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ELECTROPHYSIOLOGICAL CHARACTERIZATION OF SPINAL NEURONS IN DIFFERENT MODELS OF DIABETES TYPE 1- AND TYPE 2-INDUCED NEUROPATHY IN RATS

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Abstract—Diabetic polyneuropathy (DPN) is a devastating complication of diabetes. The underlying pathogenesis of DPN is still elusive and an effective treatment devoid of side effects presents a challenge. There is evidence that in type-I and -II diabetes, metabolic and morphological changes lead to peripheral nerve damage and altered central nociceptive transmission, which may contribute to neuropathic pain symptoms. We characterized the electrophysiological response properties of spinal wide dynamic range (WDR) neurons in three diabetic models. The streptozotocin (STZ) model was used as a drug-induced model of type-I diabetes, and the BioBreeding/Worcester (BB/Wor) and Zucker diabetic fatty (ZDF) rat models were used for genetic DPN models. Data were compared to the respective control group (BB/Wor diabetic-resistant, Zucker lean (ZL) and saline-injected Wistar rat). Response properties of WDR neurons to mechanical stimulation and spontaneous activity were assessed. We found abnormal response properties of spinal WDR neurons in all diabetic rats but not controls. Profound differences between models were observed. In BB/Wor diabetic rats evoked responses were increased, while in ZDF rats spontaneous activity was increased and in STZ rats mainly after discharges were increased. The abnormal response properties of neurons might indicate differential pathological, diabetes-induced, changes in spinal neuronal transmission. This study shows for the first time that specific electrophysiological response properties are characteristic for certain models of DPN and that these might reflect the diverse and complex symptomatology of DPN in the clinic.

Key words: diabetic peripheral neuropathy (DPN, spinal neurons), *In vivo* electrophysiology, BioBreeding/Worcester (BB/Wor) rats, Zucker diabetic fatty (ZDF) rats, streptozotocin-induced diabetic rats.

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Abbreviations: BB/Wor, BioBreeding/Worcester; BB/Wor-DP, BB/Wor diabetic prone; BB/Wor-DR, BB/Wor diabetic resistant; DPN, diabetic polyneuropathy; STZ, streptozotocin; T1D, type 1 diabetes; T2D, type 2 diabetes; WDR, wide dynamic range; ZDF, Zucker diabetic fatty; ZL, Zucker lean.

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INTRODUCTION

There are two main types of diabetes. Type 1 diabetes (T1D) results from the body's failure to produce insulin and type 2 diabetes (T2D) results from insulin resistance, where cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency. An increase in blood glucose levels represents a recognized diagnostic criterion for diabetes. A common complication resulting from diabetes is painful diabetic polyneuropathy (DPN). Analgesic therapy still represents an unmet challenge since current treatments provide unsatisfactory effectiveness, which are often associated with unwanted side effects (Woolf et al., 1992; Fox et al., 1999).

Studies characterizing the pain phenotype in diabetic patients revealed a heterogeneous population with spontaneous pain and abnormal responses to noxious and non-noxious stimuli (Brown and Asbury, 1984; Baron et al., 2009), the underlying mechanisms of which are not completely understood. There is evidence that this is initiated by hyperglycemia-induced metabolic and morphological changes, leading to degeneration of C-fibers and Aβ-fibers (Sotgiu et al., 1995; Brussee et al., 2008; Johnson et al., 2008). Pain transmission is further enhanced in the dorsal horn, where the abnormal stimulation of peripheral nociceptive afferents drives the central sensitization. Several morphometric studies have demonstrated abnormalities of the myelinated and unmyelinated fibers in painful DPN (Britland et al., 1990; Bradley et al., 1995; Malcangio and Tomlinson, 1998; Morisset and Nagy, 1998). Equally, a significant loss in dermal fiber density was observed in preclinical models of diabetes.

To assess mechanism-based therapeutic approaches to treat DPN adequate animal models are pivotal. Until recently, most preclinical studies of DPN have used streptozotocin (STZ), a cytotoxin known to ablate pancreatic β-cells to induce severe insulin deficient leading to a syndrome resembling T1D (Ahlgren and Levine, 1993; Courteix et al., 1993; Malik, 1997; Derjean et al., 2003; Wodarski et al., 2009; Otto et al., 2011b; Galloway and Chattopadhyay, 2013). Despite this model reflecting the diabetic-related symptoms observed in patients, the very rapid onset of diabetes does not reflect the slow progression of the human disease. Meaning the relevance of this model has been questioned (Galloway and Chattopadhyay, 2013). Therefore, an improved option might be a model with hereditary diabetes.

BioBreeding/Worcester (BB/Wor) rats develop T1D spontaneously due to the autoimmune destruction of insulin-producing cells, and therefore closely reflects the clinical situation. ZDF rats are a model for T2D. They develop obesity, insulin resistance and pancreatic failure with overt hyperglycemia (Chen and Wang, 2005). Adequate models for T2D are of great importance since T2D accounts for about 90% of all diagnosed cases of diabetes (Vera et al., 2012). A prominent symptom associated with DPN is spontaneous and ongoing pain. The assessment of spontaneous pain in preclinical behavioral models is challenging. Measuring spontaneous firing of wide dynamic range (WDR) neurons could therefore present a valid alternative to investigate the neuronal mechanisms contributing to spontaneous pain.

Neurons located in the dorsal horn of the spinal cord are the first integration site along the pain pathway. The mechanical response properties of spinal neurons have been shown to be altered in different models of neuropathic pain, including DPN (Chen and Pan, 2002; U.S. Department, 2011). In this study we recorded from WDR neurons, which are responsive to low-force and high-force mechanical stimuli. This is of relevance as it has been shown that an increased firing rate of rat WDR neurons correlates with diabetes-induced pain (Urch and Dickenson, 2003).

The overall aim was to investigate whether spinal neurons show altered spontaneous and mechanically evoked activity in DPN states in the rat. Furthermore, we wanted to identify common features and differences between models, which may help to explain the complexity of this pain condition.

EXPERIMENTAL PROCEDURES

Experimental animals

Experiments followed the guidelines of the directive 2010/63/EU of the European parliament and the council 2010 on the protection of animals used for scientific purposes. All *in vivo* experiments were conducted in accordance with the German Federal Law. The experimental protocols were reviewed and approved by the Animal Experimentation Ethics Committee of the Government Presidium of Tübingen, Germany.

Rats were housed in cages with soft wooden bedding material (Lignocel FS 14, J. Rettenmaier & Söhne GMBH & Co.KG) under enriched environment (Lignocel Block Large, J. Rettenmaier & Söhne GMBH & Co.KG and rat retreat in red, Plexx). The animals were kept in a temperature- and humidity-controlled (22 ± 2 °C, 55 ± 10% rH) environment, on a 12:12-h light–dark cycle and provided with food and water *ad libitum*. All animals were investigated for their sensitivity to mechanical stimulation of low (von Frey filaments) and high (pressure, Randall Sellito test) intensity at the plantar area of the hind paws (Gorodetskaya et al., 2012, 2013; Hirsch and Dickenson, 2014). In all rats blood glucose levels were always measured (OneTouch apparatus, LifeScan, Inc) in the afternoon in blood obtained from the tail vein.

BB/Wor diabetic-prone (BB/Wor-DP) and BB/Wor diabetic-resistant (BB/Wor-DR) rats were purchased from Biomedical Research Models Inc, USA at the age of four weeks and were kept in groups of four per cage (Macrolon, type IV). The BB/Wor rats were fed with a high protein/fat diet, ''M/R breeding 'GLP'Vit. Fort'' (Provimi Kliba SA, Switzerland). Blood glucose level and body weight were monitored weekly and a rat was judged to be diabetic if blood glucose concentration exceeded 250 mg/dl. When hyperglycemia was achieved plasma ketone levels were monitored weekly. Animals received a low dose of insulin only if blood glucose exceeded 450 mg/dl and ketone levels were greater than 1.5 mmol/l. For that purpose an insulin implant (LinShin Canada, Inc) releasing approximately one insulin unit per day was inserted subcutaneously under general anesthesia (Isoflurane Vol 3%, delivered by O_2–Air mixture at ~0.4 and ~2 l/min, respectively). At the age of 46–48 weeks, BB/Wor diabetic-prone rats (BB/Wor-DP; body weight 297–397 g) and aged match BB/Wor diabetic-resistant rats (BB/Wor-DR, as controls; body weight 428–474 g) male rats were anesthetized and spinal recordings were performed. All BB/Wor-DP rats showed signs of neuropathic pain for at least 15 weeks before electrophysiological recordings were performed.

Zucker diabetic fatty (ZDF; fa/fa) and Zucker lean (ZL; Fa/Fa) rats were purchased from Charles River Laboratories (Sulzfeld, Germany) at the age of seven weeks. The rats were housed in individually ventilated cages (Tecniplast, type GR900) two animals per cage. Animals were fed with M/R exp breeding ZDF rats'' produced by Provimi Kliba SA, Swizterland. Spinal recordings were conducted at the age of 32–34 weeks (ZDF fa/fa diabetic; body weight 350–450 g; (ZL) Fa/Fa; 375–470 g). All ZDF rats showed signs of neuropathic pain for at least 15 weeks before electrophysiological recordings were performed.

Wistar rats 6–8 weeks old, weighing 290–380 g (Charles River, Germany) hyperglycemia was induced by a single intraperitoneal (i.p.) administration of streptozotocin (STZ, 65 mg/kg). STZ was prepared freshly by dissolving it in 0.9% sterile saline. As an internal control for STZ rats, aged-match Wistar rats received an i.p. administration of sterile saline. Animals were judged to be diabetic if blood glucose concentration exceeded 250 mg/dl. When hyperglycemia was achieved plasma ketone levels were monitored weekly. Rats received high protein/fat diet, ''M/R breeding 'GLP'Vit. Fort'' (Provimi Kliba SA, Swizterland). Electrophysio logical recordings were performed 3 weeks after STZ or vehicle i.p. administration.

Surgical procedures

Animals were anesthetized in a chamber using 5% isoflurane in 50% O_2 and 50% air and were thereafter maintained at 2% isoflurane. An acceptable depth of anesthesia was defined by the absence of the hind paw withdrawal reflex to a forceps pinch to the plantar

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