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Upregulation of axonal HCN current by methylglyoxal: Potential association with diabetic polyneuropathy

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HIGHLIGHTS

- A metabolic derivative of glucose, methylglyoxal (MGO), has pro-excitatory effects; however, its precise effects on axonal ion channels are not well known.
- There was increased hyperpolarization-activated cation current (*I*h) in wild type mice administered with MGO.
- Upregulation of hyperpolarization-activated cyclic nucleotide-gated (HCN) currents might be also present in patients with diabetic neuropathy.

ABSTRACT

Objective: To describe functional changes of axonal ion channels by a metabolic derivative of glucose, methylglyoxal (MGO), and its potential contribution to diabetic neuropathy.
Methods: (1) In wild-type male mice, multiple excitability measurements of sensory nerves were performed at baseline and 1 week after serial administration of MGO (50 mg/kg).(2) Excitability testing in patients with diabetic neuropathy ($N = 17$) and healthy controls ($N = 12$) were also conducted, and data were interpreted using mathematical modeling.
Results: In the animal study, there was a decrease in threshold changes by long hyperpolarization and in superexcitability after administration of MGO. In the preliminary human study, the threshold changes by long hyperpolarizing current were decreased in patients with diabetes. Mathematical modeling showed increased hyperpolarization-activated cation current (*I*h) in the MGO-treated mice and in patients with diabetes.
Conclusion: *I*h was upregulated after MGO administration in normal mice.
Significance: MGO is associated with abnormal axonal excitability. Hyperexcitability in diabetic polyneuropathy may, at least in part, be caused by dysfunctional axonal hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. A future study with a large sample size of the diabetic patients would clarify this hypothesis.

1. Introduction

Distal symmetric polyneuropathy is a common complication of diabetes mellitus, and a number of underlying etiologies have been proposed, such as hypoxia (Dyck, 1989), metabolic factors (Zochodne, 2008), and disturbed nerve regeneration (Yasuda et al., 2003). Among the metabolic pathways showing abnormalities, the methylglyoxal (MGO) pathway, an offshoot of glycolysis

Abbreviations: AGEs, advanced glycation end products; CMAP, compound muscle action potential; HCN channel, hyperpolarization-activated cyclic nucleotide-gated channel; *I*h, hyperpolarization-activated cation current; *IV*, current–threshold relationship; MGO, methylglyoxal; PKC, protein kinase C; RC, recovery cycle; SDTC, strength–duration time constant; SNAP, sensory nerve action potential; TE, threshold electrotonus.

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converting glucose into MGO, has been reported to influence neuronal functions (Tomlinson and Gardiner, 2008). MGO is a highly toxic substance that activates apoptosis and damages mitochondria (Nakayama et al., 2008; Odani et al., 1999). Interestingly, MGO was recently reported to be associated with neuropathic pain by affecting the kinetics of Na^+ channels, resulting in hyperexcitable state (Bierhaus et al., 2012). Given the multiple toxic effects of MGO on multiple organs and abnormal axonal excitability in diabetic neuropathy (Horn et al., 1996; Kitano et al., 2004; Krishnan and Kiernan, 2005; Krishnan et al., 2008; Yang et al., 2001), we hypothesized that MGO has multiple effects on peripheral axons, such as modification of axonal ion channels. Therefore, the objective of the present study was to test whether MGO (or potentially its metabolites) has any *in vivo* effects on peripheral nerve excitability in animals. Of note, since diabetic individuals have been known to have increased MGO blood levels (Kalapos, 2013), normal animals treated with MGO should identify the direct neurotoxic effects of MGO, thus chosen to be tested in this study. Furthermore, the animal data were compared with data obtained from patients with diabetic neuropathy for potential clinical implications.

2. Methods

Animal study: All experiments were approved by the local animal facility in Tokushima University and conducted from October 2012 to December 2013. Ten 7-week-old male ICR mice (SLC, Hamamatsu, Japan) were divided into control and MGO groups. MGO (Tocris, Ellisville, MO) was dissolved in saline at a concentration of 50 mg/kg and was administered intraperitoneally 3 days a week 10 times in total. To our knowledge, thus far, no study has assessed axonal excitability after multiple administrations of MGO; therefore, the dosing in this experiment was arbitrary and the most effective administering schedule was still under investigation. Five mice were used as controls and were administered saline following the same protocol as the methylglyoxal group. The baseline excitability recording was performed immediately before the first injection. The post-injection excitability recording was performed 7 days after the last injection.

Electrophysiological studies were performed on the tail under 1.5% isoflurane anesthesia with the animal warmed on a heating pad to maintain a tail temperature at 32–34 °C throughout the studies. Sensory nerve action potentials (SNAPs) were recorded orthodromically by placing disposable, 30-gauge, stainless steel needle electrodes at different parts of the tail; the reference and active recording needles were placed 10 mm and 20 mm from the base of the tail, respectively; the cathode was placed 40 mm and the anode 50 mm distal to the active recording electrode, and the ground electrode was placed midway between the stimulating and recording electrodes. The tail sensory nerve was specifically selected because recording from the foot caused significant stimulus artifacts due to the short onset latency. Immediately thereafter, axonal excitability test was performed as described later.

Human study: Patients who had type 2 diabetes mellitus reporting paresthesia, neuropathic pain, or both and were referred to our department in Tokushima University Hospital were recruited for this study from January 2008 to December 2013. Serum hemoglobin A1c and random glucose levels were measured to confirm the presence of diabetes. Other causes of peripheral neuropathy were excluded based on the patient's history and blood tests (assessment of vitamin B1 and B12 levels, thyroid function tests, antinuclear autoantibody test, serum protein electrophoresis with immunofixation, and other tests were performed where clinically indicated). Informed consent was obtained from all participants before the study was conducted. The study was approved by the Institutional Review Board of Tokushima University and was performed in accordance with the principles embodied in the Declaration of Helsinki.

2.1. Axonal excitability testing

Animal study: Stimulation was controlled by a PC running the QtracS program (Institute of Neurology, London), connected via a digital I/O device (National Instruments, Austin, TX) to a pre-amplifier (MEG-1200: Nihon Kohden, Tokyo, Japan) and a stimulator (DS-4: Digitimer, Letchworth, UK). For excitability tests, the TRONDF multiple excitability recording protocol was used (Bostock et al., 1998). Stimulus–response curves were determined by using a 1-ms-duration test stimulus that decreased from supramaximal potentials to 0. To record threshold electrotonus (TE), the unconditioned threshold on one channel was tracked, while the threshold at discrete points was determined on two other channels. Briefly, during and after 100 ms of hyperpolarizing and depolarizing currents, currents were set to ±40% and −70% of the unconditioned threshold (Tomlinson et al., 2010). For the +40% depolarizing conditioning current, the difference of threshold changes between the greatest threshold reduction and at the end of the 100-ms conditioning pulse was defined as S2 accommodation. For the recovery cycle (RC), supramaximal conditioning stimuli were given 18 times with delays ranging from 200 to 2 ms before the test stimulus was provided on another channel. We defined superexcitability and refractoriness at 7 ms and 2.5 ms, respectively. The current–threshold relationship (I/V) was then recorded with a 1-ms test stimulus applied 200 ms after the onset of a long-lasting subthreshold polarizing current, the strength of which was altered in 10% steps from +50% (depolarizing current) to −100% (hyperpolarizing current) of the control threshold. The strength–duration time constant (SDTC) was determined as the stimulus strength required to excite nerves as the stimulus width is decreased from 0.5- to 0.1-ms duration. A set of excitability parameters was derived from the recordings, as previously described (Bostock et al., 1998; Nodera and Kaji, 2006), and is shown in Table 1. Of note, in the patients who received insulin therapy, axonal excitability study was performed more than 2 h after the last insulin injection, which could affect the serum potassium level.

Human study: We examined excitability of the median motor axons by recording above the abductor pollicis brevis, with the stimulation electrode placed 3 cm proximal to the wrist crease and the remote electrode at 10 cm proximal to the point of stimulation. The current required to produce certain percentage of the maximal CMAP was defined as "threshold current" (Bostock et al., 1998). Multiple excitability measurements were recorded similarly to the animal study with the exception that a DS-5 stimulator was used (Digitimer, Letchworth, UK) and the SDTC was measured by serially decreasing the stimulus width from 1.0 to 0.2 ms. Control data for nerve excitability were obtained from 12 age-matched healthy subjects (7 men and 5 women, mean age 65.2 ± 8.0, aged 52–78).

2.2. Statistical analyses

Axonal excitability data were compared using the Mann–Whitney U-test (QtracP, Digitimer) for which significance was set at $P < 0.05$. Spearman's rho was calculated by SPSS statistics 22 (IBM, USA).