4} and 10^{-4} M, respectively, and stored at 20 °C; ω-CgTx-GVIA was used at 500 nM to specifically block N-type channels and ω-Aga-IVA at 200 nM to block P and Q-type channels. Drugs were prepared daily from stock solutions, just before use. To prevent degradation during the experiments nifedipine was stored in the dark and toxins at 4 °C. T-type currents were minimized by using a holding potential (HP) of −40 mV or by using Ni^{2+} (200 μM); thus, T-current was evaluated by subtracting the resulting current from the total Ca^{2+} currents. The recording pipettes were filled with a filling pipette solution containing (mM): 150 CsBr, 5 $MgCl_2$, 10 EGTA and 10 HEPES (pH 7.2 with KOH). To test the effects of dopaminergic agonists, SKF82958 (10 μM; Sigma-Aldrich) or quinpirole (10 μM, Sigma-

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