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Specific functioning of Cav3.2 T-type calcium and TRPV1 channels under different types of STZ-diabetic neuropathy ☆

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ABSTRACT

Streptozotocin (STZ)-induced type 1 diabetes in rats leads to the development of peripheral diabetic neuropathy (PDN) manifested as thermal hyperalgesia at early stages (4th week) followed by hypoalgesia after 8 weeks of diabetes development. Here we found that 6–7 week STZ-diabetic rats developed either thermal hyper- (18%), hypo- (25%) or normalgesic (57%) types of PDN. These developmentally similar diabetic rats were studied in order to analyze mechanisms potentially underlying different thermal nociception. The proportion of IB4-positive capsaicin-sensitive small DRG neurons, strongly involved in thermal nociception, was not altered under different types of PDN implying differential changes at cellular and molecular level. We further focused on properties of T-type calcium and TRPV1 channels, which are known to be involved in Ca^{2+} signaling and pathological nociception. Indeed, TRPV1-mediated signaling in these neurons was downregulated under hypo- and normalgesia and upregulated under hyperalgesia. A complex interplay between diabetes-induced changes in functional expression of Cav3.2 T-type calcium channels and depolarizing shift of their steady-state inactivation resulted in upregulation of these channels under hyper- and normalgesia and their downregulation under hypoalgesia. As a result, T-type window current was increased by several times under hyperalgesia partially underlying the increased resting $[Ca^{2+}]_i$ observed in the hyperalgesic rats. At the same time Cav3.2-dependent Ca^{2+} signaling was upregulated in all types of PDN. These findings indicate that alterations in functioning of Cav3.2 T-type and TRPV1 channels, specific for each type of PDN, may underlie the variety of pain syndromes induced by type 1 diabetes.

1. Introduction

Peripheral diabetic neuropathy (PDN) is one of the earliest, the most frequent and troublesome complications of diabetes mellitus occurring in about 66% of patients with type 1 diabetes [1]. As in humans, development of PDN in rats is accompanied with various alterations in pain sensation (hyper-, hypoalgesia, allodynia) or leaves pain sensation unchanged (normalgesia) [2–5] and these alterations may be considered as manifestation of different PDN types. Rats with streptozotocin (STZ)-induced diabetes, a model of diabetes mellitus type 1, exhibit strong thermal hyperalgesia at 4th week, progressing to hypoalgesia after 8 weeks of diabetes development [2]. At the same time, 6–7 week STZ-diabetes represents a transition period of PDN, when the rats having the same age and terms of diabetes are expected to reveal either hyper-, or norm- or hypoalgesia [5] providing a model for PDN-type-specific remodeling of cellular and molecular systems.

Impairment of Ca^{2+} homeostasis [6–9] and remodeling of voltage- and ligand-gated ion channels [10–12] in nociceptive neurons have been implicated in development of PDN. Among ion channels modulated in STZ-diabetes T-type calcium channels [13] and TRPV1 [14,15] are both of exclusive importance for pathological nociception and directly involved in intracellular calcium signaling. T-type calcium channels are crucially involved in both acute [16–21] and neuropathic pain [22–25]. Moreover, in vivo selective block of T-type calcium channels effectively reverses thermal hyperalgesia in STZ-diabetes [13]. On the other hand, TRPV1 channels, "molecular integrators" of painful stimuli [26–30], also play a key role in variations of thermal pain sensitivity (TPS) during PDN development, that was directly demonstrated by unchanged TPS in

Abbreviations: $[Ca^{2+}]_i$, cytosolic Ca^{2+} concentration; DRG, dorsal root ganglia; HMP, holding membrane potential; IB4, isolectin B_4; LVA/HVA, low/high voltage activated; NTCN, nonpeptidergic thermal C-type nociceptive (neurons); PCD, peak current density; PDN, peripheral diabetic neuropathy; PWL, paw withdrawal latency; SSI, steady-state inactivation; STZ, streptozotocin; T-PCD, T-type PCD; TPS, thermal pain sensitivity

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STZ-diabetic vs non-diabetic TRPV1 deficient mice [15]. The changes in IB_4-positive small-sized DRG neurons [31], expressing both TRPV1 and T-type channels [32,33], are of particular interest. These neurons, being nonpeptidergic thermal C-type nociceptors (NTCN) [34], are strongly involved in TPS [35] and play a key role in neuropathic pain [36,37].

Here rats with thermal hyper-, hypo- and normalgesic PDN developed at the same age and duration of STZ-diabetes were used to determine PDN type-specific remodeling of TRPV1 and T-type calcium channels and their Ca^{2+} signaling in NTCN neurons.

2. Materials and methods

2.1. Experimental animals

Male Wistar rats were housed in the animal facility of the Bogomoletz Institute of Physiology (Kyiv, Ukraine), which was maintained at 22 °C, 55% relative humidity, with natural light/dark cycle. The animals received a standard laboratory diet and tap water ad libitum. All experimental protocols were approved by the Animal Care and Use Committee of the Bogomoletz Institute of Physiology (Kyiv, Ukraine) and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Every effort was made to minimize animal suffering and the number of animals used.

2.2. Induction of experimental diabetes

We used a well established model of streptozotocin (STZ) injections to induce diabetic neuropathy in young male Wistar rats (30–50 g, 21–23 days old) [9,38]. Experimental diabetes was induced in rats by a single intraperitoneal injection of STZ solution (80 mg/kg, i.p.). STZ was prepared in saline (0.9% NaCl adjusted to pH 4.3 with citric acid) on ice; the solution was discarded if bubbling was noted. We used intact (naive) age-matched rats for most control in vitro experiments. Blood glucose levels were checked on the 3rd day after the injection (to verify diabetes onset) and just before electrophysiological experiments (6–7 weeks after injections), using a blood glucometer (Accu-check Active; Roche Diagnostics, Indianapolis, IN, USA). Rats with values of >270 mg/dL (15 mM) were considered hyperglycemic and were included in the respective experimental group.

2.3. Assessment of thermal nociception (behavioral experiments)

Nociceptive responses to thermal stimulation were measured using Hargreaves' method [16,39]. A paw thermal stimulation system (Plantar Test (Hargreaves's Apparatus), Ugo Basile, Italy) was used in these experiments. Each animal was placed in a plastic chamber of the system to accommodate for 15 min. A radiant heat source mounted on a movable holder beneath the glass floor was positioned to deliver a thermal stimulus to the plantar side of the hind paw. When the animal withdraws the paw, a photocell detects interruption of a light beam reflection and the automatic timer shuts off. This method has a precision of ±0.05 s for the measurement of paw withdrawal latency (PWL). To prevent thermal injury, the light beam automatically discontinued at 20 s if the rat failed to withdraw its paw. PWL was obtained for each tested rat as a mean of 10 measurements with 5 min interval interleaving left and right hind paws.

2.4. Acutely dissociated dorsal root ganglia neurons

We prepared dissociated dorsal root ganglia (DRG) cells and used them within 6–8 h for whole-cell recordings as described previously [40]. In brief, we dissected two L4 and two L5 DRGs from each animal, cut each one into three to four pieces, and incubated the pieces in Tyrode's solution containing 140 mM NaCl, 4 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM glucose, and 10 mM HEPES, adjusted to pH 7.4 with NaOH and supplemented with 1 mg/ml protease Type XIV (Sigma) and 0.5 mg/ml collagenase Type I (Worthington Biochemical Corporation) water bathed at 35 °C for 18–20 min. L4 and L5 ganglia were chosen since they contain cell bodies of sensory neurons innervating peripheral receptive fields of the hind paws. Following incubation, ganglia were washed three times (Tyrode's solution, room temperature) and dissociated into single cells in three cycles of trituration with glass pipettes having progressively narrower tip diameters. For recordings, the isolated neurons were plated onto an uncoated glass coverslip, placed in a culture dish, and perfused with Tyrode's solution. All recordings were obtained from isolated DRG cells without visible processes. All experiments were done at room temperature. We routinely observed small, medium, and large sized neurons in the same preparation but, in this study, focused only on cells with a soma diameter less than 30 μm (small DRG neurons) [41].

2.5. Fluorescent imaging

TILL Photonics wide-field imaging system (TILL Photonics, Gräfelfing, Germany) containing a monochromator Polychrome V and Imago CCD camera was installed on an inverted microscope (IX71, Olympus) and controlled by TILLvision software (TILL Photonics). An oil immersion objective (40× UV, NA 1.35; Olympus) was used for fluorescent imaging. Transmitted light images were captured using DIC of the same system.

2.6. Immunohistochemistry

Cells were incubated in Tyrode's solution supplemented with 10 μg/ml isolectin B4 (IB4) [42] conjugated to Alexa Fluor® 568 dye (Invitrogen) in the dark for 10–12 min. Then a coverslip with the cells was mounted in a 500-μl recording chamber perfused with a room temperature (22 °C) Tyrode's solution at a gravity driven flow rate of 2 ml/min. Cells were visualized using a standard Rodamine Filter Set (Chroma Technology, USA). Fluorescent images were captured under standardized settings from 15 to 20 randomly selected small DRG cells on each dish before any electrophysiological recordings during the first 15 min of each experiment. Attribution of each cell to one of three classes by ability of IB4-binding (strongly IB4-positive, weakly IB4-positive and IB4-negative) and analysis of obtained distribution were performed offline following Fang and collaborators [43]. The mean intensity of halo of IB4 staining around the neuronal plasma membrane was determined for each neuron using image analysis tools of TILLvision software (TILL Photonics). Then the relative intensity was calculated separately for neurons of each coverslip. The 0 and 100% intensity values for a particular coverslip were calculated by averaging the halo intensity of the two least intensely (0%, a) and two most intensely stained cell profiles (100%, b). For each tested neuron, its halo intensity of (c) was used to determine its relative intensity (percentage of maximum) as $(100\times(c-a)/(b-a))\%$. Neurons were judged as IB4 positive (IB4+) if their relative intensities exceeded 20%, otherwise they were scored as negative. Then IB4+ neurons were classed as strongly IB4+ if their intensity was ≥40% and weakly IB4+ if their intensity was in a range of 20–40%. Fractions of neurons in the respective classes obtained for a single coverslip were then averaged for each animal group.

2.7. Fluorescent calcium imaging

To measure cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$), the intrapipette solution was supplemented with 200 μM of the high-affinity Ca^{2+} indicator Fura-2. After 10 min of intracellular dialysis, Fura-2 fluorescence reached a steady-state level. Autofluorescence of unloaded cells had signal strength of less than 5% of the fluorescence of loaded cells. Fura-2 fluorescence was recorded at 510±20 nm wavelength during alternating 340- and 380-nm excitation, and the ratio of fluorescence at 340 and 380 nm (R) was calculated on a pixel-by-pixel basis. The frame capture