Place Learning and Hippocampal Synaptic Plasticity in Streptozotocin-Induced Diabetic Rats

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Moderate impairment of learning and memory has been recognized as a complication of diabetes. The present study examined behavioral and electrophysiological measures of cerebral function in streptozotocin (STZ)-induced diabetic rats. Behavioral testing consisted of a spatial learning task in a water maze. Electrophysiological testing consisted of in vitro assessment of hippocampal long-term potentiation (LTP), an activity-dependent form of synaptic plasticity, which is believed to be related to the cellular mechanisms of learning and memory. Two experiments were performed: the first with severely hyperglycemic rats and the second with moderately hyperglycemic rats. Rats were tested in the water maze 11 weeks after induction of diabetes. Next, LTP was measured in vitro in trained animals. Both spatial learning and LTP expression in the CA1 field of the hippocampus were impaired in severely hyperglycemic rats as compared with nondiabetic controls. In contrast, spatial learning and hippocampal LTP were unaffected in moderately hyperglycemic rats. The association of alterations in hippocampal LTP with specific learning impairments has previously been reported in conditions other than diabetes. Our findings suggest that changes in LTP-like forms of synaptic plasticity in the hippocampus, and possibly in other cerebral structures, are involved in learning deficits in STZ-induced diabetes. The beneficial effect of moderate glycemic control on both place learning and hippocampal LTP supports the significance of the relation between these two parameters and indicates that the development of the observed deficits may be related to the level of glycemic control. *Diabetes* 45:1259–1266, 1996

Moderate impairment of cognitive function has been recognized as a complication of both IDDM and NIDDM (1–4). In particular, deficits of learning and impairment of memory have been noted (1,2). Furthermore, tasks involving complex reasoning result in a poorer performance by diabetic patients (3). In contrast, relatively simple tasks that assess basic attentional processes or immediate recall do not appear to be affected (2,3). As in other diabetic complications, the development of cognitive dysfunction in diabetic patients appears to be related to the level of glycemic control (3–5).

The occurrence of cognitive deficits in diabetes may reflect a central equivalent of diabetic peripheral neuropathy. Indeed, several studies provide electrophysiological and morphological evidence for neuropathic changes in the central nervous system of diabetic patients (6). Experimental studies have substantiated evidence for central nervous system involvement in diabetes. Nerve conduction velocities in cerebral and spinal pathways were found to be impaired (7,8). In addition, histological studies of the neocortex of diabetic rats showed loss of neurons in association with microvascular abnormalities (9,10). So far, the number of behavioral studies in diabetic rodents is limited, and the results have been equivocal (11–13). One-trial passive avoidance learning was enhanced in streptozotocin (STZ)-induced diabetic rats and mice (12,13). In contrast, when task difficulty was increased in an active avoidance paradigm, diabetic mice had impaired acquisition and retention compared with control mice (13). Different sensitivity of diabetic rodents to reinforcing stimuli and different response to novel environments may explain these differential findings (11,13). In the present study, cognitive function in diabetic rats was evaluated with a spatial learning task in the Morris water maze (14). This task has the advantage to enable dissociation of learning deficits from sensorimotor deficits to some extent (15).

In addition to behavioral learning, we examined synaptic plasticity in the hippocampus of the same animals in vitro. In mammals, the hippocampus appears to be involved in some specific forms of behavioral learning. In particular, the hippocampus plays a central role in declarative memory (16). This kind of memory involves associations among items that can be accessed flexibly to guide memory expression in various and even new situations (16,17). In rats, spatial learning and the use of contextual clues in behavioral paradigms are dependent on the integrity of the hippocampus (14,18). On a cellular level, learning is thought to involve activity-dependent plastic modifications of synaptic strength of specific neuronal pathways (19,20). One such activity-dependent modification is long-term potentiation (LTP) in the hippocampus, which has attracted considerable attention in the search for the mechanisms of learning and memory (19,21). LTP can be defined as a prolonged enhancement of the efficacy of excitatory synaptic transmission, which can be elicited in vivo and in vitro by brief high-frequency stimulation of excitatory afferents (19,21,22). In addition to LTP, we have measured paired-pulse facilitation (PPF) in the hippocampus. PPF is a form of short-term synaptic plasticity,

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ANOVAR, analysis of variance for repeated measures; f-EPSP, field excitatory postsynaptic potential; LTP, long-term potentiation; MNCV, motor nerve conduction velocity; PPF, paired-pulse facilitation; SNCV, sensory nerve conduction velocity; STZ, streptozotocin.

which has been used as an electrophysiological tool for detection of changes in the presynaptic processes involved in neurotransmitter release (23,24).

The combination of water maze performance and measures of hippocampal synaptic plasticity provides a tool to study cognitive deficits and possible underlying neurological deficits in diabetic rats. The following issues were addressed in the present study: *1*) Is cognitive function, as determined by spatial learning performance in a water maze, impaired in STZ-induced diabetic rats? *2*) Is hippocampal synaptic plasticity impaired in the diabetic rats? *3*) Do abnormalities in learning and hippocampal synaptic plasticity occur in parallel with peripheral neurological dysfunction (sciatic nerve conduction velocity) in the diabetic rats? *4*) Is the extent of cognitive and hippocampal dysfunction related to the severity of hyperglycemia?

RESEARCH DESIGN AND METHODS

Animals. Male Wistar rats (starting weight ~300 g, UWU-CPD, Harlan, Utrecht, The Netherlands) were used. Rats were housed on sawdust and maintained on a 12 h/12 h light-dark cycle. All rats were given food and water ad libitum and were weighed weekly. Diabetes was induced by a single intravenous injection of STZ (Serve Feinbiochemica GMBH, Heidelberg, Germany) at a dose of 40 mg/kg body weight dissolved in saline. Four days after the injection, blood glucose was determined in blood samples, obtained by tail prick by a strip-operated blood glucose sensor (Companion 2, Medisense, Birmingham, U.K.). Blood glucose levels were >15.0 mmol/l in all STZ-injected animals.

Experimental design. The study consisted of two separate experiments. Experiment one evaluated the effect of severe chronic hyperglycemia on place learning, hippocampal plasticity, and sciatic nerve conduction velocity in rats, whereas experiment two evaluated the effect of moderate chronic hyperglycemia on the same parameters. In both experiments, two groups of rats were used: a nondiabetic age-matched control group and a diabetic group (all groups $n = 10$). In experiment one, diabetic rats were left untreated. In experiment two, diabetic rats were treated with a low-dose insulin regimen to maintain a stable moderate level of hyperglycemia. Insulin treatment was initiated directly after confirmation of diabetes and continued throughout the study. Insulin was administered through subcutaneous sustained release insulin implants at a dose of 1 IU per day (Linplant, M 114 llegaard, Ejby, Denmark). In both experiments, motor (MNVC) and sensory nerve conduction velocity (SNVC) were measured 10 weeks after the induction of diabetes. At week 11, rats were tested in the water maze. Since cataract formation might affect spatial orientation in diabetic rats, the eyes of the animals were carefully examined by two observers after the final trial in the water maze. Next, hippocampal synaptic plasticity was measured. Blood glucose concentration and glycated hemoglobin (HbA_1) levels (HbA_1 test-kit, Sigma Diagnostics, St. Louis, MO) were determined.

Sciatic nerve electrophysiology. MNCV and SNCV were measured in the sciatic nerve according to the method described by De Koning and Gispen (25). In short, the sciatic and tibial nerve were stimulated at the sciatic notch and ankle, respectively. MNCV and SNCV were calculated from the latencies of the responses of the musculature of the foot and the distance between the two stimulation points.

Morris water maze. The Morris water maze is widely used to test rats' abilities to learn, remember, and go to a place in space defined only by its position to extra maze cues (14,15). The water maze consisted of a large circular black pool (210 cm diameter, 50 cm height, filled to a depth of 30 cm with water [28 ± 1°C]) in which a submerged platform was hidden. The rat could climb on the platform to emerge from the water and to escape the necessity of swimming. During a series of trials, the rat was trained to locate the platform (14,26).

On 5 consecutive days, the animals were given three acquisition trials per day. The pool was placed in a darkened room, illuminated only by sparse red light. The rat was given a maximum of 120 s to find the hidden platform (black, round, 8 cm diameter, 1 cm below surface, located 55 cm from the edge of the pool) and was allowed to stay on it for 30 s. Rats who failed to locate the platform were put on it by the experimenter. The position of the rat in the pool was automatically registered on a video computer system (26). Both latency times and distances swum to reach the platform were measured. Moreover, the percentage of time the rat spent in the border zone of the pool (within 20 cm from the edge) was calculated. The time spent in the border zone of the pool provides a measure of comprehension of the task; naive rats try to escape by the borders of the pool, and trained rats will search for the more centrally oriented platform and consequently spend less time in the border zone.

A transfer trial was performed 4 days after the final day of the training. The platform was removed, and the rat was put into the maze. The rat was allowed to swim for 60 s. A zone with a radius of 20 cm was defined around the center of the former position of the platform. The time the rat spent in this zone was compared with the time spent in a equally sized reference zone.

In an additional study, a separate group of severely hyperglycemic rats were tested in the water maze with a visible platform. Rats were tested 11 weeks after the induction of diabetes. This test with a visible platform, which does not require spatial orientation (15), was used to reveal deficits in sensorimotor processes. The rats were tested on 3 consecutive days, three trials per day. They were allowed to search for a maximum of 60 s per trial. Two groups of rats were used: a nondiabetic age-matched control group ($n = 9$) and a diabetic group ($n = 10$).

Hippocampal LTP and PPF. LTP was measured in the CA1 field of hippocampal slices according to the method described in detail by Ramakers et al. (27). Briefly, rats were decapitated, and the brains were rapidly removed from the skull. The medial part of the hippocampus was cut in 450 μm thick transverse slices. Slices were transferred into a recording chamber containing oxygenated (95% O_2, 5% CO_2) medium (composition in dH_2O [in mmol/l]: 124.0 NaCl, 3.3 KCl, 1.2 KH_2PO_4, 1.3 $MgSO_4$, 10.0 glucose, 20.0 $NaHCO_3$, and 2.5 $CaCl_2$). Bipolar stainless-steel electrodes were placed on the afferent fibers of the stratum radiatum of the CA1 region of the hippocampus. Glass microelectrodes were used to record field excitatory postsynaptic potentials (f-EPSPs) in the stratum radiatum. A stimulus-response curve to five stimulation intensities (I_1-I_5), ranging from threshold level (I_1,) to the maximum response level (I_5) was made for every slice. Only those slices in which the amplitude of the f-EPSP was 1 mV or more at maximum response level were included in the experiment. Next, the stimulus intensity was adjusted to evoke f-EPSPs of half maximal amplitude, and this intensity was used as the stimulation intensity throughout the measurements. Stimulation frequency was 0.05 Hz. The first 15 min of the recording served to obtain a baseline recording of the f-EPSP. After baseline recordings, a high-frequency train of stimuli (100 Hz for 1 s) was applied, and f-EPSPs were recorded for another 60 min. Finally, a second stimulus-response curve (post–high-frequency stimulation) was obtained using the same stimulation intensities as in the first stimulus-response curve (pre–high-frequency stimulation). The average slope of the f-EPSP at baseline was set at 100%, and changes in slope were expressed as percent change from baseline.

In experiment one, PPF in the CA1 field of the hippocampus was measured in separate slices under identical conditions as LTP. PPF consists of an increase in response to the second stimulus of two identical stimuli separated by an interstimulus interval of 20–200 ms (23,24). The stimulus consisted of a paired pulse with a 50-ms interstimulus interval. The percentage increase of the slope of the second f-EPSP as compared with the first was calculated for every slice.

Statistical analysis. Data are presented as means ± SE unless indicated otherwise. Between group differences in body weight, blood glucose, HbA_1, sciatic nerve conduction velocity, performance in the transfer trial of the water maze, stimulation intensities in the hippocampus, and effect of PPF were analyzed with two-tailed t tests for independent samples. Differences between control and diabetic groups in the baseline values of the f-EPSP slopes in the hippocampus were compared with a two-tailed Mann-Whitney U test. Between group differences in water maze performance and stimulus-response relations of the f-EPSP were compared using an analysis of variance for repeated measurements (ANOVAR). Differences in the induction and expression of LTP between groups were analyzed with a χ^2 test and an ANOVAR, respectively.

Within individual groups, effects of PPF were analyzed by comparing the absolute values of the slope of the f-EPSP of the first and second response by Wilcoxon's matched-pairs signed-ranks test.

RESULTS

Experiment one (severe hyperglycemia)

Animals, nerve conduction velocities. Diabetic animals failed to gain weight in the course of the experiment. They had significantly reduced final body weights, together with

TABLE 1
Body weight, blood glucose, HbA_1, MNCV, and SNCV: experiment one (severe hyperglycemia) and experiment two (moderate hyperglycemia)

	n	Body weight (g)	Blood glucose (mmol/l) Week 7	Blood glucose (mmol/l) Final	HbA_1 (%)	MNCV (m/s)	SNCV (m/s)
Experiment one							
Control	10	507 ± 12‡	5.3 ± 0.4‡	5.5 ± 0.5‡	4.3 ± 0.2‡	63.4 ± 0.8‡	64.3 ± 0.9‡
Diabetic	10	274 ± 13§	30.2 ± 0.7§	25.6 ± 1.0§	8.3 ± 0.8§	53.6 ± 1.1§	51.8 ± 0.9§
Experiment two							
Control	10	510 ± 8.3‡	5.7 ± 0.5‡	5.6 ± 0.2‡	4.2 ± 0.3‡	60.2 ± 1.2*	61.7 ± 0.9‡
Diabetic	10	402 ± 10.8§	17.0 ± 2.1§	18.9 ± 1.8§	6.8 ± 0.3§	54.2 ± 0.8†	54.6 ± 1.1§

Data are means ± SE and were analyzed with two-tailed t tests for independent samples; $P < 0.005$ (*versus †); $P < 0.001$ (‡versus §). At week 7, insulin implants were replaced in diabetic rats in experiment two.

raised levels of plasma glucose and HbA_1, compared with control animals (Table 1). Both MNCV and SNCV were reduced in diabetic rats (Table 1). MNCV was 85% of control levels ($P < 0.001$), and SNCV was 81% of control levels ($P < 0.001$).

Morris water maze. On the first day of training in the water maze, no statistically significant differences in performance were observed between control and diabetic rats, although the diabetic rats tended to cover less distance than the control rats. In control rats, the latency and distance swam to reach the platform and the percentage of time spent in the border zone of the pool decreased gradually during the 5 days of training (Fig. 1*A*, *B*, *C*). The decrease of these three parameters was significantly less in diabetic rats (latencies: $P < 0.005$; distance: $P < 0.05$; percentage of time in border: $P < 0.001$). The transfer trial was performed 4 days after the final day of the training. The platform was removed and the rats were allowed to swim for 60 s. Control rats spent 7.3 ± 1.0 s in the zone around the former location of the platform compared with 3.8 ± 0.8 s in the diabetic group (t test: $P < 0.05$). The control and diabetic rats spent 2.0 ± 0.3 and 1.8 ± 0.7 s, respectively, in an equally sized reference zone. On eye examination, 4 of 10 diabetic rats had no evidence of cataract, 4 rats had opacification of the lens in one eye, and in 2 rats, both eyes were affected. However, there were no apparent differences in maze performance in diabetic rats with and without cataract. An additional experiment was conducted to establish whether sensorimotor deficits might play a role in the poorer performance of diabetic rats. Separate groups of diabetic and control rats were placed in a pool with a visual platform (Fig. 1*D*, *E*, *F*). Performance was similar in control and diabetic rats. On eye examination, 6 of 10 diabetic rats had no evidence for cataract, 2 rats had opacification of the lens in one eye, and in 2 rats, both eyes were affected.

Hippocampal electrophysiology. The stimulus-response relation of the f-EPSP slope at baseline was similar in diabetic and control rats (Fig. 2*C*). Stimulation intensities were similar in control and diabetic rats; 99 ± 3 and 104 ± 5 μA, respectively. The slope of the f-EPSP in the CA1 field at baseline was not significantly different in control and diabetic rats, although slopes tended to be higher in diabetic animals (control: median 0.33 mV/ms, range 0.22–0.62; diabetic: median 0.48 mV/ms, range 0.30–0.91; Mann-Whitney U test: NS).

In 9 of 9 control rats, LTP could be induced, whereas in 4 of 10 diabetic rats, no stable LTP could be induced (χ^2: $P < 0.05$). Moreover, the increase in slope as a result of high-frequency stimulation was significantly reduced in diabetic rats as compared with controls (Fig. 2*B*; $P < 0.001$). Stimulus-response relation of the f-EPSP slope was determined again 60 min after high-frequency stimulation, using the same stimulus intensities as at baseline. The f-EPSP slope, relative to the baseline value, was higher in control than in diabetic rats at all intensities tested (Fig. 2*C*; $P < 0.001$)

PPF was measured in the CA1 field in 8 control and 10 diabetic rats. In control and diabetic rats, the slope of the f-EPSP was significantly higher in response to the second stimulus (Wilcoxon: $P < 0.02$ control, $P < 0.01$ diabetic), indicating expression of PPF in both groups of rats. The increase in slope was similar in control and diabetic rats (control 52 ± 14%, diabetic 48 ± 9%).

Experiment two (moderate hyperglycemia)

Animals, nerve conduction velocities. Diabetic animals with moderate hyperglycemia gained weight in the course of the experiment. However, weight gain was at a slower pace than in nondiabetic control animals, and weight gain ceased after 9–10 weeks at a level of ~400 g. They had significantly reduced final body weights, together with raised levels of plasma glucose and HbA_1, compared with control animals (Table 1). Blood glucose was stable throughout the experiment and was at a lower level than in the diabetic rats of experiment one. The HbA_1 level was also less elevated than in the severely hyperglycemic rats of experiment one. Both MNCV and SNCV were reduced in diabetic rats (Table 1). MNCV was 90% of control levels ($P < 0.005$), and SNCV was 88% of control levels ($P < 0.001$).

Morris water maze. In control and diabetic rats, the latency and distance swam to reach the platform and the percentage of time spent in the border zone of the pool decreased gradually during the 5 days of training (Fig. 3). Although the decrease in latency appeared to be less marked in the first 3 days in diabetic rats, the differences in performance between the two groups were not statistically significant.

In the transfer trial, control rats spent 5.1 ± 1.0 s in the zone around the former location of the platform, compared with 6.8 ± 1.7 in the diabetic group. The control and diabetic rats spent 1.8 ± 0.6 and 2.1 ± 0.4 s in an equally sized reference zone. None of the diabetic rats had evidence of cataract.

Hippocampal electrophysiology. Stimulation intensities were similar in control and diabetic rats; 98 ± 5 and 102 ± 3 μA, respectively. In the CA1 field, the slope of the f-EPSP at baseline was similar in control and diabetic animals (control: median 0.34 mV/ms, range 0.23–0.40; diabetic: median 0.33

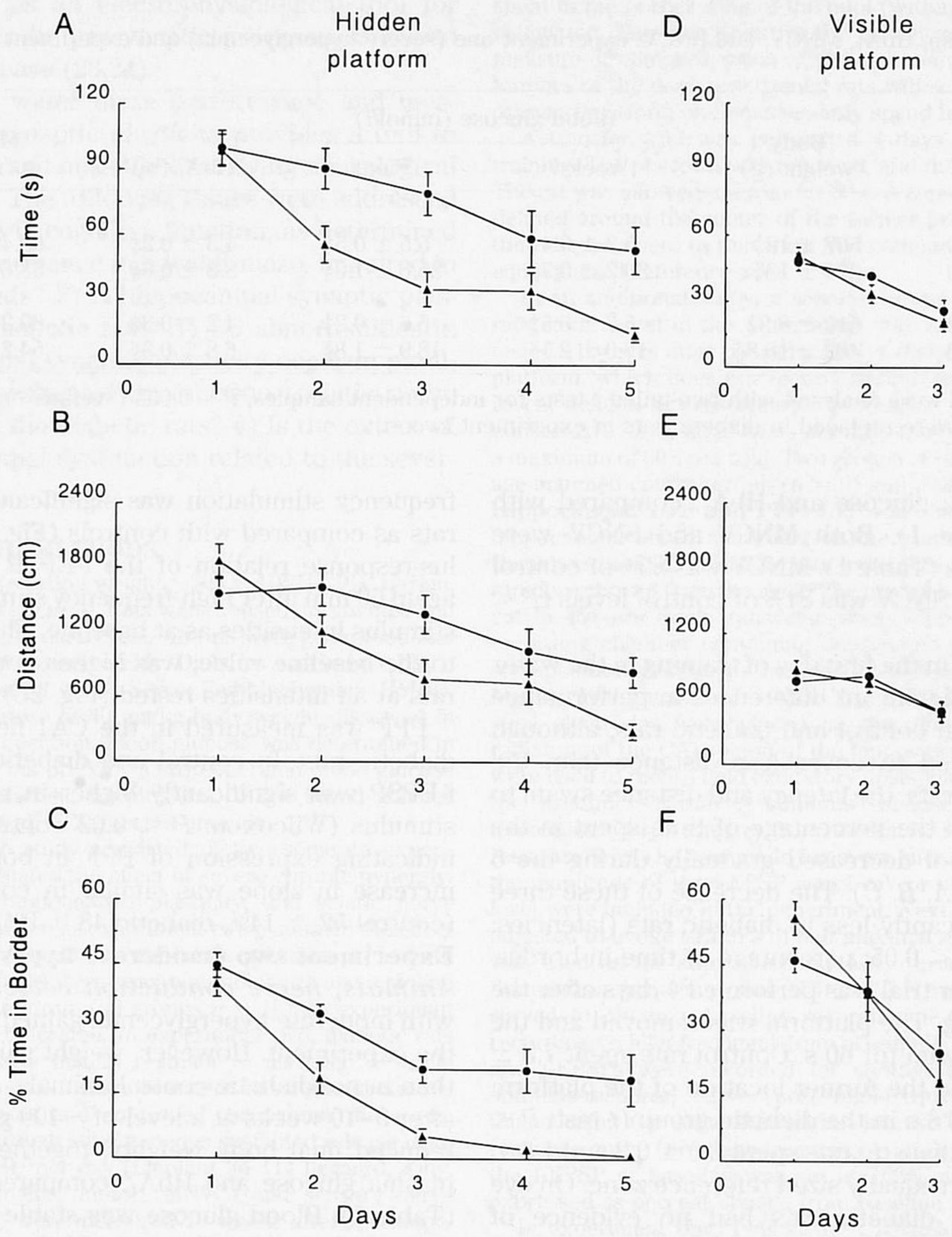

FIG. 1. Experiment one: Morris water maze; mean performance per day. *A*, *B*, *C*: test with hidden platform. Latencies to reach the platform (*A*); distances swum to reach the platform (*B*); percentage of time spent in the border zone of the pool (*C*). ▲, control rats (n = 10); ●, diabetic rats (n = 10). Performance was worse in diabetic rats: latencies: $F(1,18) = 12.7$, $P < 0.005$; distance: $F(1,18) = 4.7$, $P < 0.05$; time in border $F(1,18) = 20.3$, $P < 0.001$. *D*, *E*, *F*: test with visible platform, separate group of rats. Latencies to reach the platform (*D*); distances swum to reach the platform (*E*); percentage of time spent in the border zone of the pool (*F*). ▲, control rats (n = 9); ●, diabetic rats (n =10). Performance was similar in control and diabetic rats. Data are means ± SE.

mV/ms, range 0.30–0.70). The stimulus-response relation of the f-EPSP slope in the CA1 field at baseline was also similar in diabetic and control rats (Fig. 4*B*).

The effect of high-frequency stimulation of the Schaffer collateral commissural fibers on the slope of the f-EPSP in the CA1 field is illustrated in Fig. 4*A*. LTP could be induced by high-frequency stimulation in all six control rats. In two of seven diabetic rats, high-frequency stimulation failed to induce a stable LTP (χ^2: NS). Although the increase in f-EPSP slope as a result of high-frequency stimulation appeared to be slightly reduced in diabetic rats, this difference was not statistically significant. The stimulus-response relation of the f-EPSP slope 60 min after high-frequency stimulation in the CA1 field was also similar in diabetic and control rats (Fig. 4*B*).

DISCUSSION

In the present study, we demonstrate for the first time that deficits in cognitive function in diabetic rats, as determined with a spatial learning task, are associated with deficits in hippocampal synaptic plasticity. The deficits in spatial learning and hippocampal plasticity were observed in severely hyperglycemic rats but could not be detected in moderately hyperglycemic animals. In contrast, both severe and moderate hyperglycemia adversely affected the function of the peripheral nervous system: sciatic nerve MNCV and SNCV were reduced in severely as well as moderately hyperglycemic rats.

Reductions in nerve conduction velocity are an early manifestation of peripheral nerve dysfunction in both clinical and experimental diabetes (28,29). In the pathogenesis of peripheral diabetic neuropathy, hyperglycemia induced metabolic changes and neurovascular dysfunction have been implicated (30,31). Metabolic changes include increased polyol pathway flux, oxidative stress, and nonenzymatic protein glycation (30,32). Increased polyol pathway flux is associated with sorbitol accumulation and decreased *myo*-inositol content leading to changes in phosphoinositide

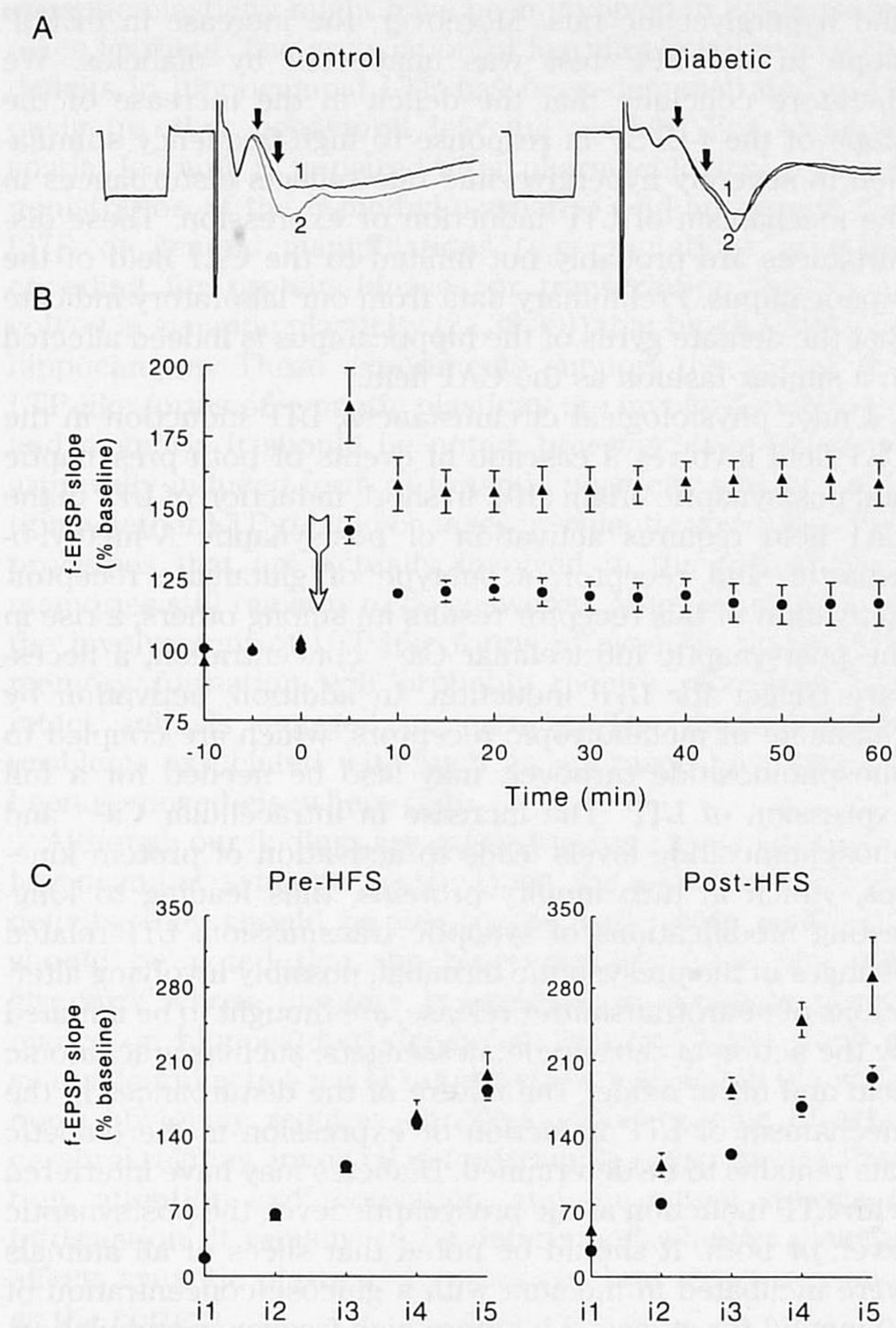

FIG. 2. Experiment one: hippocampal synaptic plasticity. *A*: typical f-EPSP recordings from the CA1 field of hippocampal slices in control and diabetic rats. Calibration pulse: vertical 1 mV, horizontal 5 ms; slope is calculated between arrows. Trace 1: baseline recording; trace 2: recording 60 min after high-frequency stimulation; note the increase in slope between the arrows. *B*: effect of high-frequency stimulation (open arrow) on the slope of the f-EPSP in the stratum radiatum of the CA1 field in slices from control (▲; n = 9) and diabetic rats (●; n = 10). The increase in slope after stimulation was significantly impaired in diabetic rats compared with controls [ANOVAR: F(1,17) = 21.4; P < 0.001]. *C*: stimulus response curves f-EPSP slope before and 60 min after high-frequency stimulation. I_1 threshold stimulus intensity; I_5 intensity to evoke f-EPSPs of maximal amplitude. The relative f-EPSP slope after high-frequency stimulation was higher in control than in diabetic rats at all intensities tested [ANOVAR: F(1,17) = 18.74, P < 0.001]. Values are expressed as percentage of the response at baseline. Data are means ± SE.

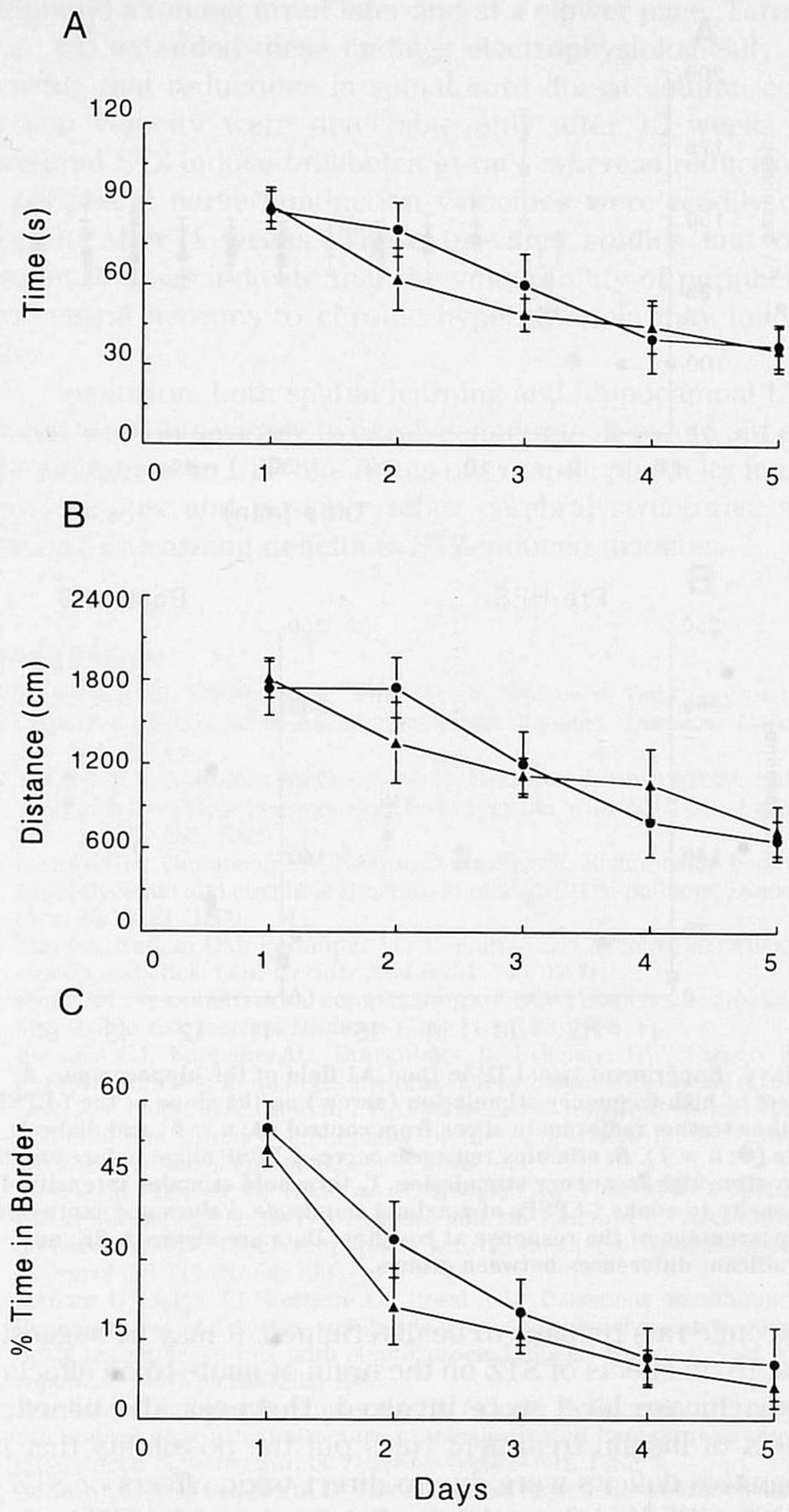

FIG. 3. Experiment two: Morris water maze. Mean performance per day. Latencies to reach the platform (*A*); distances swum to reach the platform (*B*); percentage of time spent in the border zone of the pool (*C*). ▲, control rats (n = 10); ●, diabetic rats (n = 10). No significant differences between groups. Data are means ± SE.

metabolism, protein kinase C activity, and ultimately impairment of Na^+-K^+-ATPase activity (30,33). Metabolic and vascular changes, including impairment of Na^+-K^+-ATPase activity (34), and regional reductions blood flow (35) have also been reported in the central nervous system in experimental diabetes and may adversely affect cerebral function.

Behavioral learning in the Morris water maze has been suggested to involve multiple cognitive processes as well as sensorimotor activity (36). Cognitive processes involved may include problem solving, enhanced selective attention, the formation of internal representations of the external world, such as in the case of spatial orientation, and, ultimately, the storage and retrieval of relevant information. Several factors may have been involved in the impaired water maze performance of the severely hyperglycemic rats. To examine the possible role of sensorimotor deficits, a separate group of severely hyperglycemic rats was tested in a water maze with a visible platform, a test that does not require the hippocampus-dependent spatial orientation (15). In this test, the hyperglycemic rats performed as well as age-matched control rats. Therefore, although sensorimotor deficits may have interfered with test performance in experiment one, they are unlikely to account fully for the observed deficits. Hence, we suggest that the impaired performance was, at least in part, related to cognitive dysfunction. This is supported by the observed differences in the search strategy of the severely hyperglycemic rats and control rats. Even in the final trials, severely hyperglycemic rats persisted to attempt to escape from the pool by the edges. This was reflected by increased percentage of time these animals spent in the border of the pool. This indicates that their comprehension of the task was less than that of control rats.

The cause of the cognitive deficits in the severely hyper-

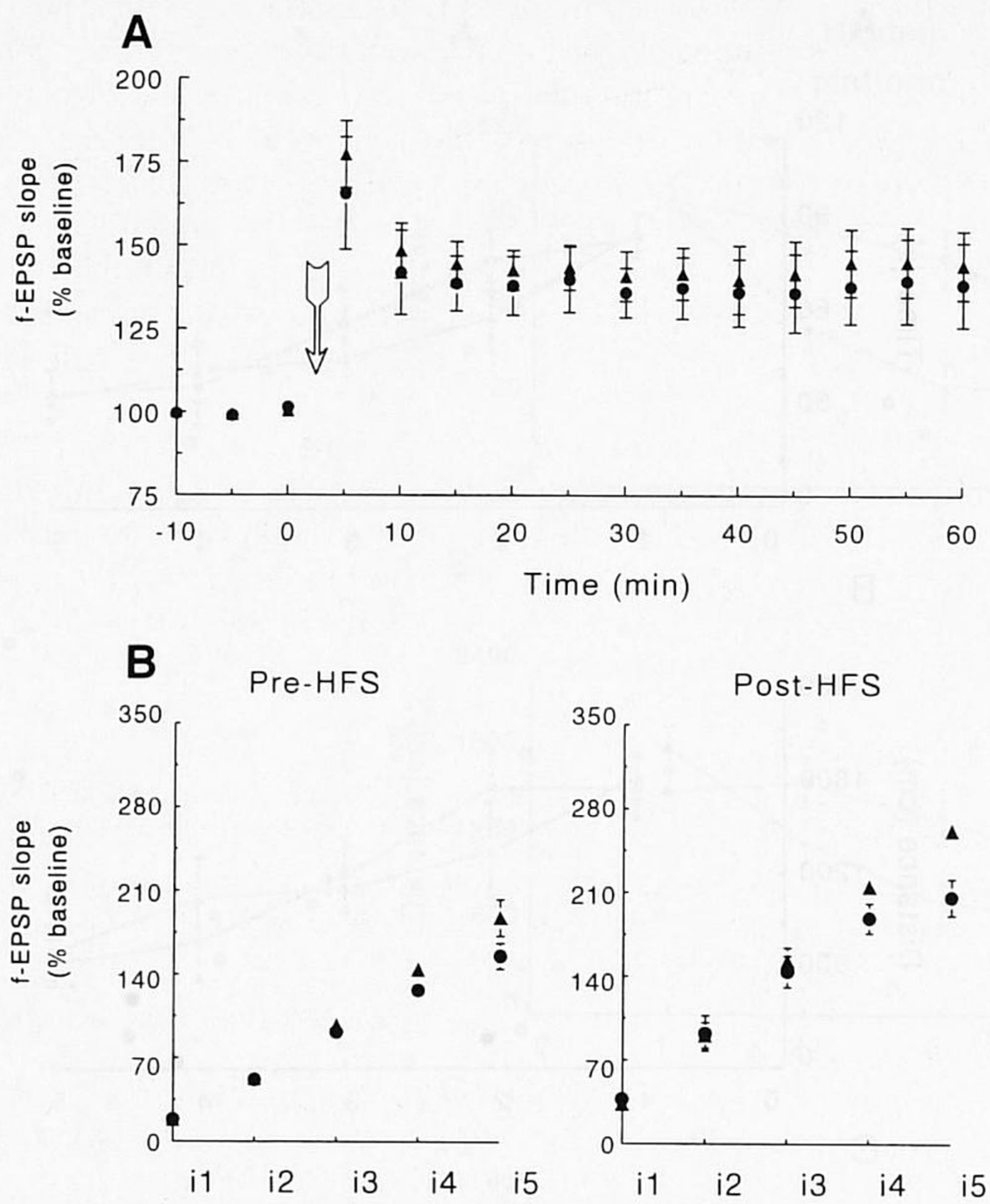

FIG. 4. Experiment two: LTP in the CA1 field of the hippocampus. *A*: effect of high-frequency stimulation (arrow) on the slope of the f-EPSP in the stratum radiatum in slices from control (▲; n = 6) and diabetic rats (●; n = 7). *B*: stimulus response curves f-EPSP slope before and 60 min after high-frequency stimulation. I_1 threshold stimulus intensity; I_5 intensity to evoke f-EPSPs of maximal amplitude. Values are expressed as percentage of the response at baseline. Data are means ± SE; no significant differences between groups.

glycemic rats remains to be determined. It may be suggested that toxic effects of STZ on the brain or acute toxic effects of high glucose level were involved. However, the beneficial effect of insulin treatment rules out the possibility that the cognitive deficits were due to direct toxic effects of STZ on the brain. Moreover, in a pilot study with STZ-induced diabetic rats that had been severely hyperglycemic for 4 weeks, water maze performance was unimpaired, suggesting that the observed deficits are due to a gradually progressive process rather than acute toxic effects of either STZ or high glucose levels. In our opinion, the cognitive deficits were related to chronic hyperglycemia and/or insulin deficiency. This is supported by the beneficial effect of moderate glycemic control.

LTP was inducible in a smaller proportion of severely hyperglycemic animals than in the nondiabetic control animals. Moreover, the increase in f-EPSP slope of the severely hyperglycemic rats in response to high-frequency stimulation was significantly less than the increase in controls. In contrast, LTP induction or expression was unimpaired in moderately hyperglycemic animals. It can be argued that the impaired ability to increase the f-EPSP slope in the severely hyperglycemic rats was related to a tendency to higher baseline f-EPSP slopes in the CA1 field of these animals as compared with the other groups. However, the potential to increase the f-EPSP slope was preserved in hyperglycemic animals, since maximal stimulation in the stimulus response curves lead to similar increments in f-EPSP slopes in control and hyperglycemic rats. Moreover, the increase in f-EPSP slope in the PPF test was unaffected by diabetes. We therefore conclude that the deficit in the increase of the slope of the f-EPSP in response to high-frequency stimulation in severely hyperglycemic rats reflects disturbances in the mechanism of LTP induction or expression. These disturbances are probably not limited to the CA1 field of the hippocampus. Preliminary data from our laboratory indicate that the dentate gyrus of the hippocampus is indeed affected in a similar fashion as the CA1 field.

Under physiological circumstances, LTP induction in the CA1 field involves a cascade of events of both presynaptic and postsynaptic origin (19). In short, induction of LTP in the CA1 field requires activation of postsynaptic *N*-methyl-D-aspartic acid receptor, a subtype of glutamate receptor. Activation of this receptor results in, among others, a rise in the postsynaptic intracellular Ca^{2+} concentration, a necessary trigger for LTP induction. In addition, activation by glutamate of metabotropic receptors, which are coupled to phosphoinositide turnover, may also be needed for a full expression of LTP. The increase in intracellular Ca^{2+} and phosphoinositide levels leads to activation of protein kineses, which in turn modify proteins, thus leading to long-lasting modifications of synaptic transmission. LTP-related changes in the presynaptic terminal, possibly involving alterations of neurotransmitter release, are thought to be initiated by the action of retrograde messengers, such as arachidonic acid and nitric oxide. The nature of the disturbances in the mechanism of LTP induction or expression in the diabetic rats remains to be determined. Diabetes may have interfered with LTP induction at the presynaptic level, the postsynaptic level, or both. It should be noted that slices of all animals were incubated in medium with a glucose concentration of 10 mmol/l for at least 2 h before high-frequency stimulation. Hence, the observed deficits in LTP were not attributable to acute effects of high glucose. Therefore, more persistent effects of antecedent chronic hyperglycemia appear to be involved. These may include changes in conduction or excitability in the presynaptic afferent fibers as a result of impaired Na^+-K^+-ATPase activity (34). Moreover, diabetes is known to affect neurotransmitter synthesis or release in several brain regions (6). Hence, glutamate synthesis or release may have been altered. However, there were no detectable changes in PPF in hyperglycemic rats. This indicates that there were no major changes in the presynaptic processes involved in neurotransmitter release (23,24). Alternatively, the function of the postsynaptic terminal may have been affected. Abnormalities in excitability related to impairment of Na^+-K^+-ATPase activity (34) or diabetes-related changes in phosphoinositide metabolism and protein kinase C activity could play a role in postsynaptic deficits. Finally, the activity of retrograde messengers involved in LTP induction, such as arachidonic acid and nitric oxide (19), may have been affected by diabetes. Disturbed metabolism of nitric oxide and arachidonic acid appear to be involved in diabetic vascular dysfunction (31). Whether diabetes also disturbs protein kinase C activity or phosphoinositide, nitric oxide, and arachidonic acid metabolism in the hippocampus remains to be determined.

The finding of impaired hippocampal LTP in hyperglycemic rats that performed poorly in a behavioral learning test and normal LTP in hyperglycemic rats that performed well in the same test suggests that impairment of LTP-like forms of

synaptic plasticity might have been involved in the impaired place learning. The association of learning impairments and deficits in hippocampal LTP has been demonstrated previously in other behavioral learning models. For example, spatial learning is impaired after pharmacological (e.g., administration of the *N*-methyl-D-aspartic acid antagonist AP5 [37]) or genetic manipulations (e.g., mutations in genes encoding for protein kinases or transcription factors involved in synaptic plasticity [22,38,39]) that block LTP in the hippocampus. These experiments support the notion that LTP-like forms of synaptic plasticity are involved in learning and memory. It should be noted, however, that LTP is an artificially induced form of synaptic plasticity and the question whether LTP-related changes in synaptic strength reflect processes that are actually involved in the formation of memories still remains to be answered. More direct proof of the involvement of LTP-like forms of synaptic plasticity in memory formation will probably require recording from intact animals engaged in learning. The methodological problems associated with such an approach have recently been reviewed elsewhere (20).

Although our findings are suggestive for a role of disturbed hippocampal synaptic plasticity in the observed learning deficits, they should be interpreted with some caution. It should be noted that the hippocampus is not the only structure whose integrity is essential to adequate performance on hippocampus-dependent learning tasks, such as spatial learning in a water maze. Spatial learning in the water maze obviously requires coordinated functioning of other cerebral regions, involved in for example sensorimotor function, attention and motivation, and the actual storage of information. It remains to be determined whether diabetes affects synaptic plasticity in other cerebral structures, such as the cortex.

One of the aims of the study was to determine if abnormalities in central neurological function occurred in parallel with peripheral neurological dysfunction in diabetic rats. Rats of the severe hyperglycemic group had signs of both peripheral and central nervous system dysfunction. In the moderate hyperglycemic group, we could not detect any abnormalities in place learning or hippocampal plasticity, although there was marked reduction of peripheral nerve conduction velocities. This finding could be due to the different sensitivity of the tests used to asses central and peripheral neurological dysfunction; it is possible that only marked abnormalities of the central nervous system lead to detectable impairments of place learning or hippocampal plasticity. Alternatively, peripheral nervous system dysfunction may precede the development of central nervous system dysfunction in diabetes. In rats that had been severely hyperglycemic for 4 weeks, water maze performance was unimpaired, despite detectable reductions in sciatic nerve conduction velocities (pilot study, data not shown). The suggestion that peripheral nervous system dysfunction precedes the development of detectable central abnormalities is supported by previous studies in diabetic rats (8,40,41). In a study of brain stem auditory evoked potentials in STZ-induced diabetic rats, 4 weeks of uncontrolled hyperglycemia lead to conduction abnormalities in the peripheral but not in the central components of the auditory evoked response (40). Moreover, Sima and Yagihashi (41) demonstrated that in the central sensory axon of the dorsal root ganglion cell, structural changes similar to those in the peripheral axon occurred later and at a slower pace. Terada et al. (8) extended these findings electrophysiologically by showing that reductions in spinal cord dorsal column conduction velocity were detectable only after 12 weeks of untreated STZ-induced diabetes in rats, whereas reductions in peripheral nerve conduction velocities were readily detectable after 4 weeks. These previous studies and our present findings indicate that the vulnerability of peripheral and central neurons to chronic hyperglycemia may indeed differ.

In conclusion, both spatial learning and hippocampal LTP are impaired in severely hyperglycemic rats. It would appear that alterations in LTP-like forms of synaptic plasticity in the hippocampus, and possibly other cerebral structures, are involved in learning deficits in STZ-induced diabetes.

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