Effect of Diabetes on Median and Dorsal Raphe Nuclei Efferent Fibers to CA3 Hippocampal Area in Rat

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Abstract- Diabetes Mellitus (DM) is one of the most important metabolic disease causes in many side effects. The decrease of mental abilities and even some form of dementia followed prolonged diabetes has been reported. There are evidences that show hippocampus might be the site of neural involvement in patients with dementia followed diabetes. As hippocampus received main serotonergic efferents from brain stem raphe nuclei include dorsal raphe nucleus (DRN) and median raphe nucleus (MRN), it is possible that these efferent affected by diabetes. We used adult male Wistar rat in this study. Diabetes was induced by IP injection of streptozotocin (STZ). After 2,4 and 6 months, HRP tracing were done for the efferent from DRN & MRN to CA3 of hippocampus.HRP Positive neuron of DRN & MRN and also the distribution of these cells were study by light microscopy. Statistical analysis was done by SPSS software. Microscopic study clearly showed significant decrease in HRP positive cells population in DRN & MRN after prolonged diabetes. Based on our finding ,the changes in the neural connection between serotonergic nuclei and hippocampus followed diabetes might be one of the reasons for dementia in these patients.

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Key words: Diabetes mellitus; raphe nuclei; hippocampus; streptozocin

Introduction

Diabetes mellitus is one of the chronic disease characterized by wide spread complications among which the peripheral and central neuropathies are well known (1). 20.8 million Children and adults- 7.0% of the population – have diabetes (2). Neuropathy, a common complication of diabetes mellitus, is generally considered to be related to duration and severity of hyperglycaemia. Usually more than 50% of patients with duration of diabetes of 25 years or more are affected, making it as one of the most common disease of the nervous system. One of the largest published series reported a prevalence of 7.5% even at the time of diagnosis of diabetes (3). Statistical analysis shows that people with diabetes had a much higher rate of developing Alzheimer's disease than those who don't have diabetes (4).

Hippocampal formation is a part of limbic system that consists of dentate gyrus, proper hippocampus, subicular complex and entorhinal cortex (5). Proper hippocampus consists of three parts in transverse section: CA1, CA2 and CA3.

Each part has three cellular layers. Middle layers consist of pyramidal cells that are the chief cells of hippocampus. Efferent projections from proper hippocampus are axons of these pyramidal cells (6). The major afferent connections of hippocampal formation are substantial inputs from the medial septum, the hippocampal formation of the opposite side, the cingulum, olfactory and neocortical association area. Recently, however, a number of reports have appeared which suggest that certain cell groups in diencephalon and brainstem may also project directly to hippocampal formation (7). Recent research indicates that ascending serotonergic projections together with ascending cholinergic projections, play an essential role in the generalized control of cerebral activity (8).

The dorsal raphe nucleus consists of rostral and caudal subdivisions. The rostral aspect of the dorsal raphe is further divided into interfascicular, ventral, ventrolateral and dorsal subnuclei. The projections of the dorsal raphe nucleus have been found to vary topographically, and thus the subnuclei differ in their projections (9). An increased number of cells in the lateral aspects

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of the dorsal raphe are characteristic of humans and other primates. The dorsal raphe is the largest serotonergic nucleus and provides a substantial proportion of the serotonin innervation to the forebrain. Serotonergic neurons are found throughout the dorsal raphe nucleus and tend to be larger than other cells (10). The median raphe nucleus is composed of polygonal, fusiform and pyriform neurons and exists rostral to the nucleus raphe pontis. The most significant projections included the ones to the interpeduncular nucleus, the mammillary bodies, the habenular nuclei and the hippocampus (11).

Since diabetes mellitus has many complications and it is reported to impair the memory function in experimental animals, and the hippocampus play a pivotal role in a diverse set of cognitive functions, and raphe nuclei has important relationship to hippocampus, we decided to investigate the effect of diabetes on median and dorsal raphe nuclei efferent fibers to hipocampus.

Patients and Methods

Wistar rats of male sex were used (210 ± 20g; n=24). They were kept in a 12 hours light/dark cycle. Prior to the study they were divided into two groups (study and control) in simple randomized manner. Diabetes mellitus was induced by injection of streptozotocin (STZ). It was intraperitonealy injected into rats (60 mg/kg). To confirm the induction of diabetes mellitus, we measured glucose levels in blood samples obtained by tail prick using a strip-operated blood glucose sensor. All STZ-injected animals showed strong hyperglycemia (blood glucose level>300mg/dl).Rats in study group were divided randomly to three groups. In first group 2 months, in second group 4 months and in third group 6 months after confirming diabetes, we used HRP to study the effects of diabetes on the efferents of dorsal and medial raphe nuclei to CA3 hippocampal area. Following injection, HRP is absorbed by axonal endings and is transferred retrogradely to perikaria that send projections to the injection site. It accumulates in several vesicles, which are HRP labelled cells. Animals were anesthesized after an intraperitoneal injection of ketamin (40 mg/kg) and xylazin (5mg/kg). We performed a stereotaxic injection of HRP (Sigma) enzyme into the CA3 hippocampal region of the two groups. After surgery, rats were allowed to recover for 48 to 72 hours and were then anesthetized deeply with ketamin and xylazin. The animals were perfused intracardiacly with fixative solution (glutaraldehyde 1.25% and paraformaldehyde %1 in 0.2 mol buffer phosphate at *p*H=7.4) followed by sucrose buffer 10%. After removal, the rat brains were cut using freezing microtome (Cryocut 1800, ELICA) in coronal sections at a thickness of 40 µm and stored in 0.1 mol phosphate buffer. In this study, after injection of HRP into CA3 hippocampal area, we counted the number of labeled cells and studied their topography in dorsal and median raphe nuclei bilaterally both in study and control groups. Sections were reacted with tetra methyl benzidin (Sigma,Mo.,USA) following the procedure of Mesolam et al(12).Sections were then mounted onto gelatinized slides, airdried and counterstained with neutral red. After assessment of the injection site, we examined slides with light microscope and took digital photographs from all dorsal and ventral raphe nuclei areas. Selected slices were traced with reference to the atlas of Paxinos and Watson (1986) and the injection site and retrogradely labeled cells were plotted with the use of a microprojectore. Topographical study on dispersion of labeled cells with HRP was performed by Adobe Photoshop 7.0 software; we used Image tool 2.0 and SPSS 13.0 (Mann-Whitney and t test) software for the analysis of findings.

Results

The age-matched control animals showed a normal increase in body weight from (210 Â± 20 to 410 Â± 5 g) without change in blood glucose level (<200 mg/dl), whereas body weight decreased in the STZ-diabetic group (from 210 Â± 20 to 130 Â± 20 g) and blood glucose levels increased to (>300mg/dl) (Figure 4).

After injection of HRP in CA3 region (Figure 7), we see that labeled cells were concentrated in median raphe nucleus (MRN) (Figure 8a) and fewer labeled cells were observed in ventral part of dorsal raphe nucleus (DRN) especially in its caudal aspect. In caudal part of DRN labeled cells are close to cerebral aqueduct (Figure 8b). In control groups number of labeled cells had a specific pattern; in rostrocaudal manner at first they increased and then decreased (Figure 1). There wasn't any significant difference in pattern of distribution and number of labeled cells of all control groups; the total mean of labeled cells of MRN was 104.5 and the mean of number of labeled cells of DRN was 69. Ipsilateral DRN has more labeled cells than controlateral DRN and in both of them ventral region has more labeled cells than dorsal region (Figure 5).

It showed that neurons of DRN send projection fiber to CA3 region of hippocampus, and ventral part of DRN has more efferent projection to CA3; and ipsilateral DRN has more efferent projection fiber to CA3 than controlateral DRN.

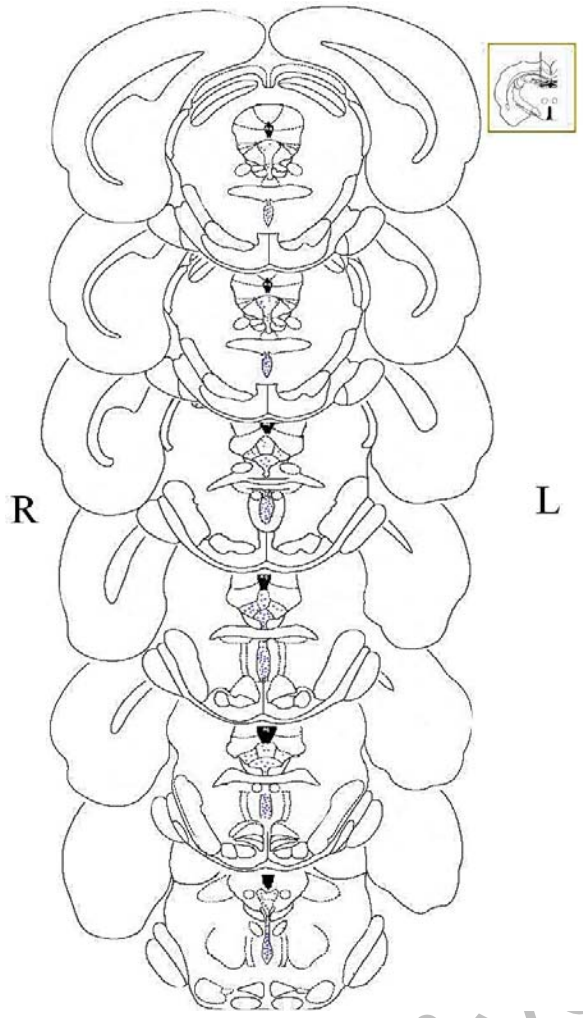

Figure 1. Dispersions of labeled cell in control group

In first study group that after 2 month STZ treated animals that connection studied there wasn't any significant differences in number of labeled cells of DRN and MRN.

In second study group" The mean of labeled cells in DRN was 47±3.6 (Figure 9a), and in contrast with control group, 36.2% was reduced, this reduction is significant (P<0.05). The numbers of labeled cells in ipsilateral side were more than contralateral and in ventral region were more than dorsal region.

In third group". The mean of labeled cells in dorsal raphe nuclei was 32.25±3.5 (Figure 10a), and in contrast with control group, 50.1% was reduced. There is significant difference between control and study group (P<0.05). There weren't any labeled neurons in contralateral DRN of study group. In second group" The mean of labeled cells in median raphe nuclei was 77±3.5 (Figure 9b), and in contrast with control group, 28.7% was reduced. This reduction is significant (P<0.05).

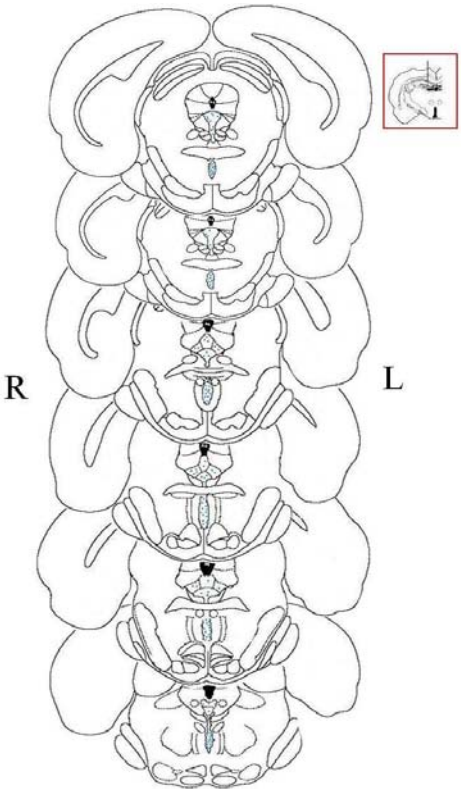

Figure 2. Dispersions of labeled cell in group 2 (4 month diabetic)

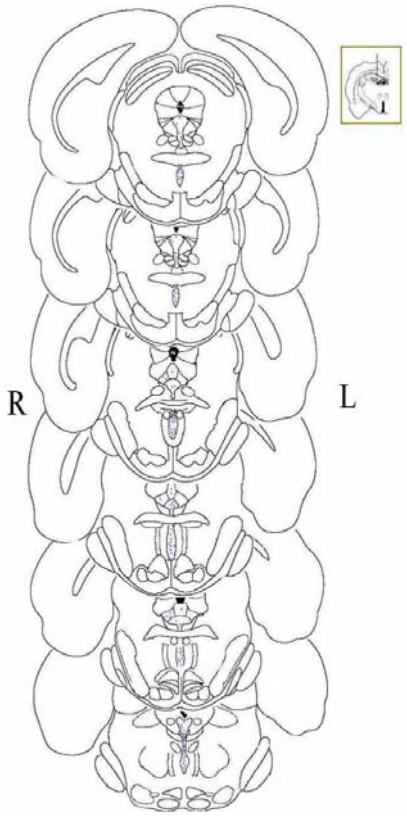

Figure 3. Dispersions of labeled cell in group 3 (6 month diabetic)

The pattern of distribution of labeled cells in MRN of control group weren't seen here (Figure 2)." In third group" The mean of labeled cells in median raphe was 54±4.1 (Figure 10b), and in contrast with control group, 48.4% was reduced (Figure 6). There is significant difference between control and study group (P<0.05). Decreasing in labeled cells in dorsal raphe was more than median raphe. The pattern of distribution of labeled cells in MRN of control group weren't seen here (Figure 3)."

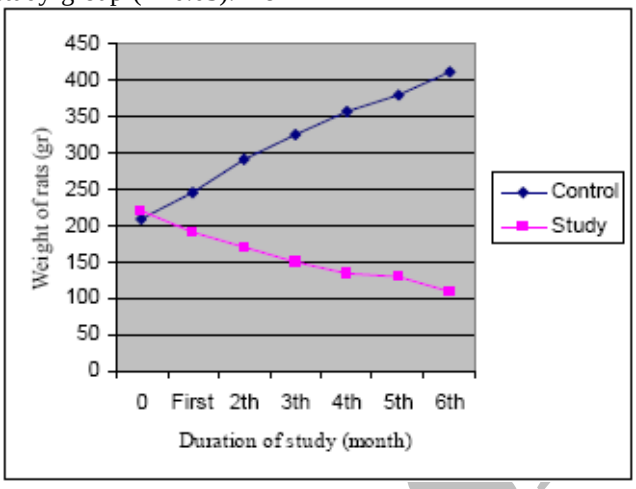

Figure 4. The relationship between weight of rats in control and study groups and duration of study

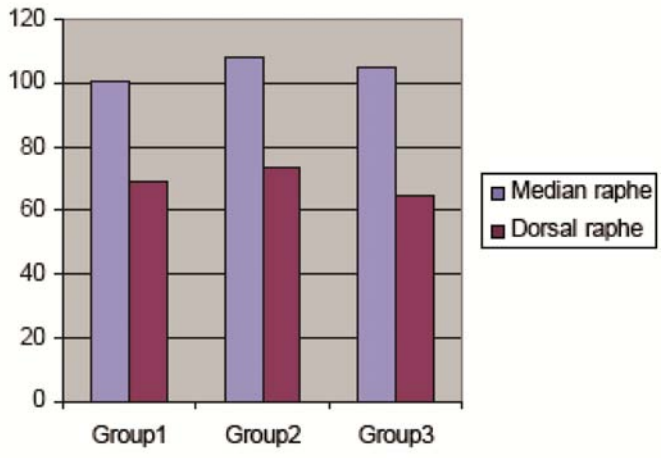

Figure 5. The number of labeled cells in median and dorsal nuclei in control groups

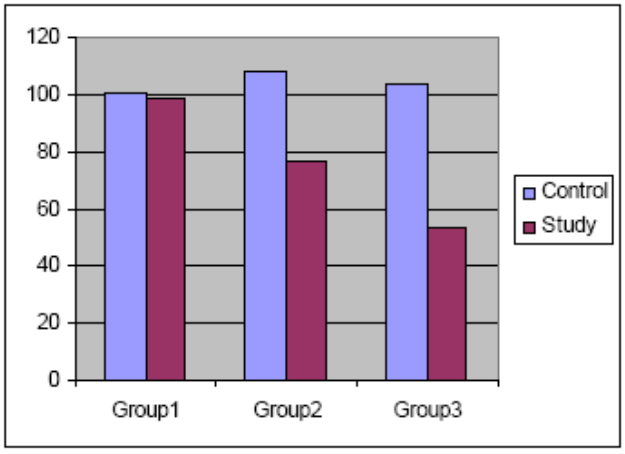

Figure 6. The mean of labeled cells in median raphe in control and study groups, 2, 4 and 6 months after diabetes

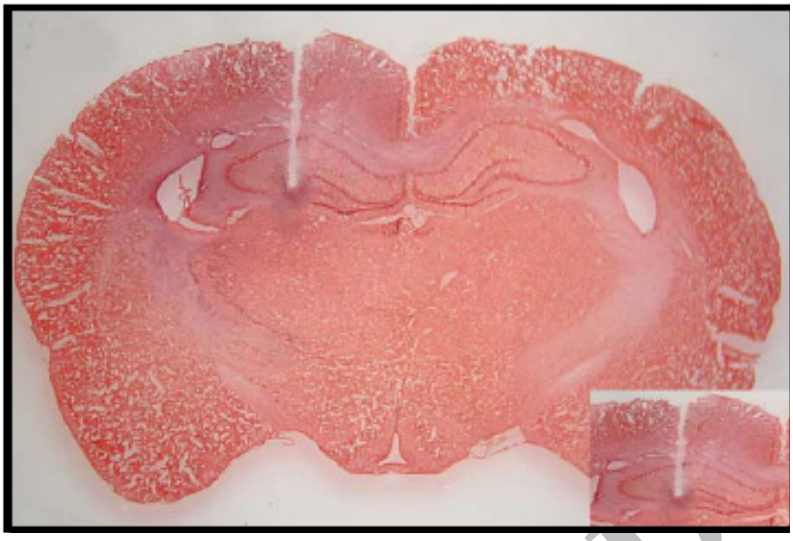

Figure 7. Injection site

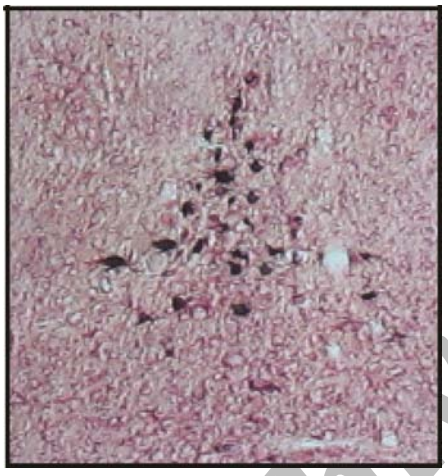

Figure 8a. Labeled cells in median raphe in control group(×100).

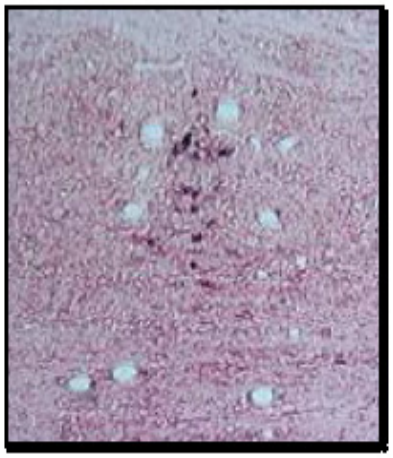

Figure 9a. Labeled cells in median raphe nuclei 4 months after diabetes(×100)

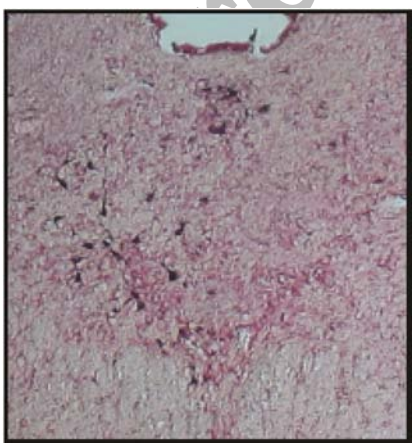

Figure 8b. Labeled cells in dorsal raphe in control group(×100)

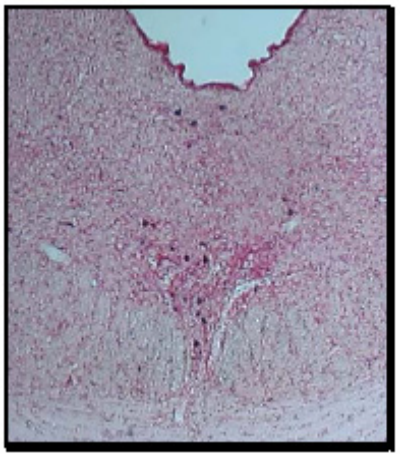

Figure 9b. Labeled cells in dorsal after 4months diabetes (×100)

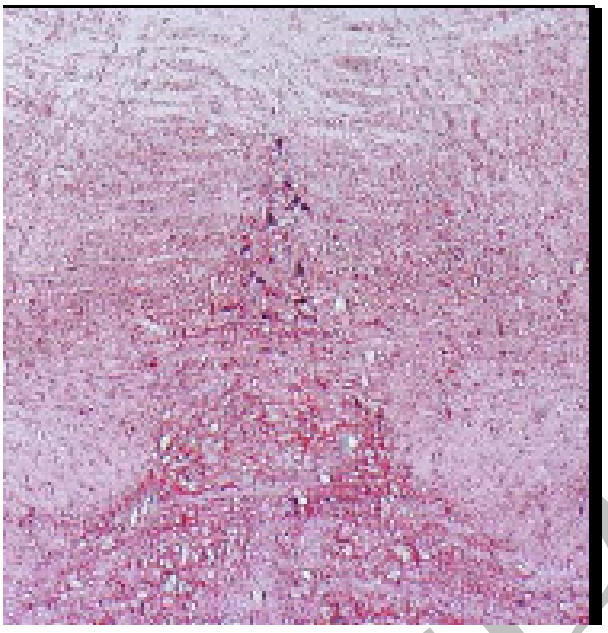

Figure 10a. Labeled cells in median raphe 6 months after diabetes (×100)

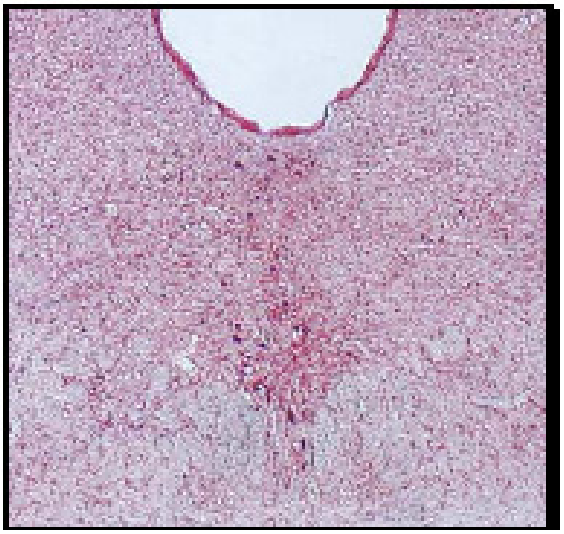

Figure 10b. Labeled cells in dorsal raphe 6 months after diabetes (×100)

Discussion

Long-term diabetes leads to a variety of discrete functional and structural disorders in the central nervous system (13). One of them is diabetic neuropathy, a frequent and serious complication of diabetes mellitus, affects sensorimotor and autonomic nerve function (14). The level of hyperglycemia and the duration of the disease are two important factors in the severity of neuropathy (1). In our study there was a significant difference between the group that only 2 months was diabetic and the groups that 4 and 6 months were diabetics. It showed that increase in duration of diabetes lead to decrease in number of labeled cells. However, long-term diabetes results in a variety of subtle cerebral disorders, which occur more frequently than is commonly believed.

Diabetic cerebral disorders have been demonstrated at a neurochemical, electrophysiological, structural and cognitive level; however, the pathogenesis is still not clear. Probably alterations in cerebral blood supply and metabolic derangements play a role, as they do in the pathogenesis of diabetic neuropathy (15).

Recent studies suggest that changes in retrograde axonal transport play an initial and important role in the

development of many axonopathies (16). Abnormalities in axonal transport have been observed in human and experimental diabetes and may be related to the pathogenesis of diabetic neuropathy (17). In our study the depletion in the number of labeled neurons in the diabetic rats compared with the nondiabetic rats is indicative of impairment of neuronal transport of the injected tracer in the diabetic rat. Our results and conclusion are quite consistent with reports obtained from similar studies in other laboratories particularly those in which other tracers have been used (18, 19). In our study the vast majority of projection neurons were concentrated in the median raphe and a few of them were observed in the dorsal raphe that is similar to previous studies (20).

Another central nervous system complication of diabetes mellitus (DM) is defects in hippocampal synaptic plasticity induction and difficulties in learning and memory (21). It was shown that hippocampal synaptic plasticity in rats is affected only in severe streptozotocin (STZ) -induced diabetes mellitus. Peripheral nerve conduction velocity and central-evoked potentials studies in both humans and animals showed progressive defects with increased duration of the disease (1). Two features of diabetes can impair memory. High blood sugar, the hallmark of the disease, depresses the function of the hippocampus (22). Results show that serotonin is important in long term memory processes(23). The dorsal raphe nucleus is the largest serotonergic nucleus and provides a substantial proportion of the serotonin innervations to the forebrain. The organization of the brainstem serotonin neuron projection to the hippocampal formation, this projection arises in the raphe nuclei of the midbrain(10). In addition in our study we find that the labeled cells in raphe nuclei in diabetic rats were less than nondiabetic groups.It showed that the retrograde axonal transport in serotoninergic neurons in diabetic groups was reduced.The distribution of HRP- containing cells retrogradely labeled from the hippocampus was similar to that shown in previous studies.

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