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ALTERATION OF THE GABAERGIC NEURONAL SYSTEM OF THE RETINA AND SUPERIOR COLLICULUS IN STREPTOZOTOCIN-INDUCED DIABETIC RAT

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INDEXING WORDS

Diabetes mellitus ; rat; retina ; superior colliculus ; streptozotocin ; GABA ; GAD ; GABA-T

SYNOPSOS

Diabetes mellitus (DM) often causes neural dysfunctions, including visual disorders. To investigate the effect of hyperglycemia on the visual system, we studied the metabolism of gamma-aminobutyric acid (GABA), a major inhibitory neurotransmitter that has an important role in visual functions. We determined the GABA content and activities of glutamate decarboxylase (GAD) and GABA transaminase (GABA-T) in the retina and superior colliculus (SC) in streptozotocin (STZ)-induced DM rats. The GABA content and activities of GAD and GABA-T were decreased in the retinas of STZ-treated rats. The GABA content and GAD activity were reduced in the SC of STZ-treated rats, whereas GABA-T activity showed no marked change. This GABA reduction in the diabetes may be correlated with visual dysfunctions.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder accompanied by hyperglycemia. DM causes various neural disturbances in the central nerve system (CNS), including abnormal visual disorders and neuropathies. Changes in neurotransmitter levels in the CNS are reported to affect neural activity in the diabetic state. [1, 12, 13, 19] Gamma-aminobutyric acid (GABA), a major inhibitory neurotransmitter in the mammalian brain and retina [3, 14] plays an important part in the control of neural activity, including visual functions. In the retina, GABA and its synthesizing and catabolizing enzymes, glutamate decarboxylase (GAD) and GABA transaminase (GABA-T), mainly are present in the inner nuclear and inner plexiform layers. [4, 17] Immunocytochemical studies have shown that in most mammals the GABA and GAD in the retina are in amacrine cells. [11] GABA in the retina has been speculated to participate in negative feedback control in bipolar cells and feedforward control in ganglion cells. [7] The superior colliculus (SC), which bears the terminals of retinal ganglion cells, functions in the integration of the visual system, in particular ocular movements. As to the regional distribution of GABA in the CNS, the SC has abundant numbers of GABA and GABAergic interneurons. [14] Previous reports showed that in streptozotosin (STZ)-induced DM rats, the GABA contents of the hypothalamus and brainstem are decreased as is GAD activity in the cerebral cortex. [6, 19] Change in the retinal GABA contents in STZ-treated animals remains controversial. [13] To clarify the effect of hyperglycemia caused by STZ on the visual system, we determined the GABA content and GAD and GABA-T activities in the retina and SC of STZ-induced DM rats.

MATERIALS AND METHODS

STZ (80mg/kg, intraperitoneal injection) was administered to male Wister rats weighing 130 ~ 170 g to induce diabetes. Blood glucose level of each animal was determined 2 to 4 weeks and just before killing the animal after STZ treatment. Blood glucose levels of specimens obtained from the caudal vein were measured using glutest-E-II (Sanwakagaku, Nagoya). We defined the diabetic state as a blood glucose level of more than 300 mg/dl and increased water intake and urine excretion. Animals showing a blood glucose level below 300 mg/dl were discarded from the experiments. Animals had free access to food and water and were housed in a 12-h light-dark cycle room under constant temperature and humidity control. Two, 12 and 16 weeks after injection of the agent, the test animals were anesthetized with sodium pentobarbitulate (40mg/kg) and killed by

decapitation. After enucleation and removal of the brain from the skull, the retina and SC quickly were isolated on ice, chilled in liquid nitrogen, then stored at -80 ℃ until the GAD, GABA-T and GABA assays. Untreated rats served as the controls. After enucleation, the retinal tissue of one eye (about 1mg protein) was homogenized with 300 μl of cold distilled water. A 5μl homogenate sample was used in the GAD and GABA-T assays. A 45μl portion of 3M perchloric acid with 6mM EDTA was added to 225μl of the residual homogenate, and the whole homogenized, then centrifuged at 2500 rpm for 15min. The supernatant was neutralized with 2M $KHCO_3$ and recentrifuged, the supernatant being used to determine the concentrations of endogenous GABA and glutamate.

GABA content and GAD activity were determined by the modified method of Okada et al. [15] Briefly, GAD activity was determined by measuring the GABA content after incubation (37℃ 60 min) in GAD assay reagent. After termination of the reaction, the GABA assay reagent was added, and the mixture incubated at 37 ℃ 30 min, then the fluorescence of the NADPH produced was measured with a fluorometer (Optical Devices, USA). The endogenous GABA content was substracted from the total GABA obtained after the GAD assay reaction to calculate the GAD activity.

GABA-T activity was determined by measuring the glutamate content after incubation (37℃ 60 min) in GABA-T assay reagent consisting of 0.2M tris-HCl buffer (pH8.9), 25mMGABA, 200μM pyridoxal phosphate and 1mMα-ketoglutarate. After incubation, the mixture was heated at 100℃ for 3 min to stop the reaction. Glutamate assay reagent consisting of 0.1M hydrazine HCl buffer (pH9.0), 2.5mM NAD, 0.3mM ADP and glutamate-dehydrogenase (100μg/ml) was added, and the mixture incubated at 37℃ 30 min to determine the glutamate concentration. NADH fluorescence was measured with a fluorometer. To calculate GABA-T activity, the endogenous glutamate value was substracted from the total glutamate value after 60min of incubation with the GABA-T assay reagent. In the case of the SC, the tissue (about 1.5mg protein) was homogenized with 500μl of cold distilled water . The GAD, GABA-T activity , GABA and glutamate in the tissue were determined in the same way as for the retina. The protein concentration was determined by the method of Lowry et al. [10] The 2-tailed Students t test was used to analyze the results.

RESULTS

To test the accuracy of the GAD and GABA-T assay used, different amounts of tissue from the retina and SC were added to the GAD or GABA-T assay reagents. The GABA or glutamate produced was measured fluorometrically. Both the GABA

and glutamate produced increased linearly with the amount of tissue tested (Fig. 1A,B).

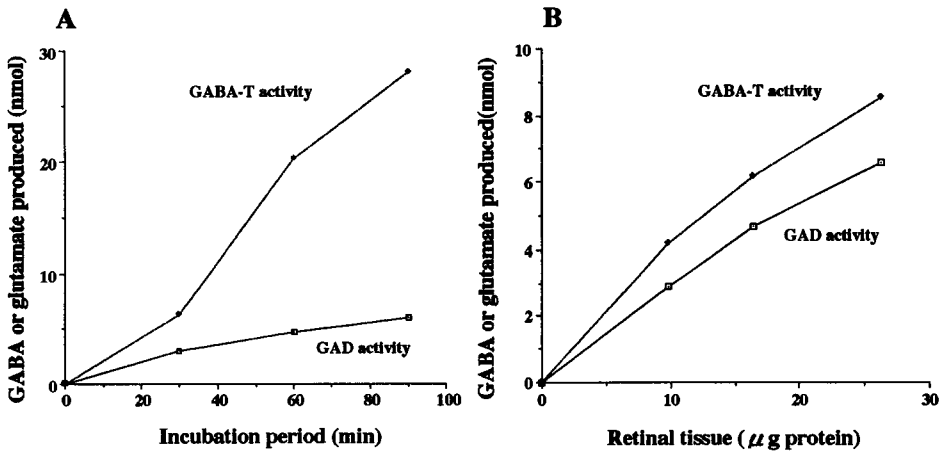

Figure 1. Effects of the incubation period and amount of retinal tissue in the assays of GAD and GABA-T. (A) GABA or glutamate produced after incubation for 30, 60 and 90 min. In each trial, 16.4 μg protein retinal tissue was added to the assay reagent. (B) GABA or glutamate produced after a 60min incubation with different amounts of retinal tissue.

In the STZ-treated animals, the GABA content of the retina decreased with the reduction in GAD activity (Fig. 2A,B). The GABA-T activity in the diabetic group decreased gradually, a significant reduction in the activity being observed after 16 weeks STZ treatment (Fig. 2C). The GABA content in the SC showed no significant change 2 and 12 weeks after STZ treatment, but after 16 weeks there was a significant decrease versus the control (Fig. 3A). Sixteen weeks after STZ treatment, GAD activity was significantly decreased, whereas GABA-T activity showed no marked change (Fig. 3B,3C).

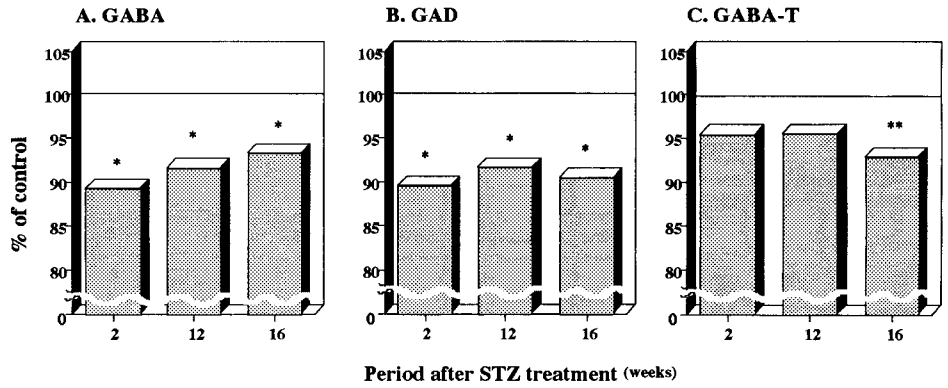

Figure 2. Change in GABA metabolism in the retina of STZ-induced diabetic rats. (A) GABA content, (B) GAD and (C) GABA-T activity in the retina 2, 12 and 16 weeks after treatment with STZ. In (A), the respective GABA contents in the control animals for 2, 12 and 16 weeks were 31.5±1.2 (S.E.M.) nmol/mg protein, 34.3±0.9 and 29.5±0.5. In (B), the respective GAD activities of the control animals for 2, 12 and 16 weeks were 151.8±3.1 nmol/mg protein, 157.3±2.3 and 152.7±3.9. In (C), the respective GABA-T activities for the control animals for 2, 12 and 16 weeks were 367.5±9.6 nmol/mg protein, 347.0±4.8 and 389.3.5±6.3. (n=3~8)
* P<0.05, ** P<0.01

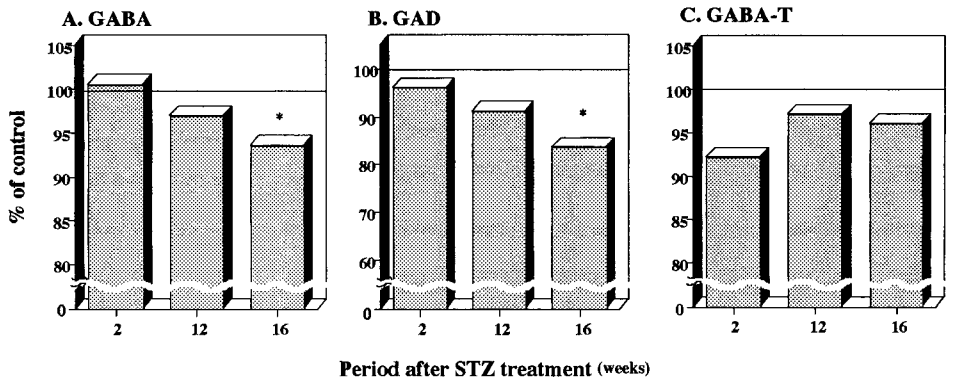

Figure 3. Change in GABA metabolism in the SC of rats with STZ-induced diabetes. (A) GABA content, (B) GAD and (C) GABA-T activity in the SC 2, 12 and 16 weeks after treatment with STZ. In (A), the respective GABA contents of the control animals for 2, 12 and 16 weeks were 65.2±3.8 (S.E.M.) nmol / mg protein, 60.5± 1.8 and 75.2±0.8. In (B), the respective GAD activities of the control animals for 2, 12 and 16 weeks were 440.3±54.9 nmol / mg protein, 482.8±43.7 and 525.3±16.4. In (C), the respective GABA-T activities of the control animals for 2, 12 and 16 weeks were 837.5±54.6 nmol / mg protein, 822.1±17.0 and 786.6±32.5. (n=3~8)
* P<0.05

DISCUSSION

In the retina GABA mainly is present in amacrine cells. [11, 16] These are considered to contribute to contrast sensitivity and color vision [9] which often are disturbed in DM, even without retinopathy. [8] In the early diabetic stage, impairment of retinal transmission is indicated by an abnormal electroretinogram showing decreased oscillatory potential waves. Nishimura et al. reported that the GABA concentration in the retina of the DM rat did not change in a period of 9 weeks after STZ treatment. [13] We found however, that the GABA concentration and GAD activity decreased in both the retina and SC of STZ-induced DM rats.

The discrepancy between their and our results may by due to the different follow-up periods used, to differences in STZ treatment, or both. We gave a single injection of 80mg/kg STZ to the rats, whereas they administered 30mg/kg STZ once a day for 5 days. In their study, the GABA content was measured only 9 weeks after STZ treatment. In our study, the GABA concentration in the SC showed no change at 2 and 12 weeks, but was reduced 16 weeks after STZ treatment, and the GABA concentration in the retina was reduced in all period. Furthermore, our results are in good agreement with the report that GABA in the brain is decreased in alloxan-induced DM. [12] It is probable that the GABA concentration in the retina, as well as in the SC, is reduced in the DM.

In the retina, GAD activity was reduced in all period, and GABA-T activity was reduced 16 weeks after treatment. In the SC, GAD activity decreased 16 weeks after STZ treatment, whereas GABA-T activity showed no marked change. The hyperglycemic state in STZ-treated animals may cause the reduction in the activities of these enzymes in GABAergic neurons which have high GAD activity and in disturbed glial cells that have abundant GABA-T. [4, 17]

The decrease in GABA is explainable as follows: One, it may be correlated with the reduction in GAD activity because Wu reported that the GABA concentration is more dependent on GAD than GABA-T activity. [20] Two, the metabolic abnormality induced by hyperglycemia may affect the response of GAD. Vitamin B6, an important coenzyme of GAD and GABA-T synthesis, is reduced in the serum of diabetic patients [5] and STZ-induced diabetic rats, [18] and this leads to a decrease in the GABA concentration in the GABAergic neurons. Three, the axonal transport of neurons is reduced in the diabetic state [2] which may produce a deficiency of the materials needed to synthesize GABA in the synaptic terminal. Although the GABA concentration in the retina was reduced from the early period after STZ treatment, it was not changed in the SC until 16 weeks after treatment, possibly because of the difference in the capacities of the Vitamin B6 pools or of transport between the retina and SC. Another possibility is that STZ itself is toxic

to GABAergic neurons in the brain and retina thereby affecting GAD and GABA-T activities. We found that 3 STZ-treated rats with no manifestation of diabetes showed no change in their GABA concentrations nor in their GAD and GABA-T activities (data not shown), suggesting that hyperglycemia is responsible for the changes in the GAD and GABA-T activities. Further investigation of whether insulin counteracts the reduction of GAD and GABA-T activity is needed to verify this. Changes in GABA metabolism in visual systems therefore may cause the impairment of the visual functions manifested in the diabetic state.

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