

実験的糖尿病ラットおよび非糖尿病ラットにおける 自発運動量に及ぼすエタノールの急性効果

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Acute Effects of Ethanol on Spontaneous Locomotor Activity in both Experimentally Diabetic and Non-diabetic Rats

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ABSTRACT

We studied acute effects of ethanol (EtOH) on the spontaneous locomotor activity in both experimentally diabetic (DM) and non-diabetic rats. Rats were rendered diabetic by an injection of streptozotocin (60 mg/kg). One-day total locomotor activity was significantly decreased in DM rats, especially in the dark period, compared with that in non-diabetic rats, suggesting that diabetes may affect the locomotor activity in rats. EtOH (0.5, 1.0, or 2.0 g/kg) was intraperitoneally injected and the locomotor activity was measured using a running wheel. All doses (0.5-2.0 g/kg) of EtOH tested significantly reduced the spontaneous locomotor activity in the dark period in both diabetic and non-diabetic rats. In the dark period, the EtOH-induced decrease in locomotor activity was more prominent in non-diabetic rats than in DM rats. The peak time of the locomotor activity in both rats gradually shifted from the dark to light period with increasing doses of EtOH. It is reported that diabetes alters functions of brain neurotransmitters, presumably thereby inducing the alteration of locomotor activity. Possible involvement of EtOH-induced alterations of neurotransmitter system in the brain is discussed.

Key words: ethanol, diabetes mellitus, spontaneous

locomotor activity, running wheel, rat

INTRODUCTION

In animals, diabetes mellitus (DM) has been reported to be associated with behavioral changes (1-4). Increased retention of passive avoidance training in mice (1,3), enhanced grooming activity in a novel environment in rats (2), and poor retention of a previously learned avoidance response in a T-maze in mice (4) have also been reported. In addition, diabetic rats showed significantly more anxiogenic activity than non-diabetic rats in open-field, elevated plus maze, zero maze, and social interaction tests (5). As with locomotion, humans with both type 1 diabetes (6) and type 2 diabetes (7) exhibit reduced maximal exercise capacity during formal testing. Likewise, severe untreated type 1 diabetes in rats also reduces spontaneous exercise (8). In type 1 diabetic mice, however, it has been reported that spontaneous locomotor activity was significantly greater than that in non-diabetic mice (9).

Alcohol is one of the most frequently consumed psychoactive drugs in society, despite well-known cognitive deficits associated with both acute and chronic consumption (10). The effects of ethanol (EtOH) on locomotor activity vary as a function of many factors, including animal species, EtOH

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dosage, and time of assessment during the course of acute intoxication (11). In general, locomotor activity in rodents is suppressed after moderate or high EtOH doses. A marked increase in spontaneous locomotor activity has been noted with relatively low (1-2 g/kg) EtOH doses (12-14), while higher doses (>4 g/kg) depress the activity (12,15). Furthermore, a biphasic relationship over time is often reported for EtOH-induced activity changes, but there is some disagreement over the direction of these changes: some studies show stimulation followed by depression (13), while others show an initial suppression of activity followed by stimulation (15). The effects of EtOH on the locomotor activity of rodents, as stated above, have been generally evaluated by open-field or plus-maze tests. In the experiments using the open-field test, locomotor activities in mice (13,16) and rats (17) were unchanged or increased by i.p. administration of EtOH (0.5-2.5 g/kg). Gulick and Gould (14) reported that EtOH (1.0-1.4 g/kg) dose-dependently increased locomotion and decreased anxiety and learning in the plus-maze test.

Evidence is now accumulating that shows a strong correlation between alcohol consumption and the development of diabetes in both male and female human populations (18). Therefore, the present study attempted to ascertain [1] the effects of type 1 diabetes mellitus on the spontaneous locomotor activity in rats, and [2] the acute effects of EtOH on spontaneous locomotor activity in the diabetic condition, using the rat as the animal species and the running wheel as the apparatus measuring locomotor activity. In addition, the effects of timing (morning or evening) of EtOH-administration were examined. Wheel running in rodents has been used as a model of physical activity dependence (19). We used streptozotocin (STZ)-treated rats as an animal model of type 1 diabetes because STZ induces pancreatic β -cell death and hyperglycemia associated with decreased insulin secretion (20). To the best of our knowledge, no report assessing the effects of EtOH on voluntary locomotor activity in rats using a running wheel has been published.

MATERIALS AND METHODS

All experiments described in this report were

performed in accordance with the specifications of the Ethical Committee of Nakamura Gakuen University and met the guidelines of the responsible governmental agency.

Animals

Male Wistar rats (4 weeks old) were purchased from Kyudo (Tosu, Japan). The rats were individually housed in a stainless-steel cage in a temperature (22°C)- and humidity (60%)-controlled room with a standard 12/12 h light/dark cycle (illumination from 7:00 to 19:00). Rats were allowed ad libitum access to food and water. The rats were divided into two groups: a control group (non-DM rats; n=15) and an experimentally diabetic group (DM rats; n=15). The experimental diabetes mellitus was induced by a single injection of streptozotocin (STZ, 60 mg/kg, i.p.) dissolved in citrate buffer (pH 4.5). The non-DM rats were injected with an equivalent amount of the buffer alone. Diabetes was verified by hyperglycemia, glucosuria, and hypoinsulinemia. The fasting blood glucose was measured by a glucose analyzer (Glutest Ace, Santa Chemicals, Nagoya, Japan), and the fasting blood insulin was measured by an insulin analysis kit (Glazyme Insulin-EA Test, Wako, Osaka, Japan) after fasting more than 12 hours. The rats were used in the experiments at 5-6 weeks old, when the body weight, fasting blood glucose, and fasting blood insulin values were 162.8 ± 14.3 g, 82.6 ± 6.7 mg/dl, and 0.978 ± 0.242 ng/ml in non-DM rats, and 144.9 ± 23.3 g ($P=0.078$ vs. non-DM rats), 363.1 ± 117.2 mg/dl ($P<0.01$ vs. non-DM rats), and 0.506 ± 0.069 ng/ml ($P<0.05$ vs. non-DM rats) in DM rats, respectively.

Voluntary wheel running

The behavioral testing protocol is shown in Fig. 1. Effects of EtOH on the locomotor activity of rats were evaluated by voluntary wheel running. Rats were placed in cages containing a free wheel (KN-79, Shinano, Tokyo, Japan) for 10 consecutive days with free access to food and water. Each revolution of the wheel was counted as one by an auto counter next to the cage and recorded daily by a computer.

EtOH administration

EtOH (99.5%) was diluted in 0.9% saline to produce solutions of 5%, 10%, and 20% w/v,

and was injected i.p. at a constant volume of 0.1 ml per 10 g body weight to produce doses of 0.5, 1.0, and 2.0 g/kg, respectively. EtOH were administered in both conditions (beginning time of dark period and light period). Five min prior to testing, subjects were injected with either 0.9% saline (control condition) or one of the three doses of EtOH, according to the experimental schedule shown in Fig. 1. First, animals were habituated to handling for three consecutive days (Days 1-3 in Fig. 1) before testing, which occurred on Day 4. On the fourth day, rats were administered 0.9% saline (an equivalent volume of the ethanol solution) intraperitoneally (i.p.) at time 19:00 (evening), and two days later, on Day 6, ethanol (EtOH) of 0.5 g/kg was administered at 19:00. Likewise, two days later, on Day 8, EtOH of 1.0 g/kg was administered at 19:00, and on Day 10 EtOH of 2.0 g/kg was administered at 19:00. On Day 5, 7, and 9, rats were free.

Blood EtOH determination

Blood samples in the presence of antioxidant, ascorbic acid (0.1%) were obtained from rats just before EtOH injection, and 1 h and 2 h after i.p. injection of 0.5 g/kg, 1.0 g/kg, or 2.0 g/kg EtOH. Blood EtOH concentrations were determined for each dose and time point using an Ethanol Assay Kit (BioChain, Hayward, CA, USA), in which dichromate is reduced by EtOH to a bluish chromic (Cr^{3+}) product. The intensity of the color, measured at 580 nm, is a direct measure of the alcohol concentration in the sample.

Drugs

Ethanol (EtOH) was obtained from Wako Pure

Chemicals (Osaka, Japan). Streptozotocin (STZ) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Statistical analysis

Data presented are expressed as means \pm S.D. Differences were analyzed by Student's t-test and a one-way analysis of variance (ANOVA). $P < 0.05$ was considered significant.

RESULTS

Blood EtOH concentrations were highest at 1 h in both DM and non-DM rats (Table 1). The blood EtOH concentrations in both rats were increased with increasing EtOH dose administered. No significant difference in the blood EtOH concentration was found in between DM and non-DM rats, at least within 2h after EtOH administration.

Figures 2A, B, C, and D show the effects of i.p. injection of saline (control), 0.5 g/kg, 1.0 g/kg, and 2.0 g/kg EtOH at the beginning time (time 19:00) of dark period on the 24-h locomotion in both DM and non-DM rats, respectively. The data are expressed as the locomotor activity per hour, and are mean \pm SD of 9 rats, respectively. In general, the locomotor activity of rats was higher during the dark period (time 19:00–7:00), but lower during the light period (time 7:00–19:00), because rats are nocturnal. In the control condition (saline administration) (panel A), the locomotor activity of non-DM rats during the dark period was more than twice that during the light period. However, the locomotor activity of DM rats was generally lower

Days 1-3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Habituation	Equivolume saline i.p.	rest	EtOH (0.5g/kg) i.p.	rest	EtOH (1.0g/kg) i.p.	rest	EtOH (2.0g/kg) i.p.

Fig. 1

Dosing schedule for ethanol (EtOH)-induced locomotor activity. Both diabetic (DM) and non-diabetic (non-DM) rats were habituated to handling for three consecutive days (Days 1-3). On the fourth day, rats were administered 0.9% saline (an equivalent volume to the EtOH solution) i.p at 19:00, and two days later, on Day 6, EtOH of 0.5 g/kg was administered at 19:00. Likewise, two days later, on Day 8, EtOH of 1.0 g/kg was administered, and on Day 10 EtOH of 2.0 g/kg was administered at the same time. On Day 5, 7, and 9, rats were untreated.

Blood EtOH concentrations in both DM and non-DM rats

non-DM rat	EtOH	0hr	1hr	2hr
	0.5 g/kg	81.6±10.9 mg/dl	78.5±17.9 mg/dl	63.6±9.0 mg/dl
	1.0	80.4±11.3	118.2±6.0 ^b	113.5±30.1
	2.0	74.7±12.2	172.1±12.8 ^{****##}	149.4±15.9 ^{****##}
DM rat	EtOH	0hr	1hr	2hr
	0.5 g/kg	100.1±32.4 mg/dl	92.5±28.4 mg/dl	68.7±49.9 mg/dl
	1.0	87.4±20.3	118.8±30.6	93.5±36.4
	2.0	67.9±23.2	140.0±44.0 ^{##}	129.4±23.1 [#]

Data are expressed as means±S.D.

** P<0.01 vs. 2hr at EtOH 0.5 g/kg, *** P<0.001 vs. 1hr at EtOH 0.5 g/kg

P<0.05, ## P<0.01, ### P<0.001 vs. 0 hr at EtOH 2.0 g/kg

^b P<0.05 vs. 1 hr at EtOH 0.5 g/kg

Table 1

than that of non-DM rats.

EtOH at 0.5 g/kg markedly decreased the total 24-h locomotor activity in both DM and non-DM rats. The decrease was more evident in non-DM rats. EtOH at 1.0 or 2.0 g/kg further affected the locomotion in both rats (panels C and D). The peak locomotor activity in both rats gradually shifted from the dark period to the light period with increasing doses of EtOH.

The effects of all three EtOH dosages (0.5, 1.0, and 2.0 g/kg) tested on locomotor activity all day, during the dark period alone, and during the light period alone are summarized in Fig. 3. Panels A, B, and C show the actual locomotor activity data (number of counts), but panels D, E, and F show the normalized data, taking the data of the control condition (saline), shown in panels A, B, and C as 100%. As stated before, the all-day locomotor activities of DM and non-DM rats were markedly suppressed in the presence of all dosages of EtOH, especially in non-DM rats. When all-day locomotor activity was divided into the locomotor activities of dark and light periods, EtOH dominantly affected the locomotor activity of both DM and non-DM rats in the dark period (panels B, C, E and F).

DISCUSSION

In this study, we tried to clarify the effects of

[1] type 1 diabetes mellitus on locomotor activity using a running wheel, and [2] EtOH on locomotor activity in rats with and without STZ-induced diabetes. Further, to what degree does the diabetic state affect EtOH-induced alterations in locomotor activity?

As shown in Figs. 2A and 3, type 1 diabetes significantly reduced total locomotor activity in 24 hours, as observed by use of the running wheel, especially in the dark period, which is the active time for rats. However, in the light period which is thought to be equivalent to rest (sleeping) time, little difference was found between DM and non-DM rats in locomotor activity (Fig. 3C). That is, DM rats have less locomotor activity during the dark period than non-DM rats. This is in good agreement with the results of Van Lunteren et al. (8) They also used STZ-induced DM rats and a running wheel to measure locomotor activity. The daily running distance of DM rats was only about 4% of the controls. Further, Merali et al. (21) observed decreased floor activity during the dark period in spontaneously diabetic Wistar BB rats, and Hilakivi-Clarke et al. (22) also reported lowered locomotor activity in STZ-diabetic mice as measured by a holeboard test.

In humans, 60-70% of diabetic patients develop neuropathy (23). Sensorimotor diabetic polyneuropathy affects both large and small

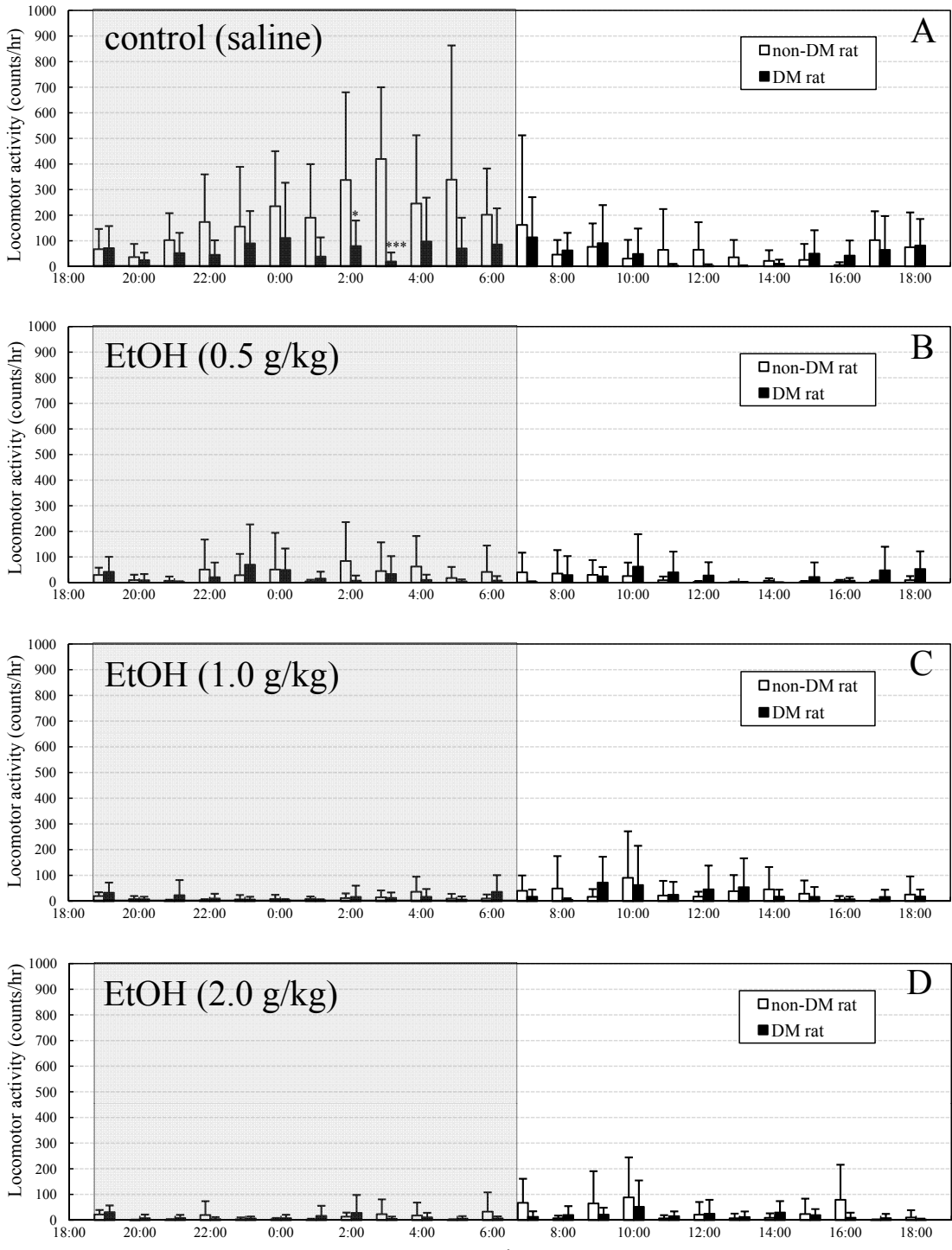


Fig. 2

Dose-dependent effects of EtOH on the circadian rhythms of locomotor activities in both DM and non-DM rats. Data were obtained from 9 non-DM rats (open columns) and 9 DM rats (closed columns), respectively. Panels A, B, C, and D show the administration of 0.9% saline, 0.5 g/kg, 1.0 g/kg, and 2.0 g/kg EtOH, respectively. Saline or EtOH was injected at 19:00. Data are expressed as means \pm S.D. Shadow area shows dark period (time 19:00–7:00). * $P < 0.05$, *** $P < 0.001$, compared to non-DM rats at the same time.

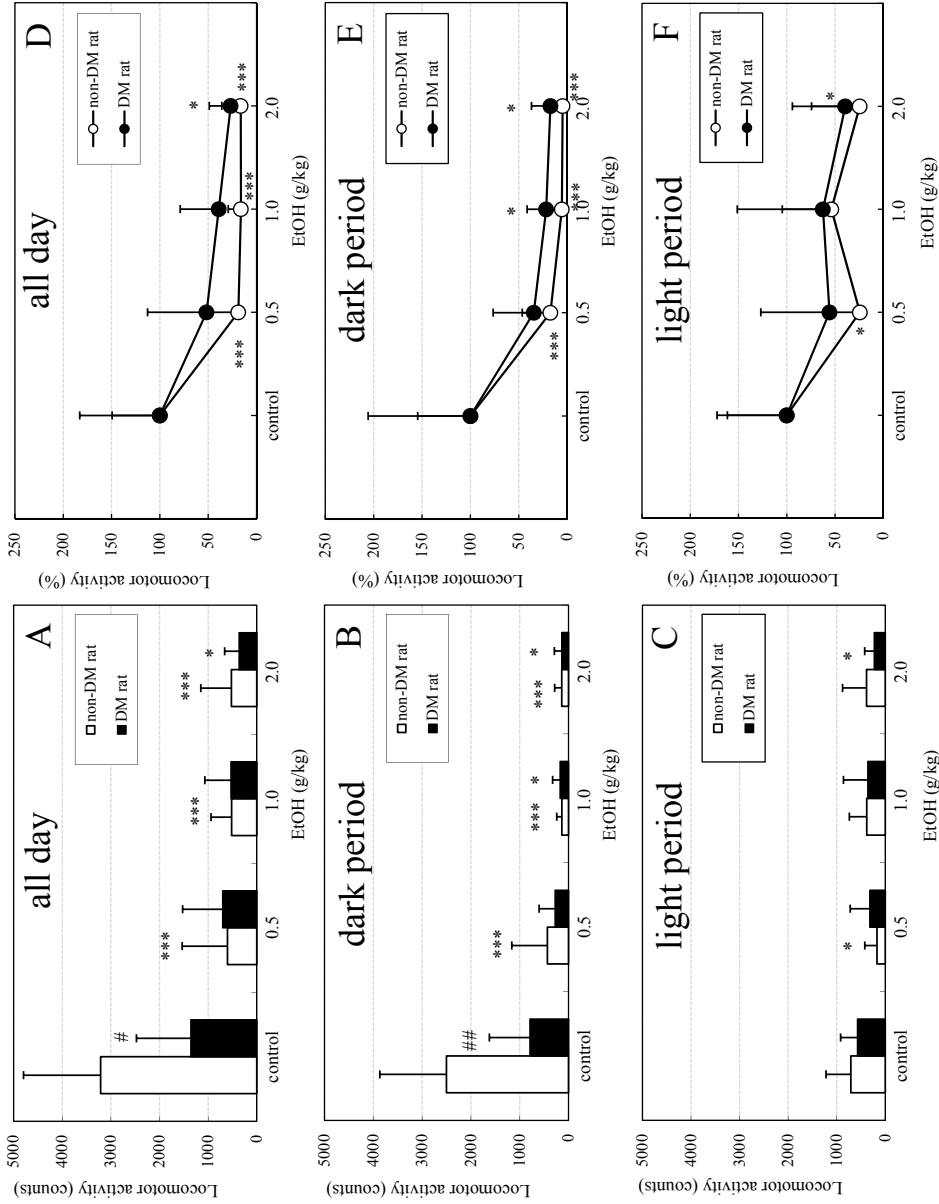


Fig. 3

Comparison of the effects of three doses of EtOH on all day, dark period, and light period locomotor activities of both DM rat and non-DM rats. Data represent mean values of 9 rats in DM and 9 non-DM rats, respectively. Panels A, B and C show the actual data of locomotor activity, but panels D, E and F show the normalized data, taking the data in control condition (saline) of DM and non-DM rats, shown in panels A, B, and C, as 100%. In these panels, closed and open symbols (column, circle) indicate DM rat and non-DM rats, respectively. Data are expressed as means \pm S.D. * $P < 0.05$, ** $P < 0.001$, compared to control. # $P < 0.05$, ## $P < 0.01$, compared to non-DM rat.

sensory afferent nerve fibers. In fact, abnormal muscle spindle innervation and large-fiber neuropathy were observed in STZ-induced diabetic mice (9). These findings suggest that the reduced locomotor activity observed in the present study might be associated with diabetes-induced neuropathy. In contrast, however, STZ-induced diabetic mice showed increased locomotor activity as measured by an ambulator (24, 25). Kamei et al. (24) suggested that this enhanced spontaneous locomotor activity in diabetic mice may result from increased dopamine (DA) neurotransmission, which might be due to an increase in DA release in the mesolimbic DA system. The resultant differences between our experiment and the experiments by Kamei et al. might be due to the differences in animal species, severity of diabetic state, and/or apparatus used to measure locomotor activity. Uncontrolled diabetes mellitus causes abnormalities of the daily temporal variations of several behavioral and physiological processes in both humans (26) and rodents (27-29). In the present study, the locomotor activity in the dark period was significantly reduced by the diabetic state, while the locomotor activity in the light period was almost unchanged. This finding suggests that diabetes may affect, at least in part, the circadian rhythm of locomotion in rats. Studies of the endocrine rhythms in diabetic rodents reveal that daily variations of plasma corticosterone can be either phase advanced (27) or masked by continuous hypercortisolemia (28). The locomotor activity rhythm of DM rats exhibits an advanced phase angle of entrainment (28) as well as a decreased amplitude (28, 29). In mice, abnormalities of daily temporal organization associated with diabetes can result from altered circadian responses to the daily variation in ambient light (30).

Acute administration of EtOH (0.5-2.0 g/kg) obviously decreased the spontaneous locomotor activity in both DM and non-DM rats. The decrease in locomotor activity was greater in the dark period than in the light period, although this may be due to the decreased concentration of blood EtOH in the light period than in the dark period by the metabolism of EtOH. The peak time of the locomotor activity in both rats gradually shifted

from the dark to the light period with increasing EtOH dose (Fig. 2). Acute EtOH selectively attenuates the phase-shifting effects of late-night light pulses in hamsters (31).

A close relationship between neurotransmitters and specific behavioral and other effects of EtOH has been observed in many studies. EtOH modifies neural function and thereby produces intoxication, memory impairment, reinforcement, and dependence. In vivo microdialysis data from our laboratory show that acute administration of EtOH (0.5-2.0 g/kg) significantly increased serotonin release from rat hippocampus dose-dependently (32). Alterations in brain neurotransmitter systems will, as a matter of course, cause alterations in behavioral activity. Pastor and Aragon (33) have reported that injection of EtOH (64-256 μ g) into the hypothalamic arcuate nucleus induces behavioral stimulation in rats.

Differential effects of EtOH on locomotor activity between DM and non-DM rats were observed. Namely, during the dark period, the EtOH-induced decrease in locomotor activity was more prominent in non-DM rats than in DM rats. This may not be due to the difference of the metabolizing process of EtOH in between DM and non-DM rats, because the blood EtOH concentrations in DM rats at 1 and 2 h after EtOH administration were not significantly different from those in non-DM rats. The present study is the first, to our knowledge, to compare the acute effects of EtOH administration on the locomotor activity of DM and non-DM rats. Diabetes is associated with several behavioral changes in animals. We previously reported significant decreased level of serotonin in the hippocampus of diabetic rats (34). Therefore, the findings in the present study might be, at least in part, related to changed level of neurotransmitters in diabetic brain.

CONCLUSION

The present results indicate that type 1 diabetes mellitus significantly reduced total 24-hour locomotor activity as observed on the running wheel. The decrease in locomotor activity was more evident during the dark period than during the light period. Next, acute administration of

EtOH (0.5-2.0 g/kg) significantly decreased the spontaneous locomotor activity in the dark period in both DM and non-DM rats. However, during the dark period, the EtOH-induced decrease in locomotor activity was more prominent in non-DM rats than in DM rats. Recently, attention has been directed towards the detrimental effects of type 1 diabetes on the central nervous system, and the results suggest that altered functions of brain neurotransmitters may be involved.

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