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Neuroscience Letters 389 (2005) 152–156
Neuroscience Letters
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# Functional alteration of inhibitory influences on spinal motor output in painful diabetic neuropathy in rats

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Received 1 June 2005; received in revised form 11 July 2005; accepted 21 July 2005

**Abstract**

Diabetes is frequently accompanied by painful polyneuropathies that are mediated by enhanced neuronal excitability in the spinal cord, partly because of decrease in spinal intrinsic inhibitory influences. Changes in spinal excitatory–inhibitory balance may alter spinal segmental motor output. In the study presented here, the mono- and disynaptic (the fastest polysynaptic) reflexes (MSR and DSR, respectively) were recorded from L5 ventral roots in response to stimulation of the ipsilateral L5 dorsal root in spinalized streptozotocin (STZ)-induced diabetic rats with a reduced withdrawal threshold to mechanical stimuli. The diabetic rats generally exhibited larger spinal reflex amplitudes, the DSR being influenced in particular. We addressed whether recurrent and presynaptic inhibition of the spinal reflexes were altered in STZ-treated animals. The recurrent inhibition of the MSR and DSR elicited by preceding antidromic conditioning stimulation delivered to the recorded L5 ventral root was markedly suppressed in diabetic rats. By contrast, the presynaptic inhibition of the MSR and DSR elicited by preceding conditioning stimulation to the ipsilateral L4 dorsal root was not impaired. Thus, in diabetic painful neuropathy, reduced spinal intrinsic inhibition in the ventral horn contributes to an enhanced spinal segmental motor output.


*Keywords:* Tactile allodynia; Diabetes; Spinal reflexes; Recurrent inhibition; Presynaptic inhibition; Central sensitization

Spinal plasticity and sensitization following peripheral nerve injury or inflammation are essential phenomena that play a major role in the induction and/or maintenance of neuropathic pain and inflammatory pain [14,18,19,24]. As the primary target of nociceptive afferent inputs, and therefore, the first to undergo central sensitization, the importance of the superficial layers of the spinal dorsal horn is widely accepted. Hypersensitivity of dorsal horn neurons and increased efficacy of nociceptive transmission are driven partly by the removal of inhibitory influences [1,10,15,16,21]. Disinhibition in the dorsal horn may change nociceptive behaviors, as evidenced by decreases in the withdrawal threshold to thermal or mechanical stimuli. The withdrawal behaviors, initiated by the arrival of nociceptive signals via small diameter fibers, are mediated via the polysynaptic reflex pathways. However, whether much faster spinal segmental reflexes elicited by the activation of large diameter fibers (group Ia afferent fibers) are affected in hyperexcitable states remains to be established. The goal of this study was to determine if the recurrent inhibition mediated by Renshaw cells [5] and presynaptic inhibition that influence the spinal reflexes [11,13] were changed in rats suffering from painful diabetic neuropathy with a reduced withdrawal threshold to mechanical stimuli. We have demonstrated that the recurrent inhibition but not the presynaptic inhibition is functionally reduced, presumably resulting in increased segmental mono- and disynaptic (the fastest polysynaptic) reflexes (MSR and DSR, respectively).

All experimental protocols used here were approved by the Animal Care and Use Committee of Nagoya City University, and were conducted according to the guidelines of the National Institutes of Health and the Japanese Pharmacological Society.

Male Wistar/ST rats (4 weeks old, SLC, Shizuoka, Japan) were treated with streptozotocin (STZ, 50 mg/kg, dissolved in

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doi:10.1016/j.neulet.2005.07.039

0.9% (w/v) physiological saline and administered intraperitoneally (i.p.)). Blood glucose concentrations and the 50% withdrawal threshold against mechanical stimuli applied to the plantar surface of the hindpaw with von Frey filaments were measured before, and 2 (blood glucose level was assessed only in some rats) and 4 weeks after STZ treatment. An ACCU-CHEK Active blood-glucose monitoring system (Roche Diagnostics, Indianapolis, IN, USA) was used to measure the glucose concentration in a blood sample obtained by tail prick. The up–down method of Dixon [4] was used to determine the withdrawal threshold, and the resultant scores were used to calculate the 50% threshold [2]. Four weeks after STZ treatment, rats underwent spinal reflex measurements. Control experiments were carried out using age-matched, 8-week-old animals. Rats were anesthetized with α-chloralose (25 mg/kg, i.p.) and urethane (1 g/kg, i.p.). Cannulae were inserted into the trachea for ventilation and into the femoral vein for drug administration. To make spinalized preparations, the spinal cord was transected at the level of the C1 vertebra under lidocaine anesthesia (4%, 50 μl) and a dorsal laminectomy was performed in the lumbosacral region of each rat. The ventral and dorsal roots below L4 were cut distally at their points of exit from the vertebral column, and the entire exposed surgical area was covered with liquid paraffin kept at 36 ± 0.5 °C by radiant heat. Rectal temperature was maintained at 36 ± 0.5 °C. For the recording of the spinal reflex, the dorsal and ventral roots of the L5 segment were placed on bipolar Ag–AgCl wire electrodes for stimulation (test stimulation with a single rectangular pulse, 0.05 ms in duration, at 5 V, a supramaximal voltage that is approximately twice that required to evoke a maximal reflex response) and recording, respectively. The spinal reflex employed in the present study consisted of the MSR and the DSR, which were elicited by monosynaptic excitation and disynaptic excitation of the α-motoneurons via one interneuron, respectively [7]. The MSR and the DSR were amplified, and displayed on an oscilloscope. The test stimulation was applied every 5 s (0.2 Hz) to average eight consecutive responses, and the amplitudes of the MSR and the DSR were measured. To understand the differential influences of diabetes on the MSR and the DSR, the DSR/MSR ratio in each animal was also calculated. After establishment of stable averaged responses, inhibitory influences on the spinal reflex recorded from the L5 ventral root were assessed. To elicit the recurrent inhibition to the MSR and the DSR, an antidromic conditioning stimulus (a 0.05 ms single pulse at 5 V) prior to the test stimulation (conditioning-test intervals of 5, 10, 20, 30 and 100 ms) was delivered to the L5 ventral root via another bipolar Ag–AgCl wire electrode placed proximally to the recording electrodes. When the presynaptic inhibition to the MSR and the DSR was assessed, a conditioning stimulus prior to the test stimulation (conditioning-test intervals of 5, 10, 20, 30 and 100 ms) was delivered to the ipsilateral L4 dorsal root using another bipolar Ag–AgCl wire electrode [13]. The MSR and the DSR, which were affected by the conditioning stimulation, were normalized with respect to those induced by the test stimulation alone, and the inhibitory curves for the MSR and the DSR were generated with conditioning-test intervals of between 5 and 100 ms. All data are expressed as the mean ± S.E.M. Student's *t*-test (two-tailed) was employed to compare values between age-matched control and STZ-treated groups. When the population variances were unequal, Welch's procedure was employed. Differences at $p < 0.05$ were considered significant.

Two weeks after STZ treatment, the diabetic rats exhibited high-blood glucose levels and low 50% thresholds, both of which were maintained for at least 2 more weeks (Fig. 1A; $p < 0.01$ versus age-matched control). The 50% withdrawal thresholds before and 4 weeks after STZ treatment were 12.7 ± 1.2 g and 3.1 ± 0.3 g ($n = 26$), respectively. By contrast, age-matched 8-week-old rats exhibited a 50% threshold of 12.5 ± 0.5 g ($n = 28$). Thus, rats treated with STZ exhibited a reduced withdrawal threshold to mechanical stimuli. The MSR and DSR amplitudes recorded in STZ-treated rats were generally larger compared to those obtained from age-matched animals (Fig. 1B1). The MSR and DSR amplitudes of age-matched rats ($n = 28$) were 0.80 ± 0.05 mV and 0.20 ± 0.02 mV, respectively, and those of STZ-treated rats ($n = 26$) were 0.97 ± 0.10 mV and 0.43 ± 0.08 mV ($p < 0.05$ versus age-matched DSR), respectively. The enhanced

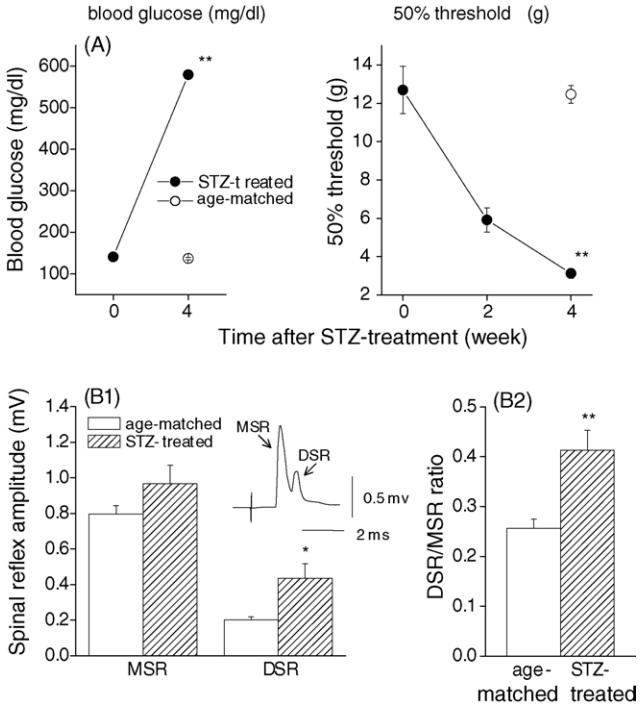


Fig. 1. Reduced withdrawal threshold to mechanical stimuli and enhanced spinal mono- and disynaptic reflexes after treatment of streptozotocin (STZ). STZ was injected to 4-week-old rats, and the spinal reflexes were measured 4 weeks later in spinalized preparations. Data are presented as the mean ± S.E.M. of STZ-treated ($n = 26$) and age-matched control (8 weeks old, $n = 28$) rats. The statistical significance of differences between age-matched control and STZ-treated groups was determined by two-tailed Student's *t*-test or Welch's procedure. $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. age-matched control.