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Research report

Pronociceptive changes in the activity of rostroventromedial medulla (RVM) pain modulatory cells in the streptozotocin-diabetic rat

M. Silva[a,1], D. Amorim[b,1], A. Almeida[b], I. Tavares[a], F. Pinto-Ribeiro[b,2], C. Morgado[a,*,2]

[a] Department of Experimental Biology, Faculty of Medicine of Porto and IBMC, University of Porto, Rua Dr Plácido Costa, 4200-450 Porto, Portugal
[b] ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Universidade do Minho, Campus Gualtar, 4710-057 Braga, Portugal

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ABSTRACT

Neuropathic pain is one of the most frequent complications of diabetes. The increased neuronal activity of primary afferents and spinal cord neurons in streptozotocin (STZ)-diabetic rats increases the recruitment of the nociceptive ascending pathways, which may affect the activity of pain control circuits in the brain. This study aimed to characterize the electrophysiological responses of neurons of the rostroventromedial medulla (RVM), a key brainstem area involved in descending modulation of nociceptive neurotransmission at the spinal cord, in STZ-diabetic rats. Spontaneous and noxious-evoked activity of ON-like cells (pain facilitatory cells) and OFF-like cells (pain inhibitory cells) in the RVM were analyzed by single cell extracellular electrophysiological recordings in STZ-diabetic rats with behavioral signs of diabetic neuropathic pain 4 weeks after diabetes induction and in age-matched non-diabetic controls (CTRL). The electrophysiological analysis revealed an increase in the spontaneous activity of RVM pronociceptive ON-like cells in STZ-diabetic rats when compared to CTRL. On the contrary, the number of active antinociceptive OFF-like cells was significantly lower in the STZ-diabetic rats and their spontaneous activity was decreased when compared with CTRL. Overall, the changes in the activity of RVM pain modulatory cells in STZ-diabetic rats point to enhancement of descending pain facilitation. Based on similar results obtained at the RVM in traumatic neuropathic pain models, the changes in the electrophysiological responses of RVM in STZ-diabetic rats may account for exacerbated pain-like behaviors in diabetic neuropathy.

1. Introduction

Neuropathic pain is a common and high impact complication of diabetes that greatly affects patients' quality of life (Quattrini and Tesfaye, 2003). Diabetic neuropathic pain (DNP) is characterized by spontaneous pain, mechanical hyperalgesia and tactile allodynia (Boulton et al., 2004; Tesfaye et al., 2010; Pertovaara et al., 2001; Morgado et al., 2010). These altered pain sensations have been attributed to damage of peripheral nerves. More recently, functional impairments of the central areas involved in pain modulation/transmission have also been proposed to account for DNP. Streptozotocin (STZ)-diabetic rat, an animal model of diabetes that develops DNP, displays spontaneous hyperactivity and hyperexcitability of neurons in the spinal cord and thalamus (Pertovaara et al., 2001; Morgado et al., 2010; Chen and Pan, 2002; Morgado and Tavares, 2007; Fischer et al., 2009; Fischer and Waxman, 2010). Neuronal hyperactivity has been attributed to increased peripheral input (Chen and Levine, 2001), which is likely to potentiate the recruitment of the nociceptive ascending pathways, and to altered mechanisms of pain control at the spinal cord (Morgado et al., 2008, 2011a; Jolivalt et al., 2008). Another possibility that needs to be evaluated is impairment in pain modulation from the brain. This hypothesis is likely since we have recently demonstrated neurochemical and structural changes in the rostroventromedial medulla (RVM), a key pain control area of the brain (Morgado et al., 2011b). It should be recalled that the RVM works as the gateway to directly convey to the spinal cord the descending pain modulatory influences from the periaqueductal gray matter (PAG), which collects modulatory input from several higher brain centers like the cortex, amygdala and hypothalamus (Gonçalves et al., 2007; Porreca et al., 2001; Kincaid et al., 2006; Xu et al., 2007). Since in traumatic and inflammatory pain models, the activity of RVM neurons is altered toward descending pain facilitation (i.e. pronociception) (Millan, 2002; Vanegas and Schaible, 2004; Heinricher et al., 2009; Ossipov

* Corresponding author at: Department of Experimental Biology, Faculty of Medicine of Porto, Alameda Hernani Monteiro, 4200-319 Porto, Portugal. Tel.: +351 220426745; fax: +351 225513655.
E-mail addresses: martalvesilva@gmail.com (M. Silva), dianaamorim@ecsaude.uminho.pt (D. Amorim), aalmeida@ecsaude.uminho.pt (A. Almeida), isatav@med.up.pt (I. Tavares), filiparibeiro@ecsaude.uminho.pt (F. Pinto-Ribeiro), cmorgado@med.up.pt (C. Morgado).
[1] These authors contributed equally for this work.
[2] These authors contributed equally for the supervision of this work.

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et al., 2010; Khasabov et al., 2012), it urges to study the electrophysiological features of RVM neurons in animals models of DNP. As referred above, the serotoninergic descending pain pathway from the RVM is impaired in DNP, possibly accounting to the spinal cord neuronal hyperexcitability and exacerbated pain-like behaviors in STZ-diabetic rats (Morgado et al., 2011b). Besides the serotoninergic neurons, it is widely accepted that the RVM comprises three types of pain modulatory cells, characterized by their electrophysiological activity and role in nociceptive modulation, the ON-, OFF- and NEUTRAL-cells. ON-cells have been associated with increased pain behavioral responses and were, therefore, ascribed a pronociceptive role. On the contrary, OFF-cells decrease their activity before the pain behavioral response and are considered antinociceptive cells. NEUTRAL-cells do not appear to correlate their activity with pain behavioral responses and their role in pain modulation remains controversial (Fields et al., 1983, 2006). Imbalance in the activity of pronociceptive ON- and antinociceptive OFF-cells was shown to occur in traumatic neuropathic pain, with increase in "ON-cell" firing and the reverse for "OFF-cell", which facilitates nociceptive transmission at the spinal cord (Gonçalves et al., 2007; Porreca et al., 2001; Carlson et al., 2007). The electrophysiological activity of RVM neurons was never studied in DNP. Taking into account that diabetes affects RVM actions (Morgado et al., 2011b) and considering the role of ON- and OFF-cells in the RVM in pain modulation, this study aimed to evaluate the electrophysiological activity of ON- and OFF-cells of the RVM in STZ-diabetic rats with behavioral signs of DNP.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (Charles River; Barcelona, Spain), weighing 250–350 g at the beginning of the experiments were housed two per cage, in a room with a constant temperature (22 ± 2 °C) and humidity (55 ± 5%) and under a 12-h light/dark cycle. Food and water were provided ad libitum. Experiments were performed in accordance with the ethical guidelines for the study of pain in conscious animals (Zimmermann, 1983) and the European Community Council Directive 86/609/EEC.

2.2. Induction of diabetes

Type-1 diabetes was induced by an intraperitoneal (i.p.) injection of STZ (60 mg/kg body weight; Sigma–Aldrich, St. Louis, USA) freshly dissolved in 0.1 M citrate buffer, pH 4.5 (n = 9). Age matched control rats (n = 12) were injected with equal volume of the vehicle (CTRL). Three days after the injection, glucose concentration was measured, using Accu Chek Sensor Comfort (Roche Diagnostics, Germany), in blood samples collected from the tail vein. Only STZ-injected rats with glucose concentration higher than 270 mg/dl were considered diabetic and included in the STZ group. The levels of hemoglobin A1C were also measured using A1cNow+ (Bayer Diabetes Care, USA).

2.3. Behavioral evaluation of nociception

Behavioral evaluation of nociception was performed before STZ or vehicle injection and 4 weeks later, using the paw pressure test (Randall–Selitto test, Ugo-Basile, Comerio, Italy). In this behavioral test, which is a validated test of mechanical hyperalgesia, increasing mechanical forces are applied to the dorsal surface of right hindpaw with a cone-shaped plunger. The mechanical force (in grams) that induces hindpaw withdrawal was recorded. The paw withdrawal threshold (PWT) of each animal was considered to be the average of three consecutive measurements, taken with 5 min intervals. For habituation purposes, all the animals were handled daily by the experimenter 8 days prior to the testing period.

2.4. Electrophysiological recordings in the RVM

The electrophysiological recording of neurons in the RVM was performed in STZ-diabetic (n = 9) and CTRL (n = 12) rats at the day after the last behavioral evaluation (29 days after diabetes induction) following a methodology previously described by Pinto-Ribeiro et al. (2008). Animals were initially anesthetized with pentobarbitone (50 mg/kg, i.p.). During the recording sessions, anesthesia was maintained by the administration of additional reinforcements (15–20 mg/kg/h, i.p.), every hour until the end of the experiments. During the recording, the level of anesthesia was monitored every 15 min by verifying dilation of the pupils and the general muscle tone. A warming blanket was used to maintain the body temperature within physiological range. The anesthetic protocol used in our experiments is not compatible with classical recording of RVM ON- and OFF-cells in which cell activity is directly correlated with tail flick reflexes of the animal (Fields et al., 1983). The RVM neurons were characterized based on their responses to peripheral noxious stimulation and, accordingly, are referred as ON-like (if their activity increases during noxious stimulation) and OFF-like (if their activity decreases during noxious stimulation) cells. RVM neurons displaying no or only a negligible (<10%) alteration in discharge rates during noxious stimulation were considered NEUTRAL-like cells. This classification of ON-like neurons, OFF-like neurons and NEUTRAL-like neurons has previously been used in several studies (Pertovaara et al., 2001; Pinto-Ribeiro et al., 2008; Miki et al., 2002; Sanoja et al., 2010; Sugino et al., 2012).

After placing the animal in a standard stereotaxic frame, the skull was exposed to allow the placement of the electrode in the RVM, according to the coordinates of the Paxinos and Watson atlas (Paxinos and Watson, 2007) (1.92 mm caudal to the interaural line, 0 mm lateral from the midline, and 10 mm ventral from the dura mater; Fig. 1). Single neuron activity was recorded extracellularly with lacquer-coated tungsten electrodes (tip impedance 3–10 MΩ at 1 kHz; FHC Inc., Bowdoinham, ME, USA) and then amplified and filtered. Data sampling was performed with a computer connected to a CED Micro 1401 interface and using Spike 2 software (Cambridge Electronic Design, Cambridge, UK). In our experimental protocol RVM neurons were first identified upon peripheral noxious stimulation of the tail and then evaluated upon the stimulation of the hind paw previously tested by the paw pressure test. In case the response was present also for the hind paw we proceeded with the stimulation protocol. About 90% of cells responding to mechanical stimulation of the tail also responded to the same type of stimulation of the hind paw.

The recordings of RVM neurons were performed in two phases. In the first phase, the activity of RVM neurons was recorded after the application of the stimulation protocol described below. In the second phase, three dorsoventral descents of the recording electrode were performed at 0.2 mm intervals during brief (3 s) paw noxious pinching.

The stimulation protocol comprised the recording of (i) the spontaneous activity of RVM cells, by recording during 20 s the basal activity of the neurons before any stimulus, (ii) the noxious-evoked activity of RVM cells, by recording the activity of the neurons in response to the pinching of the hindpaw with a surgical clamp (with a strength that produced a painful sensation when applied to the experimenter's hand) during 5 s, and (iii) the post-stimulation activity during 60 s after the noxious stimulus was applied. If cells displayed aberrant activity for more than 5 min after the stimulus was applied, no more recordings were performed in that animal. Pinching was chosen as the noxious mechanical stimulus taking into account our previous experiments demonstrating that this modality of stimulation induced a significant increase in the activity of spinal nociceptive neurons, which was exacerbated in the STZ-diabetic rats (Morgado and Tavares, 2007).

At the end of each recording session, electrolytic lesions were made in the recording sites and the animals were given a lethal dose of pentobarbitone. The brains were removed for posterior histological verification of the recording sites.

2.5. Statistical analysis

Analysis of the differences in the distribution of each type of cell was performed using Fisher's exact test. The potential effect of diabetic neuropathy upon the spontaneous activity and noxious-evoked responses of RVM pain modulatory cells was examined by using an independent-sample t-test. Statistical significance was settled at $p < 0.05$. Data is presented as mean ± SD.

3. Results

3.1. Metabolic and behavioral characterization of experimental groups

At 4 weeks post-injection, STZ-diabetic rats presented significantly lower body weights and higher blood glucose and hemoglobin A1C levels than the age-matched non-diabetic CTRL animals. STZ-diabetic rats displayed mechanical hyperalgesia as shown by the significant decrease in PWTs when compared with CTRL animals (Table 1).

3.2. Effects of diabetes on the distribution of RVM cells

Overall the percentage of each type of RVM pain modulatory cells was altered in STZ-diabetic animals (Table 2), with the Fisher's exact test demonstrating that the proportion of neuronal types of RVM cells was significantly different between CTRL and STZ-diabetic rats (p = 0.0126).