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Changes in serotoninergic and noradrenergic descending pain pathways during painful diabetic neuropathy: The preventive action of IGF1

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ABSTRACT

Painful diabetic neuropathy (PDN) induces neuronal hyperactivity at the spinal cord and periaqueductal gray (PAG), a key area in descending nociceptive modulation. Since the PAG uses relay stations at serotoninergic and noradrenergic brainstem areas, we determined the serotonin and noradrenaline levels at the spinal cord of streptozotocin-diabetic rats and at those brainstem areas (serotoninergic rostroventromedial medulla and noradrenergic A_5 and A_7 cell groups). Since, during diabetes, the levels of insulin growth factor 1 (IGF1) decrease, reducing its neurotrophic effect in the brain, we also studied the effects of IGF1 treatment. One week after diabetes induction, subcutaneous injections of IGF1 (2.5 mg/kg) were performed during 3 weeks. Body weights, glycemia, and mechanical nociception were weekly evaluated until the end of the study, the time when the animals were subjected to a modified formalin test to study chemical allodynia. Serotonin and noradrenaline levels were quantified by ELISA at the spinal cord, whereas at the brainstem, the quantification was performed by immunohistochemistry against, respectively, tryptophan hydroxylase (TpH) or tyrosine hydroxylase (TH). STZ-diabetic rats exhibited mechanical hyperalgesia and chemical allodynia, along with higher spinal levels of serotonin and noradrenaline and higher numbers of neurons expressing TpH at the RVM and TH at the A_5 noradrenergic cell group. Treatment with IGF1 prevented the behavioral signs of PDN and reversed the neuronal hyperactivity at the spinal cord and ventrolateral PAG and the neurochemical changes at the spinal cord and at the brainstem. Based on the facilitatory role of serotoninergic and noradrenergic descending modulation during chronic pain, the increased serotonin and noradrenaline innervation of the dorsal horn in STZ-diabetic rats may probably account for enhanced pain during PDN. The benefits of IGF1 in PDN are probably due to blockade of the increased peripheral input to the somatosensory system, but direct central actions cannot be discarded. The value of IGF1 in PDN treatment deserves further evaluation.

Introduction

Neuropathy affects a large portion of diabetic patients. Their quality of life is further reduced due to pain complaints, which occurs in about one quarter of patients with diabetic neuropathy (Quattrini and Tesfaye, 2003; Tesfaye, 2009). Painful diabetic neuropathy (PDN) is characterized by spontaneous pain, mechanical hyperalgesia, and tactile allodynia (Tesfaye, 2009). It is accompanied by functional and neurochemical changes at peripheral nerves, spinal cord, and supraspinal pain control areas (Sima, 2003; Selvarajah et al., 2006, 2008; Morgado and Tavares, 2007; Morgado et al., 2010). Streptozotocin-diabetic rats (STZ-diabetic rats) present spontaneous activity of spinal dorsal horn neurons (Pertovaara et al., 2001; Chen and Pan, 2002; Morgado and Tavares, 2007; Morgado et al., 2010). Increased neuronal activity was recently demonstrated in a key brainstem center of pain modulation—the periaqueductal gray matter (PAG; Morgado et al., 2010). The PAG does not project directly to the spinal cord but uses relay stations located at the brainstem, with a well-known involvement of the rostroventromedial medulla (RVM; Millan, 2002; Heinricher et al., 2009). Descending nociceptive modulation is performed by the release of serotonin and noradrenaline at the spinal cord (Millan, 2002). Serotoninergic innervation of the spinal dorsal horn is mainly originated from the RVM. Noradrenergic innervation derives mainly from the A5, A6, and A7 cell groups of the brainstem, with variable contributions of each area in different rat strains (Proudfit, 2002). Whereas descending modulation is known to inhibit acute pain, during chronic pain situations, an imbalance of inhibitory and facilitatory actions occurs (Porreca et al., 2002). Impairment of descending inhibition and enhancement of facilitation are proposed to increase nociceptive transmission at the spinal cord during chronic pain (Tracey and Mantyh, 2007). The participation of the RVM and noradrenergic cell groups in potentiating chronic pain was demonstrated in inflammatory (Howorth et al., 2009) and traumatic neuropathic pain models (Ma and Eisenach, 2003;

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Hayashida et al., 2008), but with variable degrees depending on the pain condition (Porreca et al., 2002). During PDN, the role of serotoninergic and noradrenergic areas in descending modulation was never studied. Inconsistent results were obtained as to the levels of serotonin in the brainstem and spinal cord of STZ-diabetic rats (Sandrini et al., 1997; Padayatti and Paulose, 1999; Gallego et al., 2003). As to the noradrenergic system, diabetes-induced increases in the levels of noradrenaline were reported at the brainstem and spinal cord (Bitar et al., 1999; Bitar and Pilcher, 2000). However, the brainstem areas that are affected by diabetes and account for those changes were never evaluated. Supporting a role of descending serotoninergic and noradrenergic systems in PDN, antidepressant drugs that act on the reuptake of serotonin and noradrenaline are effective in PDN treatment (Tesfaye, 2009).

Hyperglycemia is no longer considered the exclusive etiological factor of PDN (Sima, 2003; Pierson et al., 2003). It has been suggested that insulin growth factor 1 (IGF1) deprivation, which occurs in type 1 diabetes, may account to the higher severity of PDN in this type of diabetes, in comparison to type 2 (Ishii, 1995; Schmidt et al., 1999; Sima and Kamiya, 2006; Zhuang et al., 1996). Further supporting a role of IGF1 in diabetic neuropathy, it was shown that the reduction of circulating levels of IGF1 is greater in patients with neuropathy than in those without neuropathic complaints (Migdalis et al., 1995; Guo et al., 1999). IGF1 exhibit glycemia-independent neuroprotective effects, preventing peripheral nerve damage and promoting nerve regeneration, which may account for the analgesic ability of IGF1 during PDN (Ishii, 1995; Schmidt et al., 1999; Zhuang et al., 1996; Piriz et al., 2009). Suppression of IGF1 actions and downregulation of IGF1 receptors was demonstrated in the brain of diabetic rats (Li et al., 2005; Zhang et al., 2008; Sima et al., 2009), but no studies were ever directed to the specific brainstem areas involved in descending pain control. Considering the aforementioned information and the occurrence of IGF1 receptors at the brainstem (Folli et al., 1994), we performed a double-goal study. First, we wanted to measure the levels of serotonin and noradrenaline in the spinal cord of STZ-diabetic rats and the respective contribution of the serotoninergic and noradrenergic brainstem centers referred above. Second, we aimed to evaluate if treatment with IGF1 doses that do not reverse hyperglycemia affects the behavioral signs of PDN, neuronal hyperactivity at the PAG and at the spinal cord, and serotoninergic and noradrenergic brainstem systems.

Materials and methods

Animals

A total of 40 male Wistar rats (Charles River Laboratories, Barcelona, Spain), weighing 280–300 g at the beginning of the experiments, were used. The experiments were performed in accordance with the ethical guidelines of the European Community Council Directive 86/609/EEC and of the International Association for the Study of Pain in conscious animals (Zimmermann, 1983).

Animals were housed 2 per cage, in a room with constant temperature (22 ± 2 °C) and humidity (55% ± 5%) and a 12-h light/dark cycle, and received food and water ad libitum. The animals were daily handled by the experimenter for habituation purposes and were used according to the general experimental procedures summarized in Fig. 1.

Induction of diabetes

Diabetes was induced in 20 rats by an intraperitoneal (i.p.) injection of STZ (60 mg/kg body weight) dissolved in 1 M citrate buffer (pH = 4.5). Twenty rats received equal volumes of the vehicle (control group). Three days after the injections, glucose concentration was measured in tail vein blood samples using a glucose oxidase impregnated test strip (Accu Chek Sensor Comfort, Roche Diagnostics, Germany). Only rats with glucose concentration higher than 270 mg/dl were considered diabetic and included in the STZ group. All the animals were weekly weighed and daily observed during the study.

Treatment protocol

One week after vehicle or STZ injection, the rats were injected subcutaneously with 2.5 mg/kg body weight of recombinant human IGF1 (STZ + IGF1 group; $n = 10$; control + IGF1 group; $n = 10$) or 300 μl of saline (STZ + saline group; $n = 10$; control + saline group; $n = 10$). All animals were injected 3 times per week during 3 weeks. The injections were performed at the same time of the day (10 h, a.m.) and were evenly distributed throughout the week (Mondays, Wednesdays, and Fridays). Immediately before the injections of IGF1 or saline, blood glucose concentrations were measured as above

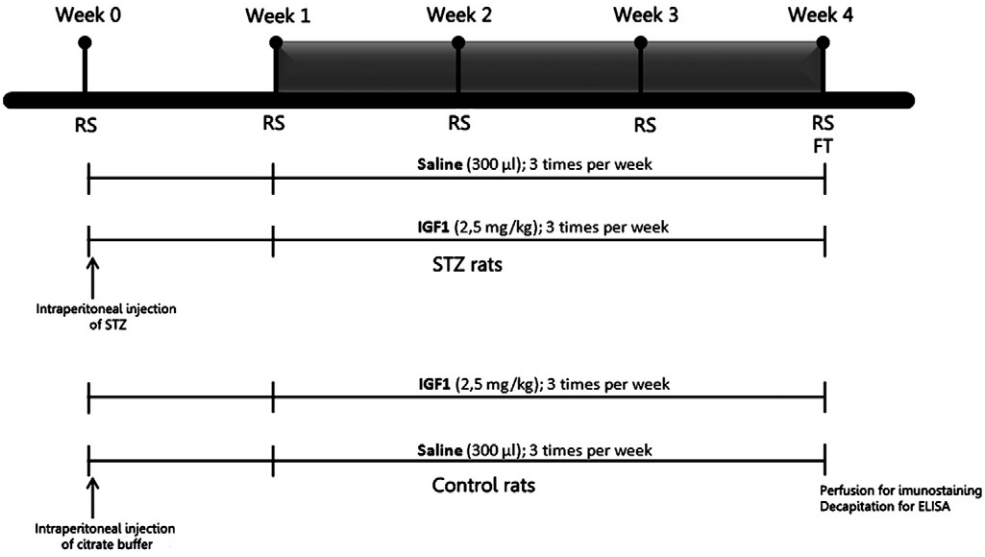

Fig. 1. Schematic overview of the experimental procedures. Each experimental group included 5 animals. Behavioral evaluation was performed using the Randall–Selitto (RS) and formalin (FT) tests. Animals were sacrificed at 4 weeks post-injection by vascular perfusion or decapitation. Spinal cord and brainstem sections were immunoreacted against Fos protein, TpH, or TH and noradrenaline and serotonin contents were quantified in lumbar spinal cord homogenates.