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Research paper

Reduction of spinal glycine receptor-mediated miniature inhibitory postsynaptic currents in streptozotocin-induced diabetic neuropathic pain

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HIGHLIGHTS

- Spinal disinhibition may represent a pathophysiological mechanism contributing to diabetic neuropathic pain.
- STZ injection reduces spinal GlyR-mediated synaptic activity.
- STZ injection reduces spinal glycine levels.
- Intrathecal glycine injection attenuates diabetic neuropathic pain.

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ABSTRACT

Diabetic neuropathic pain (DNP) is a common clinical problem, and the mechanisms underlying the onset and progression of this complication are poorly understood. The present study examined the glycine receptors (GlyR) in the control of synaptic input to dorsal horn neurons in diabetes. Male Sprague-Dawley rats with or without streptozotocin (STZ) intraperitoneal injections were used. Tactile sensitivities were assessed by measuring paw withdrawal thresholds to von Frey filaments for four weeks. The extent of GlyR-mediated inhibition controlling primary afferent-evoked excitation in dorsal horn neurons was examined by using the whole cell patch clamp recording technique in isolated adult rat spinal cord slices. The content of the spinal dorsal horn glycine levels was measured by microdialysis. An intrathecal glycine agonist injection was used to test whether mimicking endogenous glycine-receptor-mediated inhibition reduces DNP. We found that persistent hyperglycemia induced by the administration of STZ caused a decrease in the paw withdrawal latency to mechanical stimuli. The miniature inhibitory post-synaptic current (mIPSC) rise, decay kinetics and mean GlyR-mediated mIPSC amplitude were not affected in DNP. The mean frequency of GlyR-mediated mIPSC of lamina I neurons from DNP rats was, however, significantly reduced when compared with neurons from control rats. Principal passive and active membrane properties and the firing patterns of spinal lamina I neurons were not changed in DNP rats. Spinal microdialysis rats had a significantly decreased glycine level following its initial elevation. The intrathecal administration of glycine diminished tactile pain hypersensitivity in DNP rats. In conclusion, these results indicate that long-lasting hyperglycemia induced by STZ injections leads to a reduced glycinergic inhibitory control of spinal lamina I neurons through a presynaptic mechanism.

1. Introduction

Diabetes mellitus is one of the most rapidly growing health concerns in the developed world [31]. Patients with diabetes report a significantly decreased quality of life secondary to diabetic neuropathic pain (DNP) [3]. Despite the high morbidity associated with DNP, the treatment is relatively ineffective. The current therapeutic options are limited to symptomatic treatment [25] and are associated with adverse side effects. The development of better treatment modalities for DNP should be based on pathogenetic mechanisms.

Multiple postulated mechanisms contribute to the stimulus-evoked pain hypersensitivity that may be experienced after hyperglycemia in response to normally innocuous and noxious stimuli. One potential mechanism that has not been studied to

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a large extent is a loss of inhibition (disinhibition) [6,26]. Inhibition plays a prominent role in controlling nociceptive transfers in the dorsal horn. There is a very high density of GABAergic and glycinergic inhibitory interneurons in the superficial dorsal horn that synapse both with nociceptor primary afferent central terminals and the neurons they synaptically contact [16,27]. Functionally, dorsal horn neurons have been shown to be under very powerful tonic and phasic inhibition, arising both locally in the spinal cord and from descending pathways originating in the brain [13]. Pharmacologically removing inhibition by blocking GABA or glycine receptors with bicuculline or strychnine, for example, produces a pattern of behavioral pain hypersensitivity, flexor reflex hyperexcitability and dorsal horn excitation that closely resembles certain features of neuropathic pain, particularly tactile allodynia [10,21,23,29].

Based on the accumulated findings from these earlier studies, we set out to investigate whether DNP results in a loss of synaptic inhibition.

2. Materials and methods

2.1. Animals

The study was reviewed and approved by the Chung Gang Memorial Hospital Animal Care and Use Committee (Kaohsiung, Taiwan). Male Sprague-Dawley rats (National Science Council, Taipei, Taiwan) weighing 200–250 g were studied. The rats were housed in standard cages in climate- and light-controlled rooms (with a 12-h light/dark cycle) at a temperature of 22 °C with free access to water and commercial regular chow. With the rats under 2.5% isoflurane anesthesia, the intrathecal microdialysis catheter was implanted into each rat's intrathecal lumbar (L3–4) space through a cisternal incision as previously described [12]. The in vivo experimental design is presented in Fig. 1.

2.2. Drugs

All of the drugs were obtained from Sigma, RBI or Tocris.

2.3. STZ-induced DNP

STZ Model (type I diabetic model): The DNP rat model was produced by intraperitoneally (i.p.) injecting streptozotocin (STZ, 60 mg/kg) dissolved in saline. The control group received normal saline [24]. Diabetes was confirmed 6 days and 12 days post-injection (glucose > 12 mmol/l). Only rats with confirmed diabetes were then tested for mechanical allodynia.

The paw withdrawal threshold in response to a mechanical stimulus was determined using the up–down method with a series of von Frey filaments (Stoelting, Wood Dale, IL, USA), ranging from 0.23 to 59.0 g. The animals were placed in a plastic cage with a metal mesh floor, allowing them to move freely. They were allowed to acclimatize to this environment for at least 20 min before the experiment. For analysis purposes, all response latencies were expressed as von Frey test pain indices (vFPI) where: vFPI = the pain threshold by the von Frey test/the pain threshold before receiving the drug by the von Frey test.

2.4. Electrophysiology

The lumbar spinal cord was removed from adult naïve or diabetic rats under urethane anesthesia (1.5 g/kg, i.p.) and placed in pre-oxygenated ice cold Krebs (in mM: NaCl 117, KCl 3.6, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25, and glucose 11). A 650-

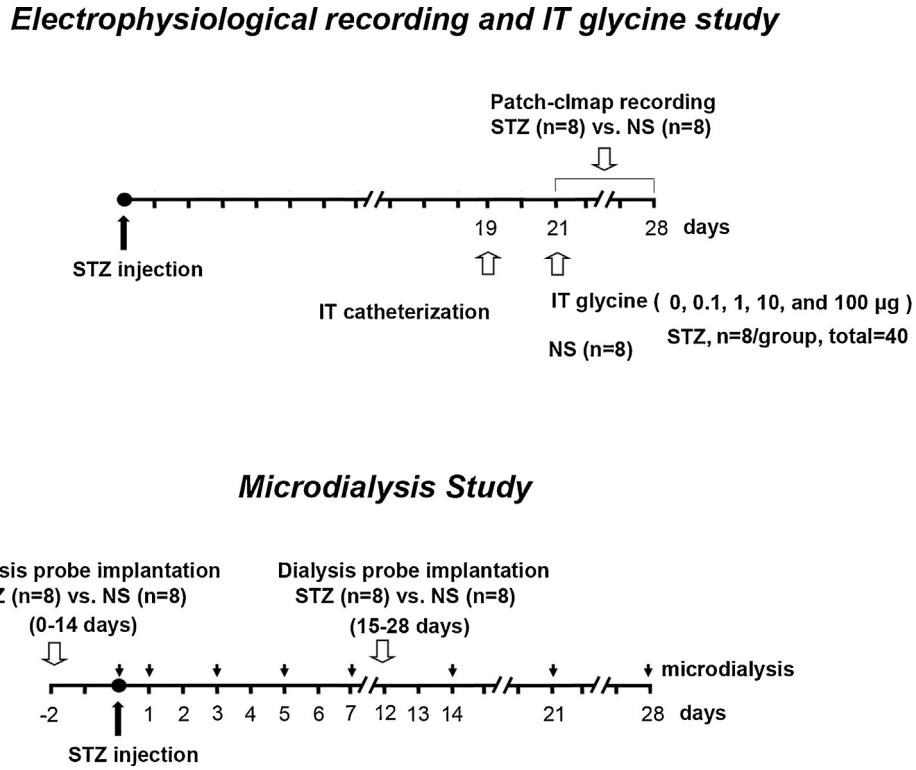

Fig. 1. Flowchart of electrophysiological recordings, the intrathecal glycine study, and the microdialysis study of streptozotocin-induced diabetic neuropathic pain. Diabetes was induced by the intraperitoneal injection of 60 mg/kg streptozotocin (STZ). Two groups (STZ rats vs. saline-treated controls) of Sprague-Dawley rats (8 per group) were used in the electrophysiological recordings (0–14 days), the electrophysiological recordings (15–28 days) and the microdialysis study. Five groups (control, 0.1, 1, 10, and 100 μg) of rats (8 per group) were used in the intrathecal (IT) glycine study. In the electrophysiological recordings, a whole cell patch was performed in weeks 3–4. In the microdialysis study, dialysis probes were inserted at days 2 and 12, and the glycine concentrations were measured at the indicated times.