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Pioglitazone Inhibits the Development of Hyperalgesia and Sensitization of Spinal Nociresponsive Neurons in Type 2 Diabetes

Ryan B. Griggs,* Renee R. Donahue,* Braxton G. Adkins,* Katie L. Anderson,† Olivier Thibault,† and Bradley K. Taylor*

*Departments of *Physiology, and †Pharmacology and Nutritional Science, College of Medicine, University of Kentucky Medical Center, Lexington, Kentucky.*

Abstract: Thiazolidinedione drugs (TZDs) such as pioglitazone are approved by the U.S. Food and Drug Administration for the treatment of insulin resistance in type 2 diabetes. However, whether TZDs reduce painful diabetic neuropathy (PDN) remains unknown. Therefore, we tested the hypothesis that chronic administration of pioglitazone would reduce PDN in Zucker Diabetic Fatty (ZDF$^{fa/fa}$ [ZDF]) rats. Compared with Zucker Lean (ZL$^{fa/+}$) controls, ZDF rats developed: 1) increased blood glucose, hemoglobin A1c, methylglyoxal, and insulin levels; 2) mechanical and thermal hyperalgesia in the hind paw; 3) increased avoidance of noxious mechanical probes in a mechanical conflict avoidance behavioral assay, to our knowledge, the first report of a measure of affective–motivational pain-like behavior in ZDF rats; and 4) exaggerated lumbar dorsal horn immunohistochemical expression of pressure-evoked phosphorylated extracellular signal-regulated kinase. Seven weeks of pioglitazone (30 mg/kg/d in food) reduced blood glucose, hemoglobin A1c, hyperalgesia, and phosphorylated extracellular signal-regulated kinase expression in ZDF. To our knowledge, this is the first report to reveal hyperalgesia and spinal sensitization in the same ZDF animals, both evoked by a noxious mechanical stimulus that reflects pressure pain frequently associated with clinical PDN. Because pioglitazone provides the combined benefit of reducing hyperglycemia, hyperalgesia, and central sensitization, we suggest that TZDs represent an attractive pharmacotherapy in patients with type 2 diabetes-associated pain.

***Perspective:** To our knowledge, this is the first preclinical report to show that: 1) ZDF rats exhibit hyperalgesia and affective–motivational pain concurrent with central sensitization; and 2) pioglitazone reduces hyperalgesia and spinal sensitization to noxious mechanical stimulation within the same subjects. Further studies are needed to determine the anti-PDN effect of TZDs in humans.*

***Key words:** Zucker Diabetic Fatty rat, peroxisome proliferator-activated receptor gamma, painful diabetic neuropathy, neuropathic pain.*

Approximately one-third of patients with diabetes experience pain,[1,17,47] commonly referred to as painful diabetic neuropathy (PDN).[102] Most preclinical PDN studies focus on the streptozotocin (STZ) model of type 1 diabetes.[8] However, 90% of diabetic patients have type 2 diabetes, the prevalence of PDN is greater in patients with type 2 diabetes,[1,100] and pain mechanisms in type 1 versus type 2 diabetes likely differ.[39,85-87] Therefore, we chose to study a genetic model of type 2 PDN, the Zucker Diabetic Fatty (ZDF$^{fa/fa}$ [ZDF]) rat.[13]

Hyperglycemia develops within 6 to 10 weeks of age[6,13,49,92] in ZDF but not Zucker Lean (ZL$^{fa/+}$ [ZL]) controls, and is followed by behavioral correlates of PDN, including hypersensitivity to mechanical[6,25,70,78,92,101,112] and thermal[25,49,78,80] somatosensory stimulation. However, conflicting studies report either hypoalgesia[89,92] or hyperalgesia[78,112] in similarly aged ZDF rats. This raises concerns regarding examination of sensory thresholds at just a single age without regard to the developmental stage of diabetes,[6,49,78,89,112] or omission of ZL control rats when

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Address reprint requests to Bradley K. Taylor, PhD, Department of Physiology, University of Kentucky Medical Center, 800 Rose Street, Lexington, KY 40536-0298. E-mail: brad.taylor@uky.edu
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multiple ages are assessed.[70] These deficiencies in the literature led us to rigorously evaluate multiple pain-like behaviors in ZDF and ZL rats at various ages in a well-controlled study.

We hypothesized that spinal cord plasticity contributes to pain in type 2 diabetes because: 1) hyperalgesic db/db mice exhibit dorsal horn increases in phosphorylated extracellular signal-regulated kinase (pERK),[16,109] a marker of nociresponsive neuron activation[26] in the spinal dorsal horn,[37,63,113] and the astrocyte activation marker glial fibrillary acidic protein[16,52,77,109]; 2) intrathecal injection of either the extracellular signal-regulated kinase phosphorylation inhibitor U0216[109] or the astrocyte toxin L-α-aminoadipate[52] reverses hyperalgesia; and 3) augmented N-methyl-D-aspartate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor expression and function in the spinal cord[50] might contribute to hyperalgesia in ob/ob mice.[44] A recent report indicated that spinal neurons in ZDF exhibit central sensitization in response to non-noxious hind paw stimulation.[87] However, concurrent evaluation of pain-like behavior was not performed. We go beyond these studies by evaluating spinal plasticity in PDN using a clinically relevant noxious pressure stimulus to evoke not only expression of pERK in the spinal dorsal horn but also behavioral hyperalgesia in the same subjects.

Thiazolidinedione drugs (TZDs) such as pioglitazone (Actos; Takeda Pharmaceuticals) are approved by the U.S. Food and Drug Administration for the treatment of type 2 diabetes. TZDs also reduce the molecular and behavioral sequelae of neurological disease,[22,40] as well as neuropathic pain associated with brain,[84,111] spinal cord,[58,71] or nerve[11,30,55,63,95] injury. For example, we recently reported that pioglitazone reduced hypersensitivity to non-noxious mechanical stimulation in rats with traumatic nerve injury.[63] However, it still remains unclear whether TZDs reduce the neuropathic pain associated with type 2 diabetes.[97] In addition, behavioral signs of PDN in rodents are more frequently associated with hypersensitivity to noxious, rather than non-noxious stimulation. To address these gaps, we tested the hypothesis that chronic administration of pioglitazone would prevent the development of noxious pressure-evoked hyperalgesia and spinal central sensitization in ZDF rats.

Methods

Subjects

Experiments were carried out in accordance with the Institutional Animal Care and Use Committee at the University of Kentucky (Approved Protocol 2009-0429). All efforts were made to minimize animal suffering, to reduce the number of animals used, and to use alternatives to in vivo techniques, in accordance with the International Association for the Study of Pain[115] and the National Institutes of Health Office of Laboratory Animal Welfare Guide for the Care and Use of Laboratory Animals.

Male ZL and ZDF rats (http://www.criver.com/products-services/basic-research/find-a-model/zucker-diabetic-fatty-(zdf)-rat; Charles River, Wilmington, MA) aged 4 to 19 weeks were used for all experiments. "Obese" ZDF rats are homozygous for the loss of function "fatty" mutation in the leptin receptor (*fa*/*fa*) that results in the development of type 2 diabetes.[13] "Lean" ZL rats are heterozygous (*fa*/+), do not develop a diabetic phenotype, and are genetic control rats for ZDF rats. All rats were housed in a temperature- and humidity-controlled room on a 12-hour light–12-hour dark cycle with lights on from 7:00 AM to 7:00 PM. Before any behavioral or dietary manipulations, all rats were provided water and Formulab 5008 (TestDiets; Purina Mills, Richmond, IN) chow ad libitum. Formulab 5008 (formerly Purina 5008) food yields reliable diabetic symptoms including hyperglycemia, hyperlipidemia, impaired glucose tolerance, and insulin insensitivity as reported by Charles River.

Measurement of Blood Glucose and Hemoglobin A1c

Blood was collected at sacrifice for the measurement of methylglyoxal-derived (MG) advanced glycation end-products (AGE) and insulin at 19 weeks of age in the Characterization of PDN study, and for the measurement hemoglobin (Hb) A1c before (12 weeks) and after (19 weeks) drug treatment in the Pioglitazone Administration study. Blood glucose was measured at weekly intervals in both studies. The details for these studies are described in the Experimental Design section.

Rats were lightly restrained in a towel and the distal tail wiped with an alcohol swab. A small nick was made at the distal tip of the tail using a number 11 scalpel blade. Initial bleeding was wiped clean with gauze and subsequent drops of blood were either loaded into a room temperature HbA1c cartridge and analyzed using a DCA Vantage Analyzer (Siemens, Munich, Germany), or placed on a glucose test strip in triplicate and inserted into a glucose monitor (TrueTrack; Walgreens, Deerfield, IL). To avoid perturbations in pain-like behavior elicited by fasting or exogenous glucose administration in a tolerance test,[18,19] nonfasting blood glucose was measured. A random blood glucose level >200 mg/dL (11.1 mmol/L) was defined as hyperglycemia.[81] We measured blood glucose at the same time each week to minimize circadian-induced fluctuations.

Pain-Like Behavior: Stimulus-Evoked

Fluctuations in noise, vibrations, temperature, and other distractors in the behavioral testing room were minimized to optimize reliable measurements between cohorts of animals tested during different behavioral sessions. To further reduce variability and acclimate animals to the different testing apparatuses, 2 weeks of training were performed before behavioral measures reported at 8 weeks of age.

Heat hyperalgesia was assessed by placing the animals on a heated surface (52.5 ± 1°C) within an acrylic enclosure (Hotplate; Columbus Instruments, Columbus, OH). The time until a hind paw withdrawal response (eg,