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Effects of aldose reductase inhibitor on microneurographically assessed peripheral sympathetic nerve activity in rats

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ABSTRACT

Autonomic neuropathy, one of the serious complications of diabetes, decreases quality of life. Aldose reductase inhibitor (ARI) blocks sorbitol production, and results in prevention of damage of nerve fibers. Beneficial effects of ARI have usually been confirmed through nerve conduction velocity tests in motor and sensory nerves. On the other hand, few reports have dealt with the effects of ARI on the small fiber activity such as sympathetic nerve one. In the present study, we administered eparlestat, ARI orally for 3 weeks, to streptozotocin-induced diabetic (STZ + ARI) rats, and then recorded peripheral sympathetic nervous signal detected with microneurographic technique. Action potentials (APs) and bursts of APs were detected from the recorded signal, and their rates and incidences (= rates/heart rate) were compared with those in non-diabetic control (normal) and ARI-untreated streptozotocin-induced diabetic (STZ) rats. While streptozotocin and/or epalrestat did not influence burst parameters in all the three groups, AP parameters in the STZ + ARI and normal groups were higher than those in the STZ group. However, response of AP parameters to the intravenous glucose administration (IVGA) was not large in the STZ + ARI group, similar to that of the STZ group and different from that of the normal group in which AP parameters increased after IVGA. The results suggest that epalrestat may prevent sympathetic nerve activity (SNA) from reduction under hyperglycemic and insulin-depleted conditions, that enhancement of SNA was not induced after IVGA under that condition, and that AP parameters might be useful to assess the degree of neuropathy.

1. Introduction

Neuropathy is well known as one of the severe complications of diabetes mellitus. The development of diabetic neuropathy partially attributes to hyperglycemia and accumulation of sorbitol in neuronal cells due to aldose reductase which is a part of the polyol pathway (Schmidt et al., 1989, 1991, 1998, 2001). In the polyol pathway, sorbitol is produced from glucose by aldose reductase, and is transferred to fructose by sorbitol dehydrogenase. Because inhibition of sorbitol dehydrogenase leads to development of the neuropathy in diabetes (Schmidt et al., 2005), aldose reductase is believed as one of the main factors to induce diabetic neuropathy.

For treatment of the neuropathy, epalrestat (ONO-2235), aldose reductase inhibitor (ARI), was developed in the 1980s, and its efficacy has been confirmed via nerve conduction velocity tests mainly on large fibers such as motor and sensory ones (Hotta et al., 2006, 2008; Schemmel et al., 2010) in spite of diabetic neuropathy that usually commences in small fibers (Malik et al., 2005; Smith et al., 2001; Sumner et al., 2003) such as autonomic ones. As for autonomic effects of ARI, several reports demonstrated beneficial effects of epalrestat on cardiac autonomic nerve function (Hu et al., 2014; Ikeda et al., 1999; Kiyono et al., 2005; Nakayama et al., 2001). In addition, epalrestat prevented norepinephrine turnover (one of the parameters representing sympathetic nerve activity [SNA]) from reduction in brown adipose tissue, heart, and pancreas in diabetic rats (Yoshida et al., 1987). However, few studies have been reported so far regarding direct measurement of autonomic nerve activity in ARI-administered animals or humans.

We previously reported that streptozotocin lowered peripheral SNA recorded microneurographically in rats; action potential (AP) rate was a more sensitive parameter than conventional burst rate

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for the evaluation of SNA (Shinzawa et al., 2013). In the present study, therefore, we administered epalrestat for 3 weeks to streptozotocin-induced diabetic (STZ) rats, and then measured SNA microneurographically in the sciatic nerve under anesthetic condition.

2. Materials and methods

2.1. Animal treatment

All of the surgical and experimental procedures described below have been approved by the Yamagata University Animal Research Committee and followed the Guide for the Care and Use of Laboratory Animals issued by the National Institutes of Health.

Male Wistar rats, obtained from an in-house breeding colony, were divided into STZ, epalrestat-administered STZ (STZ + ARI) and normal groups (n = 6, respectively). At 8 weeks of age, rats in the STZ and STZ + ARI groups were intraperitoneally injected with streptozotocin (80 mg/kg) dissolved with 0.1 M citric acid (pH 4.6), while the normal rats were with the citric acid solution only. From the day after injection, epalrestat (150 mg/kg/day suspended in 0.5% sodium carboxymethyl cellulose (vehicle)) was orally administered to the STZ + ARI group rats, and the vehicle only to the STZ and normal groups. All the animals, allowed free access to a standard laboratory chow (CE-2; CLEA Japan, Tokyo Japan) and tap water, were housed in a room illuminated daily from 8:00 AM to 8:00 PM (a 12:12-h light/dark cycle) at 21 ± 1 °C for 3 weeks.

2.2. Surgical and recording procedures

The rats were anesthetized with intraperitoneal administration of sodium pentobarbital (50 mg/kg) after overnight fast, and placed in the supine position. Body temperature was concerned to keep 37 °C by continuous monitoring of rectal temperature. The right jugular vein and common carotid artery were cannulated with catheters filled with heparinized saline (20 U/mL). Additional doses of sodium pentobarbital were intermittently administered at a rate around 20 min via the venous line to keep the animals under stable condition as done in our previous work (Shinzawa et al., 2013), and blood was sampled via the arterial line. The blood pressure was monitored via the left common carotid artery with an extracorporeal pressure transducer (DX-360, Nippon Becton Dickinson, Tokyo, Japan), and heart rate (HR) was calculated from the signal.

The sciatic nerve was exposed at the thigh level, and unilateral muscle sympathetic nerve signal was detected with microneurography (Nakamura et al., 2003). In brief, the sympathetic nervous signal was detected using a fine tungsten microelectrode (approximately 5 MΩ; FHC, ME) with a 1 μm tip diameter and 250 μm shaft diameter inserted into a sympathetic fascicle of the sciatic nerve. The signal is identifiable by typical characteristics of SNA, i.e., spontaneous and intermittent burst. The sympathetic signal was recorded on a personal computer (PC) using LabVIEW Signal Express (National Instruments Japan, Tokyo, Japan) with a 16 bit A/D converter at a sampling rate of 16 kHz.

After the blood glucose level became stable, intravenous glucose administration (IVGA; 50%, 0.25 ml) was conducted in all groups, based on the fact that SNA is increased after IVGA in the normal but not in STZ rats (Shinzawa et al., 2013), and the nerve signal was stored for 60 min.

2.3. Blood and SNA analyses

Blood glucose (BG) was measured with standard enzymatic method (Glutest Ace, Sanwa Kagaku Kenkyusho, Nagoya, Japan). Sampled blood was then centrifuged, and plasma insulin (PI) was determined with ELISA method (Rat Insulin ELISA Kit; AKRIN-010, Shibayagi, Gunma, Japan).

Recorded data on a PC was analyzed on MATLAB (MathWorks, MA). The recorded signal was filtered and integrated to clarify the bursts by visual inspection as described previously (Shinzawa et al., 2013). The burst rate was determined by detecting bursts in 1 min. APs were manually detected from the sympathetic signal after the denoising procedure proposed by Diedrich et al. (2003). The AP number detected in each 30 s period was doubled to obtain that per minute.

Burst incidence (bursts/100 cardiac cycles), another popular parameter to assess SNA, was calculated from the burst rate and HR. In the same manner, AP incidence (spikes/100 cardiac cycles), a new parameter, was also calculated.

The data obtained immediately before the glucose shot was used as that at time 0 (baseline).

2.4. Statistical analyses

Data are expressed as mean ± SEM. Statistical significance of differences in the burst and AP parameters was tested after log-transformation of them for higher classification capability. The significance of change in temporal data of each group was tested by the one-way ANOVA, followed by Dunnett's post-hoc test for comparisons with the data at 0 min as the reference. Comparisons among the STZ, STZ + ARI, and normal groups were conducted with the one-way ANOVA, followed by Tukey's post-hoc test. The significance level was set at $P < 0.05$.

3. Results

3.1. Blood glucose and insulin

BG in the STZ (n = 6) and STZ + ARI (n = 6) groups was markedly higher than that in the normal group (n = 6) (Fig. 1). In contrast, PI in

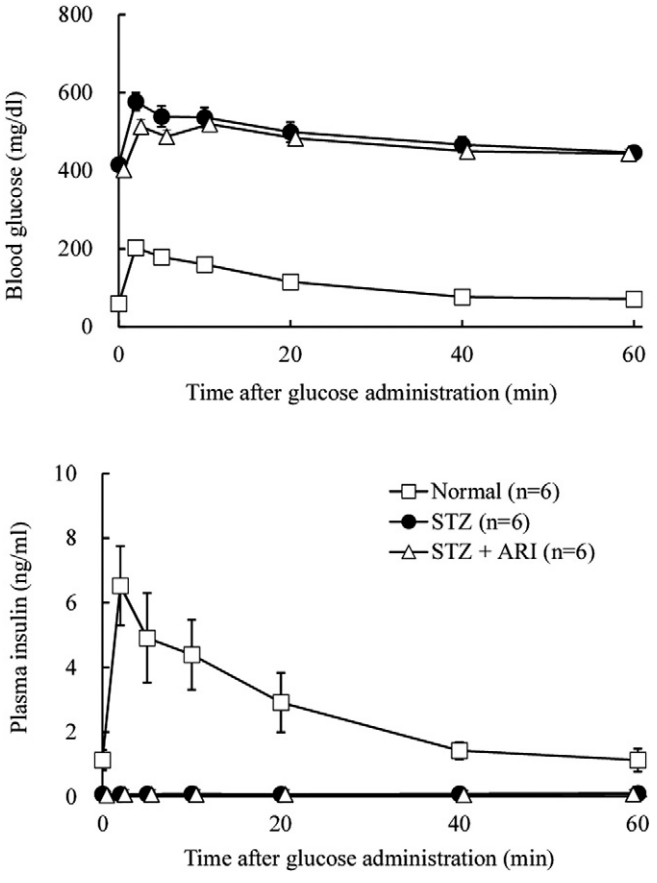

Fig. 1. Time course of blood glucose and plasma insulin levels after intravenous glucose administration (mean ± SEM). The data shown at time 0 was acquired immediately before glucose load. STZ, streptozotocin-induced diabetic rats; STZ + ARI, epalrestat-administered STZ rats.