GDNF differentially regulates neuronal excitability in DRGs from normal and diabetic mice

Ciglieri E., Ferrini F., Salio C.

INTRODUCTION
Both type 1 and type 2 diabetes are associated with complications impairing a wide range of systems and leading to pathologies such as neuropathy, retinopathy, vasculopathy, cardiomyopathy, dermatopathy, and nephropathy. One of the main features of diabetic neuropathy is pain, which arises as a direct consequence of a lesion or a disease affecting somatosensory system. Several studies postulated the involvement of dorsal root ganglia (DRGs) in this pathology considering both the development of sensory abnormalities in early diabetic patients and the particular vulnerability of sensory neurons, due to the lack of the blood-brain barrier and the high metabolic requirements.

DRGs are a group of pseudo-unipolar cells with two branches acting as a single axon, a distal and a peripheral process, transmitting information from a variety of specialized peripheral receptors. Nociceptors, the sensory neurons specialized in the transmission of nociceptive stimuli, can be recognized for their small size (small to medium cells) and the binding of specific markers such as calcitonin gene-related peptide (CGRP), peptidergic nociceptors. Nociceptors are specialized for encoding mechanical, thermal or chemical stimuli, but they can also encode more than one modality (polymodal nociceptors).

Small-diameter nociceptors in DRGs of adult animals require specific neurotransmitters for the maintenance of their phenotype: peptidergic neurons are under the control of nerve growth factor (NGF) via trkA receptor, while non-peptidergic neurons are under the control of glial cell line-derived neurotrophic factor (GDNF) via GFRα1 signaling. Moreover, GDNF is expressed both in a population of DRG neurons and in DRG satellite cells and plays a role in modulating nociceptive processing.

We focused our attention on the analysis of primary sensory neurons of the DRGs in a streptozotocin-induced model of type 1 diabetes by combining electrophysiological and morphological approaches.

The specific aims are:
1. To compare physiological parameters of primary sensory neurons in control and diabetic mice
2. To analyse the effect of GDNF in control and diabetic mice
3. To analyse the morphological alterations of DRGs due to diabetes

ACKNOWLEDGMENTS
This work was supported by Progetto Avenza-Conmpagnia di San Paolo 2012 (Torino, Italy in C3).

Contact: elisa.ciglieri@unito.it

EFFECT OF DIABETES ON DRG NEURONS

I. Small-sized neurons showed different membrane properties than medium-sized ones
II. Small-sized neurons were more affected by alterations induced by diabetes
III. Diabetes could affect voltage-sensitive mechanisms

EFFECT OF GDNF ON DRG NEURONS

VII. GDNF may reduce the excitability of DRG neurons from control mice, especially affecting firing properties in small-size neurons

MATERIALS AND METHODS

- Adult CD-1 male mice
- Single i.p. injection of Streptozotocin or vehicle in one month old mice
- Measurement of glycemia at two months of age to assess diabetes (300 mg/dL)

Electrophysiology
- Recordings were performed in whole-cell configuration, with current and voltage clamp protocol
- DRGs were incubated at 38°C with collagenase for 1 hour to remove the outer connective layer
- GDNF (100 ng/µL) was bath-applied for 5 minutes

Immunofluorescence
- Immunofluorescence staining was performed on whole floating DRGs, fixed in 4% PFA, and on sections from paraaffin embedded DRGs
- Fluorescent images were obtained using a confocal fluorescence microscopy

Statistical analysis
- Paired data were analyzed by Wilcoxon signed rank test and independent groups were analyzed by Mann-Whitney U test. Differences were considered significant for P<0.05.

CONCLUSIONS
- Diabetes alters different parameters in both small (until 490 um) and in medium-to-large (between 490 and 1100 um) DRG neurons. Diabetes seems to increase cellular excitability mostly in small size neurons.
- Diabetes seems to increase K CURRENTS, mostly in small-sized neurons.
- Administration of GDNF on control animals has a relevant impact on voltage-sensitive mechanisms, as shown in presence of hyperpolarized conditions. This effect is lost in diabetic animals.
- Applying depolarizing steps, GDNF acts on both sustained and transient components of K CURRENTS and this effect is almost lost in diabetic conditions.
- GDNF seems to exert its effect mostly on small-sized neurons: applying hyperpolarizing steps in voltage clamp mode, it induces a block of inward currents likely mediated by voltage-dependent potassium channels. The effect is lost in medium-sized neurons.

Our data indicate that GDNF acts with multiple mechanisms among which stands out an inhibitory effect on small DRG neurons. In addition, changes of holding potential highlighted an impairment of the voltage-dependent response of small-sized neurons in diabetic conditions. Restoring this inhibitory control in sensory neurons from diabetic patients may represent a novel strategy for mitigating the symptoms of painful diabetic neuropathy.