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Short communication

Reduction of the spinal nucleus of the bulbocavernosous volume by experimental diabetes

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Abstract

The sexually dimorphic nuclei, spinal nucleus of the bulbocavernosus (SNB) and dorsolateral nucleus, are located at the lumbar segment of the rat spinal cord. These nuclei innervate perineal muscles involved in penile erection and ejaculation. Testosterone levels modulate their size in adult male rats. Because diabetes is associated with low levels of testosterone, we have evaluated morphological changes on spinal cord of diabetic animals. Significant reduction in the SNB volume was observed 4 weeks after diabetes induction accomplished by a reduction on the motoneuronal size. Insulin prevents the morphological alterations. No significant changes were observed on other dimorphic nucleus. The altered sexual behavior of diabetic rats could be consequence of the detected reduction in the SNB volume.

Keywords: Spinal cord; Motoneuron; Spinal nucleus of the bulbocavernosus; Testosterone; Insulin; Erectile dysfunction; Rat

Erectile dysfunction is the most common form of sexual disorder observed in men with advanced stages of diabetes [13]. Vascular alterations and autonomic dysfunctions are the major factors that cause this disorder [30]. However, the changes in the central nervous system control of sexual arousal, the endocrine function and the motor spinal reflex may also play an important role [17]. Some of these altered parameters have been assessed in diabetic animal models [9].

The lumbosacral segment of the spinal cord of male rats contains clusters of sexually dimorphic motoneurons [16]. The spinal nucleus of the bulbocavernosus (SNB) innervates two sexually dimorphic muscles, namely the bulbocavernosus and the levator ani, and the non-sexually dimorphic anal-sphincter muscle [3,16,24]. A second cluster of motoneurons, the dorsomedial nucleus (DLN) innervates the sexually dimorphic ischiocavernosus and the non-dimorphic uretral sphincter muscles [16]. The bulbocavernosus and the ischiocavernosus muscles have an important role in copulatory behavior stimulating intracavernosus pressure to increase penile rigidity [16]. Besides, in this spinal cord region there is a non-sexually dimorphic motor nucleus called retrodorsolateral (RDLN) that innervates muscles of legs and feet [19]. In humans, the sexually dimorphic motoneurons are organized in a single cluster localized bilaterally in the anterior horns of the sacral 1–2 segment of the spinal cord [20].

Circulating androgens are essential for differentiation, development, maintenance and survival of the sexually dimorphic motoneurons and their innervated muscles [6,10].

Considering that both vascular and perineal muscular systems are involved in erectile mechanisms and that 50% of men with late stages of diabetes suffer from erectile dysfunction [13], the aim of this work was to explore the morphology of the motoneurons of the lumbosacral spinal cord in diabetic animals.

Adult males rats (Sprague–Dawley, 75 days old), born and raised in the animal facilities of the IByME according to

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the international rules of Federation of European Laboratory Animal Science Associations (FELASA), were housed in a temperature-controlled room on a 12:12-h light/dark cycle (lights on at 0700 h). Food and tap water were provided 'ad libitum'. All procedures concerning animal care and use were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised in 1996. Diabetes was induced by a single injection of streptozotocin (60 mg/kg body weight, STZ, Zanosar, Pharmacia & Uphjon, Kalamazoo, MI, USA; STZ group) dissolved in 0.9% Sodium Chloride (pH 3.5–4.5) into the femoral vein, under ketamine anesthesia (50 mg/kg, Holliday, Argentine). The control group was injected with vehicle containing 2.2% of ascorbic acid. Two separate experiments were performed. In the first experiment, STZ effect was evaluated ($n = 8$/group). In a second experiment, another set of 18 animals was divided in three experimental groups. Twelve rats were injected with STZ and six were injected with vehicle as it was previously described. One week after STZ injection half of the STZ-treated animals were daily subcutaneously injected with insulin (4 IU, Betasint Insulina NPH; Laboratorios Beta, Argentine; INS group). The other animals were injected with vehicle (0.9% NaCl). In both experiments, the animals were sacrificed four weeks after STZ injection. Diabetic rats showed glycosuria 48 h after STZ injection (higher than 2 g/100 ml) determined by Diastix reactive strips for glucose (Bayer, Argentine). Serum glucose was also determined by a colorimetric enzymatic method (Glaxo, Argentine) and testosterone levels by radioimmunoassay as it was described by Dorfman et al. [8].

Animals were anaesthetized with ketamine (50 mg/kg), auricular blood collected and then perfussed transcardially with 100 ml of 0.9% NaCl solution containing 50 IU of heparin followed by 200 ml of a fixative solution (4% paraformaldehyde in 0.1 M sodium phosphate buffer). Lumbar spinal cords were removed by dorsal laminectomy. Tissue blocks were post-fixed in the same fixative solution during 2 h followed by overnight cryoprotection in 30% sucrose in 0.1 M phosphate buffer. Lumbar spinal cords were frozen with powdered dry ice and stored at −70 °C. Consecutive transversal sections (16 μm) from regions 5 to 6 of the lumbar spinal cord were cut in a cryostat at −16 °C, thaw-mounted onto gelatin coated microscope slides, air-dried at room temperature and stored at −70 °C. All serial sections were stained by the Nissl technique. Slides were coded and the morphometric analysis was done by two independent investigators who were blind to the treatments. The number and the size of motoneurons were determined under 200× magnification using a video camera (CCD Sony-XC77) attached to a BH2 Olympus microscope, coupled to a Macintosh computer equipped with a video card (Data Translation) and NIH-Image software (developed by Wayne Rasband 1995, NIH, Research Services Branch, NIMH, Bethesda, MD). Approximately 52–56 sections per animal were evaluated and the morphological characteristics of 150 cells per rat were analysed for the motoneuronal nuclei. Anatomical identification of the spinal cord segments was carried out according to the Rexed nomenclature [22].

Volume changes (expressed as mean ± S.E.M.) were evaluated using one-way analysis of variance (ANOVA) and comparisons among the three groups were made by Fisher and Bonferroni–Dunn tests. Frequency distribution histograms were analyzed by Kolmogoroff–Smirnoff non-parametric test. Differences were considered significant when $p < 0.05$.

Diabetic rats showed four times higher glycemia than control animals, meanwhile insulin-treated rats showed similar glycemia values to that controls (Control = 89.9 ± 5.2 mg/dl, STZ* = 450.4 ± 31 mg/dl, INS = 102 ± 15 15 mg/dl, *$p < 0.001$). Treatment with STZ also produced a significant decrease in serum testosterone levels (Con-

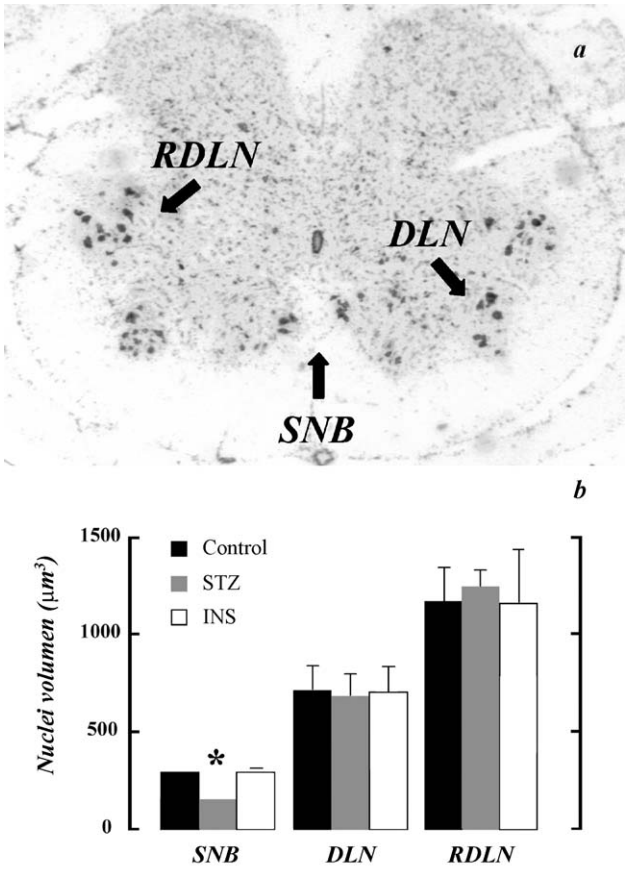

Fig. 1. (a) Representative image of a coronal section of lumbar 6 spinal cord of a control rat stained by Nissl (50×). (b) Changes in the volume of the motoneuronal nuclei determined by integration of the cell area from 52 to 56 consecutive coronal sections of the lumbar spinal cord region from Control, STZ group and STZ insulin-treated animals (INS), $n = 6$/group, values are expressed in μm^3. SNB = Control: 291 ± 19; STZ*: 148 ± 7; INS: 288 ± 24; DLN = Control: 711 ± 141; STZ*: 681 ± 134; INS: 704 ± 156; RDLN = Control: 1170 ± 188; STZ: 1243 ± 104; INS: 1159 ± 299. *$p < 0.05$ by ANOVA followed by Fisher test.

trol = 11.3 ± 2.1 ng/ml, STZ* = 2.3 ± 0.7 ng/ml, INS = 12.3 ± 2.4 ng/ml, *$p < 0.05$).

The morphology of the two sexually dimorphic nuclei SNB and DLN and the non-sexually dimorphic nucleus RDLN was studied (Fig. 1a). The volume of these nuclei was evaluated by integration of the cell area of consecutive coronal sections of the lumbar region. A significant decrease in the SNB volume of STZ rats was observed with respect to the control group. STZ insulin-treated animals (INS) showed similar values to the control group (ANOVA $F(2,15) = 142.6$ $p = 0.001$, Fig. 1b).

The changes observed in the SNB volume were due to a decrease in the percentage of large motoneurons and an increase in the percentage of small motoneurons. The motoneurons size distribution of the SNB, expressed as cell area of all studied sections, was altered in the STZ group as shown in Fig. 2. The Control group showed 54% of motoneurons between 1400 and 2200 μm^2 while STZ rats presented the majority (75%) of their neurons in the range of 1000–1800 μm^2. Besides, low number of big neurons was observed in STZ group. The Control group showed 39% of large neurons distributed from 2600 to 4200 μm^2, and the STZ group presented only 8% of large motoneurons, with the largest size at 3000 μm^2 (Fig. 2). The frequency distribution pattern of neuronal size in the INS group had similar values to that observed in the Control group (data not shown).

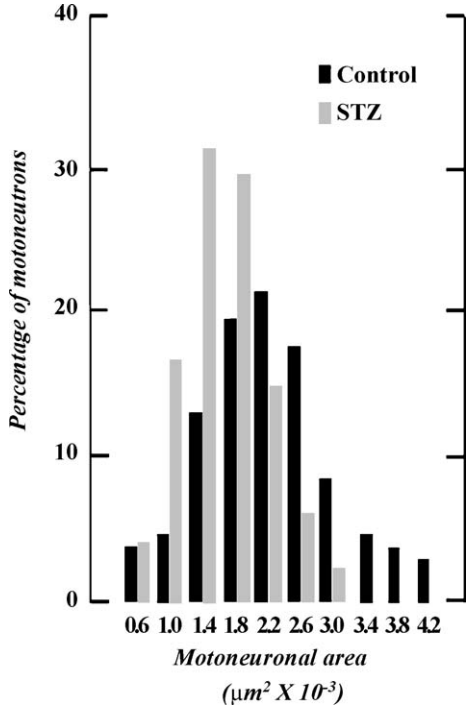

Fig. 2. Frequency histograms of SNB motoneurons distribution according with its size for Control and STZ rats ($n = 8$/group). The frequency indicates the number of motoneurons with a given area distributed in increasing ranges of 400 μm^2, from 200 to 4200 μm^2. A significant shift to the left was observed for the diabetic group ($p < 0.05$; Kolmogoroff–Smirnoff).

The volume of the other two nuclei present in the lumbar spinal cord sections (DLN and RDLN) did not show significant differences among the three experimental groups. Their motoneurons size distribution was between 1000 and 1800 μm^2 (64%) and a few large motoneurons which measured from 3000 to 3800 μm^2 were observed (3%). No significant changes in the frequency of motoneuronal size distribution were observed in both nuclei of the three groups (data not shown).

The described STZ effects on the copulatory behavior [7] may be due to the variety of changes occurring in the central and peripheral nervous systems. The endocrine system is also altered by STZ showing a significant decrease on testosterone levels [29,12,28]. Because the motor nuclei that innervate the striated muscles involved in the control of penile erection and ejaculation are affected by the testosterone levels [4,6], the objective of the present study was to examine the morphology of these pudendal nuclei in the STZ-induced diabetic rats.

The SNB volume of diabetic animals without insulin treatment showed a significant decrease with respect to Control rats. The morphological alteration is due to a simultaneous increase in the number of small motoneurons and a decrease in the number of the large ones, without changes in the total cell number. This effect is evident as the increase in the percentage of motoneurons from 1000 to 1800 μm^2 was between 1.6- and 4.2-fold; meanwhile, larger motoneurons (2600–4200 μm^2) decreased three to five times (Fig. 2).

It is unlikely that the observed differences between Control and STZ groups may be due to direct effect of STZ on the motoneurons. This drug, an analog of glucose, is transported by GLUT2. Although this transporter is widely expressed at low levels in the nervous tissue, its presence in the blood–brain barrier has not been documented [2,23]. Moreover, the morphological changes observed can be prevented by insulin treatment and other authors have shown that the altered sexual behavior of STZ-induced diabetic rats can be reverted by insulin replacement [26,27].

The maintenance of SNB neuronal morphology depends on circulating testosterone levels, including somatic size, dendritic arborizations and neuronal survival [10,23]. A significant reduction in the motoneuronal size of the sexual dimorphic nuclei was also showed in castrated male rats [7]. This effect could be reverted by testosterone treatment [4,7,11]. On the other hand, reduced serum testosterone levels have been described in rats in both STZ-induced [12,25] and spontaneously occurring diabetes [18]. The significant decrease in testosterone levels in STZ animals could be one of the causes of the observed morphological alterations.

STZ treatment affects both pituitary–testicular axis and testicular functions which are reverted by insulin [1,21]. Other studies from our laboratory showed significant decrease in serum testosterone levels after 3 weeks of STZ

injection although they were non-significant during the first week after STZ administration (data not shown). It is likely that at the time insulin treatment was started, motonerurons had not been affected by the endocrine condition. Then, insulin effects seem to be directly related to the reestablishment of the testicular function.

In diabetic rats, we have observed that only the SNB motoneurons showed an altered morphology, without significant changes in the DLN or in the RDLN among the three groups. Considering that these nuclei are within the same segment of lumbar spinal cord and assuming that the circulating androgens have an equal access to them, the observed modifications could be related to the different hormonal sensitivity of each nucleus. Almost all SNB cells (95.8%) accumulate testosterone or its metabolite after testosterone injection [5]. Besides, the SNB is more sensitive to testosterone than DLN [26,31]. The non-significant morphological changes of the DLN could be then a direct consequence of its hormonal dependence.

The RDLN shows androgen receptors during development [14], but it has been well described that its morphology is independent of testosterone levels. Then this nucleus could be considered a good inner control of the effects produced by this steroid and in this study no morphological changes were observed in the RDLN.

Although testosterone levels seem to play an important role in the morphological changes observed in this animal study, clinical evidences show that diabetic patients with normal level of androgens suffer erectile dysfunction. Moreover, testosterone treatment to diabetic and non-diabetic patients with low levels of testosterone and sexual dysfunction has no effect [15]. So, androgens are necessary but not sufficient to have a normal sexual function.

The same could be considered with the insulin treatment. Although insulin modulates testosterone secretion, the hormone alone is unable to improve the erectile dysfunction when it is established in diabetic patients.

The morphological alterations observed in the SNB motoneurons of diabetic rats would be in part responsible for their altered sexual behavior. The study of pudendal motoneurons morphology and their alterations in a short-term diabetic animal model is a substantial contribution to understand the early-occurring events in the diabetic erectile dysfunction.

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