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Effects of cytidine 5′-diphosphocholine (CDP-choline) on the thermal nociceptive threshold in streptozotocin-induced diabetic mice

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ABSTRACT

Neuropathy accompanied by abnormal sensory perception is the most common complication in insulin-dependent and -independent diabetes mellitus. Since there are very few effective therapeutic regimens for sensory abnormalities in diabetes, we examined the effect of cytidine 5′-diphosphocholine (CDP)-choline on the thermal nociceptive threshold in streptozotocin-induced diabetic mice using the tail-flick test. Diabetic mice showed a shorter tail-flick latency at 1–4 weeks after streptozotocin treatment and a longer tail-flick latency after 8–12 weeks. This hyper- and hypoalgesia in diabetic mice was almost completely inhibited by daily treatment with CDP-choline (100mg/kg/day, p.o.) beginning on the day of streptozotocin treatment. Daily treatment with CDP-choline beginning 5 weeks after streptozotocin treatment attenuated the development of hypoalgesia. Diabetic mice showed a significant increase in Na^+-K^+-ATPase activity at 3 weeks after streptozotocin treatment, whereas Na^+-K^+-ATPase activity was decreased at 12 weeks after treatment. These alterations were normalized by daily treatment with CDP-choline (100mg/kg/day, p.o.) beginning the day of streptozotocin treatment. These results provide evidence to support the therapeutic potency of CDP-choline on the development of thermal hyper- and hypoalgesia and the progression of thermal hypoalgesia in diabetic mice. Moreover, these effects of CDP-choline may result from the normalization of Na^+-K^+-ATPase activity.

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1. Introduction

Diabetic neuropathy accompanied by anomalies in pain perception is the most common peripheral neuropathy and one of the most frequent complications in insulin-dependent diabetes (Guy et al., 1985; Le Quesne and Fowler, 1986; Ziegler et al., 1988; Watkins, 1990). Many clinical and experimental studies have suggested that diabetes or hyperglycemia alters pain sensitivity (Morley et al., 1984). In humans, diabetic neuropathy can be associated with burning tactile hypersensitivity (Bays and Pfeifer, 1988). Hyperalgesia has been well described in animal models of diabetes (Forman et al., 1986; Lee and McCarty, 1990; Kamei et al., 1991b; Courteix et al., 1993). Although the pathogenesis of diabetic neuropathy remains unclear, morphofunctional abnormalities in experimental diabetic neuropathy have been shown to be caused by decreased Na^+-K^+-ATPase activity (Greene et al., 1990; Low et al., 1990), decreased microcirculation in nerve tissue (Williamson et al., 1993; Tesfaye et al., 1994; Dyck and Giannini, 1996), and abnormalities of lipid metabolism in nerve cell membrane (Horrobin, 1998).

Diabetic rodents develop behavioral disorders that have prompted the use of these animals for modeling the influence of diabetes on sensory perception. These disorders include allodynia in response to light touching and hyperalgesia in response to noxious chemical stimuli (Calcutt et al., 1996). Behavioral responses to thermal stimuli have also been studied in diabetic rodents, but no clear picture has emerged. With the use of the tail-flick test to examine spinally mediated reflexes, diabetic mice have been reported to have increased (Levine et al., 1982) response times. On the other hand, diabetic rats have been reported to show increased (Apfel et al., 1994) or decreased (Lee and McCarty, 1992; Courteix et al., 1993) response times. Recently, the duration of diabetes has been suggested to be a possible major cause of the different nociceptive thresholds in diabetic rodents. Indeed, hyperalgesia was observed at an early stage of diabetes in the hot-plate test, whereas hypoalgesia was observed in a late stage of diabetes (Pabbidi et al., 2008). We found similar changes using the tail-flick test (Ohsawa et al., 2008). These results suggest that short-term diabetes may be associated with thermal hyperalgesia while long-term diabetes is associated with thermal hypoalgesia in rodents.

A large body of clinical evidence indicates that cytidine 5′-diphosphocholine (CDP)-choline, injected intravenously or into the subarachnoid space of the spinal cord, is effective for restoring brain activity in patients with trauma, apoplexy, and other disorders of the brain (Shimamoto and Aramaki, 1975; Hazama et al., 1980). CDP-

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choline is an essential intermediate in the biosynthesis of the structural phospholipids of cell membranes, especially phosphatidylcholine (Julio and Guillermo, 1995). CDP-choline restores membrane Na^+-K^+-ATPase activity based on the results obtained with a model of cryogenic cerebral edema in rabbits (Julio and Guillermo, 1995). CDP-choline has also been reported to slightly increase the cerebral blood flow (Watanabe et al., 1975). After oral administration, CDP-choline is absorbed almost completely, and its bioavailability after oral administration is approximately the same as that after intravenous administration (Agut et al., 1983). However, no previous report has examined the effect of CDP-choline on peripheral nervous system disorders, and especially on diabetic peripheral neuropathy. To clarify the therapeutic potency of CDP-choline on diabetic peripheral neuropathy, we examined the effects of CDP-choline on the thermal nociceptive threshold in streptozotocin-induced diabetic mice. Moreover, we also examined the effect of chronic treatment with CDP-choline on the sciatic nerve Na^+-K^+-ATPase activity in diabetic mice.

2. Materials and methods

2.1. Animals

Male ICR mice (Tokyo Laboratory Animals Science, Tokyo, Japan), weighing about 20g at the beginning of the experiments, were used. They had free access to food and water in an animal room which was maintained at 24 ± 1°C with a 12-h light–dark cycle. Animals were rendered diabetic by a single injection of streptozotocin (200mg/kg, i.v.) prepared in 0.1N citrate buffer at pH 4.5. Age-matched non-diabetic mice were injected with vehicle alone. Mice with serum glucose levels above 400mg/dl were considered diabetic and used for the investigation. Serum glucose levels were measured at the end of the experiments. Animals were used only once in all experiment. This study was carried out in accordance with the guide for the care and use of laboratory animals adopted by the committee on the care and use of laboratory animals of Hoshi University, which is accredited by the Ministry of Education, Sports and Culture.

2.2. Assessment of the nociceptive response

The nociceptive response was evaluated by recording the latency to withdrawal of the tail in response to noxious skin heating. Briefly, the tails of mice were exposed to a focused beam of light from a 50-W projection bulb. The heat intensity was set by adjusting the source voltage of the bulb to 50V. When a withdrawal response occurred, the stimulus was terminated and the response latency was measured electronically. In the absence of a response up to a predetermined maximum latency (30s), the test was terminated to prevent tissue damage. All measurements were performed by an investigator who was unaware of the treatment group of individual animals.

2.3. Na^+-K^+-ATPase activity assay

To measure Na^+-K^+-ATPase activity, mice were killed by cervical dislocation, and sciatic nerves were taken and homogenized in 1ml of a prechilled solution of 10mM Tris–HCl, pH 7.5. Aliquots were frozen and stored at − 80°C until use. Na^+-K^+-ATPase activity was determined spectrophotometrically by the coupled enzymatic method (Hermenegildo et al., 1993) as the difference between total and ouabain-insensitive ATPase activity. The assay mixture contained 10mM Tris–HCl, pH 7.5, 5mM $MgSO_4$, 2.5mM phosphoenolpyruvate, 50μM NADH, 2.5mM ATP, 0.2U of pyruvate kinase, 0.2U of lactate dehydrogenase, and the sample (200μl of crude homogenate). Ouabain, when added, was used at 3mM. Incubations were conducted at 37°C, and the absorbance at 360nm was followed for 15min. The activity is expressed as micromoles of NADH oxidized per hour per milligram of protein.

2.4. Drugs

Streptozotocin was purchased from Sigma (St. Louis, MO). Cytidine 5′-diphosphocholine monosodium salt (CDP-choline) was synthesized at Yamasa Corporation. CDP-choline was dissolved in saline. A feeding probe was used when CDP-choline and its vehicle were given p.o. All administrations were completed 23h before measurement of the thermal nociceptive threshold. Treatment with CDP-choline was started on the day of streptozotocin treatment to evaluate the effect of CDP-choline on the development of thermal nociceptive hyper- and hypoalgesia. To evaluate the effect of CDP-choline on the development of thermal hypoalgesia, the treatment was started 5 weeks after the streptozotocin treatment. All treatments were continued once per day.

2.5. Statistical analysis

The data are expressed as the mean ± S.E.M. The statistical significance of differences between groups was assessed with Student's *t*-test (comparison of two groups) or an analysis of variance (ANOVA) followed by the Bonferroni test (comparison among multiple groups). A level of probability of 0.05 or less was considered significant.

3. Results

3.1. Time-dependent changes in the thermal nociceptive threshold in diabetic and non-diabetic mice

As shown in Fig. 1, after the administration of streptozotocin, diabetic mice exhibited a transient hyperalgesic response followed by a prolonged hypoalgesic response. ANOVA with repeated measures revealed that streptozotocin treatment significantly affected the tail-flick latency in mice [Diabetes × Duration after streptozotocin treatment; $F(12, 240) = 25.827$; $P < 0.001$]. The tail-flick latencies in diabetic mice were significantly shortened at 1–4 weeks after streptozotocin treatment and significantly prolonged at 6–9 weeks after streptozotocin treatment compared with those in non-diabetic mice.

3.2. Preventive effects of CDP-choline on the development of thermal hyper- and hypoalgesia in diabetic mice

To examine the preventive effects of CDP-choline, p.o. treatment with CDP-choline was started beginning on the day of streptozotocin injection and continued daily for 8 weeks. As shown in Fig. 2, chronic treatment with CDP-choline (100mg/kg, p.o.) almost completely inhibited thermal hyper- and hypoalgesia in diabetic mice. Two-way ANOVA with repeated measures revealed that CDP-choline treatment significantly affected the tail-flick latency in diabetic mice [Drugs ×

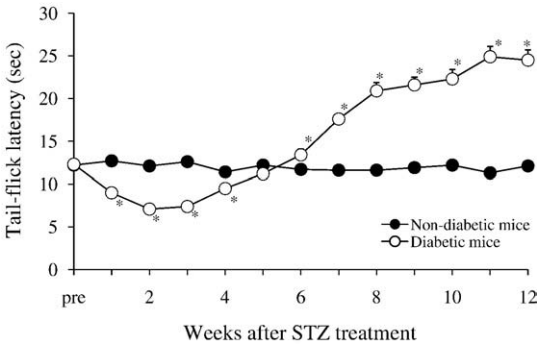

Fig. 1. Time-course of the changes in the tail-flick latency in diabetic and non-diabetic mice. Tail-flick latency was measured once a week. Heat intensity was set at 50 V. Each point represents the mean ± S.E.M. for ten mice. *$P < 0.05$ vs. respective vehicle-treated non-diabetic mice (Student's *t*-test).

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