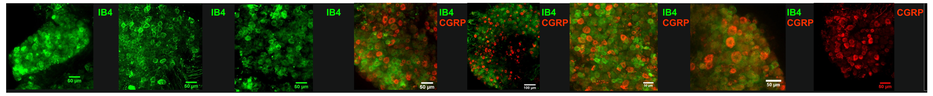
GDNF differentially regulates neuronal excitability in DRGs from normal and diabetic mice



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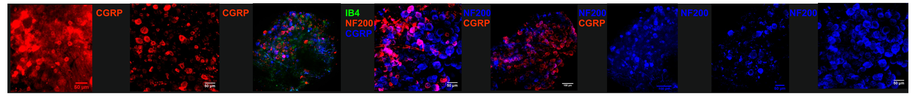

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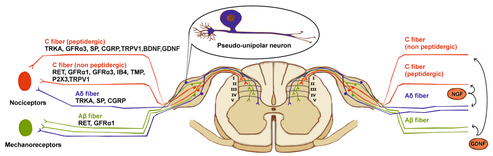
INTRODUCTION

Both type 1 and type 2 **diabetes** are associated with complications impairing a wide range of systems and leading to pathologies such as nephropathy retinopathy, vasculopathy, cardiomyopathy, dermatopathy, and neuropathy. 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, transducing 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 isolectin B4 (**IB4**; non-peptidergic nociceptors) or the expression of peptides, such as the 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 neurotrophins 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 **GFRα-1/Ret** 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:

- **to compare physiological parameters of primary sensory neurons in control and diabetic mice**
- **to analyse the effect of GDNF in control and diabetic mice**
- **to analyse the morphological alterations of DRGs due to diabetes**

ACKOWLEDGMENTS

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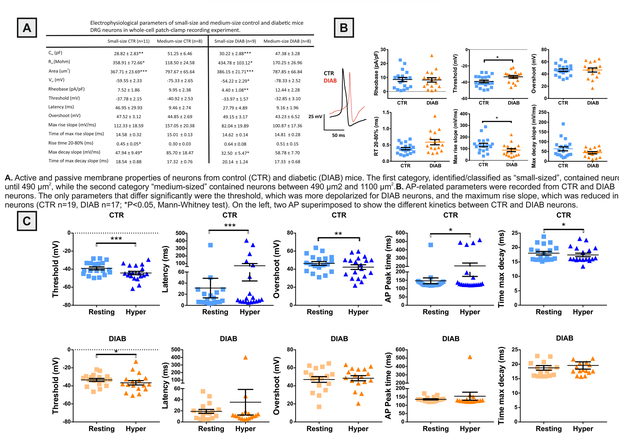
EFFECT OF DIABETES ON DRG NEURONS

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

Regular Na^+ & Ca^{2+} - CC



A. Active and passive membrane properties of neurons from control (CTR) and diabetic (DIAB) mice. The first category, identified/classified as "small-sized", contained neurons until 490 μm^2, while the second category "medium-sized" contained neurons between 490 um2 and 1100 μm^2. **B.** AP-related parameters were recorded from CTR and DIAB neurons. The only parameters that differ significantly were the threshold, which was more depolarized for DIAB neurons, and the maximum rise slope, which was reduced in DIAB neurons (CTR n=19, DIAB n=17; *P<0.05, Mann-Whitney test). On the left, two AP superimposed to show the different kinetics between CTR and DIAB neurons.

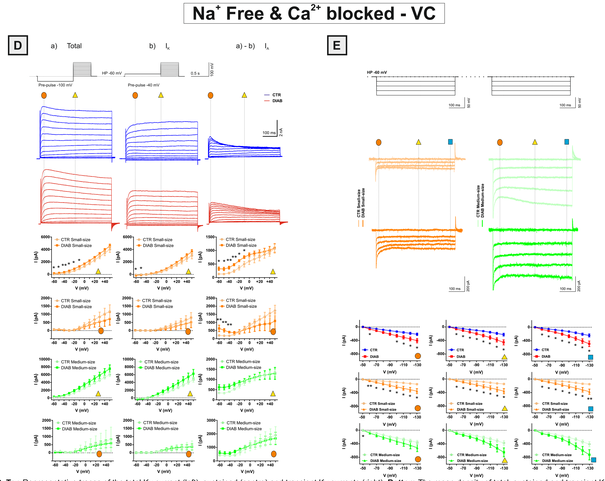
C. AP related parameters were recorded from CTR (**top**) and DIAB (**bottom**) neurons. For each neurons, the same protocols were repeated in aCSF and hyperpolarized (i.e. -85 mV) conditions, to analyze voltage-dependent differences. Interestingly, only CTR neurons showed significant differences in hyperpolarized condition in parameters as threshold, latency, overshoot, AP peak time and time of max decay (CTR n=19, DIAB n=17, *P<0.05, **P<0.01, ***P<0.001, Wilcoxon test).

IV. Diabetes induced changes in K^+ currents in small-sized neurons

V. DIAB neurons showed higher sustained K^+ currents (I_K) and transient K^+ currents (I_A) after depolarizing protocols

VI. DIAB neurons showed increased outward K^+ currents after hyperpolarazing protocols

Na^+ Free & Ca^{2+} blocked - VC

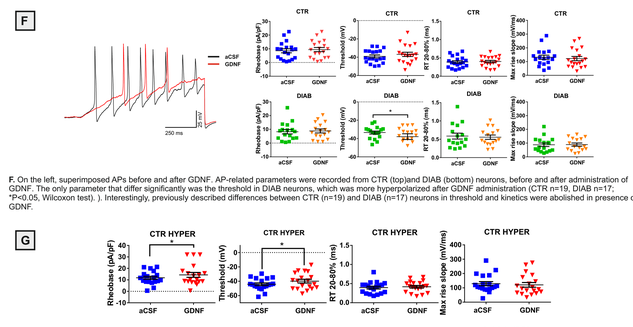


D. Top Representative traces of the total Kv current (left), sustained (centre) and transient Kv currents (right). **Bottom** The mean density of total, sustained and transient Kv currents was significantly higher in DIAB small-sized neurons than in CTR small-sized one, while there was no difference between CTR and DIAB neurons in the medium-sized class. This difference was particularly high measuring the current at the middle point (yellow triangle) (CTR small-sized n=9, medium-sized n=5;DIAB small-sized n=10, medium-sized n=5; *P<0.05, **P<0.01, Mann-Whitney test). **E. Top** Representative traces of the current elicited by the hyperpolarizing protocols from -50 mV to -130 mV (500 ms, Δ=20 mV). Currents were measure at the start (orange circle), the middle (yellow triangle) and at the end (blue square). **Bottom** The mean density currents in DIAB neurons were significantly greater than in CTR neurons, mostly due to the effect in small-sized cells (CTR n=15, DIAB n=15; *P<0,05, Mann-Whitney test).

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

Regular Na^+ & Ca^{2+} - CC



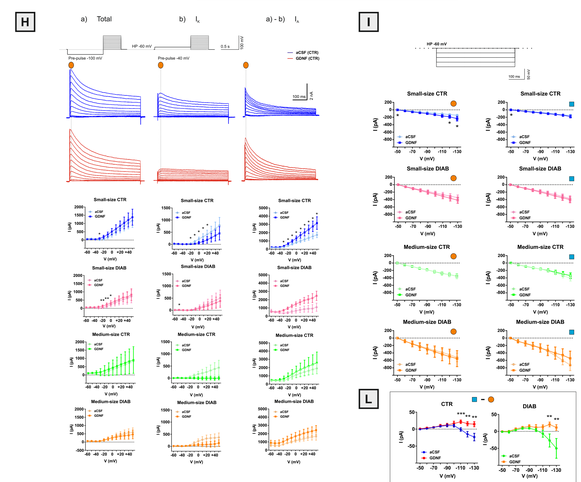
F. On the left, superimposed APs before and after GDNF. AP-related parameters were recorded from CTR (top)and DIAB (bottom) neurons, before and after administration of GDNF. The only parameter that differ significantly was the threshold in DIAB neurons, which was more hyperpolarized after GDNF administration (CTR n=19, DIAB n=17; *P<0.05, Wilcoxon test).). Interestingly, previously described differences between CTR (n=19) and DIAB (n=17) neurons in threshold and kinetics were abolished in presence of GDNF.

G. AP-related parameters were recorded from CTR (top)and DIAB (bottom) neurons, before and after administration of GDNF in hyperpolarizied condition (i.e. -85 mV). In comparison to resting condition, hyperpolarization had a major effect on neurons before GDNF treatment and, mostly, on CTR neurons. The effect recorded in DIAB neurons were lost after reconsidering the samples based on their sizes (CTR n=19, DIAB n=17; *P<0.05, Wilcoxon test).

VIII. GDNF exerted its major effect on small-sized CTR neurons

IX. GDNF seemed to act via multiple mechanisms involving K^+ currents

Na^+ Free & Ca^{2+} blocked - VC



H. Top Representative traces of the total Kv current (left), sustained (centre) and transient Kv currents (right). The mean density of sustained and transient Kv currents of CTR small-sized neurons was significantly different after GDNF administration, with a decrease of the sustained component and an increase of the transient component. Contrariwise, the total Kv current did not vary significantly. This effect was not measured in small-sized DIAB neurons or in medium-sized both CTR and DIAB (CTR small-sized n=9, medium-sized n=5;DIAB small-sized n=10, medium-sized n=5; *p<0.05, Wilcoxon test). **I.** The mean density current was significantly increase after GDNF administration, but only in small-sized CTR neurons and at high negative voltages (CTR small-sized n=9, medium-sized n=5;DIAB small-sized n=10, medium-sized n=5; *p<0.05, **p<0.01, Wilcoxon test). **L.** Hyperpolarization-activated inward current is calculated as the difference between the steady state currents at the end (blue square) and at the start (orange circle) of the traces. A significant difference was noticed at high negative voltages, both in CTR and DIAB animals. GDNF seemed to block the inward currents (CTR small-sized n=9, medium-sized n=5;DIAB small-sized n=10, medium-sized n=5; *p<0.05, **p<0.01, Wilcoxon test).

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 outern connective layer
- GDNF (100 ng/μL) was bath-applied for 5 minutes

Immunofluorescence

- Immunofluorescence staining was performed on entire floating DRGs, fixed in 4% PAF, and on sections from paraffin embedded DRGs
- Fluorescent images were obtained using a confocal fuorescence microscopy

Statistical analysis

Paired data were analyzed by Wilcoxon signed rank test and indipendent 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^2) and in medium-to-large (between 490 and 1100 um^2) 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 condition.
- 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-dependant 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.