Altered circadian responses to light in streptozotocin-induced diabetic mice

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Challet, Etienne, Olivier Van Reeth, and Fred W. Turek. Altered circadian responses to light in streptozotocin-induced diabetic mice. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E232–E237, 1999.—Diabetes mellitus affects the daily expression of many behavioral and metabolic processes. Recent studies indicate that changes in brain glucose metabolism alter the entraining effects of light of the circadian pacemaker. To test whether diabetes-associated diurnal changes are related to alterations in the responses of the circadian pacemaker to light, photic phase resetting of the circadian rhythm of locomotor activity was analyzed in diabetic mice housed in constant darkness. Multiple low doses of streptozotocin, which damages pancreatic β-insulin-producing cells, were used to render C57BL/6j mice mildly diabetic. In those mice treated with streptozotocin, serum glucose was increased by 25% and circadian responses to light either were increased by 40% for phase delays or were close to those observed in control animals for phase advances. Furthermore, insulin-induced hypoglycemia normalized light-induced phase delays in diabetic animals, without altering those in nondiabetic mice. These results show that abnormalities of daily temporal organization associated with diabetes can result from altered circadian responses to the daily variation in ambient light. Such alterations could be normalized with appropriate insulin therapy.

suprachiasmatic nucleus; circadian rhythm; diabetes; hyperglycemia; insulin; hypoglycemia

UNCONTROLLED DIABETES mellitus is usually associated with abnormalities of the daily temporal variations of several behavioral and physiological processes in both humans (24, 28) and rodents (19, 22, 29). Among endocrine rhythms studied in diabetic rodents, daily variations of plasma corticosterone can be either phase advanced (19) or masked by continuous hypercortisolemia (29). The locomotor activity rhythm in diabetic rats exhibits an advanced phase angle of entrainment (29) as well as a decreased amplitude (22, 29). Alterations of the daily variations of important physiological parameters, such as glucose tolerance (28) or cardiovascular events (24), are also found in diabetic patients. Therefore, a detailed understanding of the impact of diabetes on circadian rhythmicity could lead to new insights for the diagnosis and management of diabetes.

The daily variation in ambient light provides the primary temporal cues for entrainment of the main circadian pacemaker, located in the hypothalamic suprachiasmatic nuclei (14). Changes in daily organization occurring with diabetes may be related to alterations of the pacemaker itself and/or alterations of circadian input or output signals. Because the aforementioned changes have been reported in diabetic individuals exposed to light-dark cycles, it is not possible to unequivocally assess whether the effects described are due to effects on the circadian pacemaker or to complex masking processes that would dampen or blunt circadian outputs. Changes in period or phase of a circadian rhythm studied in constant dim light or darkness are considered to more directly reflect alterations of the circadian pacemaker (1).

Fos expression in response to light pulses is altered in the suprachiasmatic nuclei of diabetic rats (31), indicating that some diabetes-related changes can occur upstream of or at the level of the pacemaker itself. Moreover, circadian responses to light pulses are reduced in several situations of decreased glucose availability (4). This latter finding raises the possibility that the phase-shifting effects of light are increased in hyperglycemic diabetic animals. Therefore, the present study was designed to test possible effects of chronic hyperglycemia and acute insulin treatment on phase-resetting responses to a light pulse in diabetic mice housed in constant darkness.

MATERIALS AND METHODS

Animals and laboratory conditions. Forty-eight 8-wk-old male C57BL/6j mice (Jackson Labs, Bar Harbor, ME) were initially maintained in a temperature-controlled room (23 ± 1°C) with a 12:12-h light-dark cycle. During daytime, light intensity was ~300 lux at the level of the cages. Food (standard laboratory chow, Harlan Teklad) and water were available ad libitum, unless otherwise stated. A fan provided constant fresh airflow and background noise. After at least 2 wk of exposure to this light-dark cycle, animals were randomly divided into two groups injected intraperitoneally with either 40 mg/kg streptozotocin (STZ; Sigma Chemical, St. Louis, MO) or the vehicle, citrate buffer (0.05 M sodium citrate, pH 4.5; Sigma), at the end of the light period for 5 consecutive days. One day later, all mice were transferred to constant darkness for 3 wk. Mice were housed individually in cages equipped with a running wheel (diameter: 11 cm), and each revolution of the wheel activated a microswitch. Continuous wheel-running activity was acquired and analyzed with Chronobiology Kit (Stanford Software Systems, Stanford, CA).

Experimental design. After 10 days in constant darkness, STZ- and saline-injected mice received a subcutaneous injection of saline or insulin (5 IU/kg body mass; Sigma) 30 min before a 10-min light pulse (50 lux of white light; n = 6/group) given at circadian time (CT) 15 (with CT12 defined as the onset of locomotor activity) or CT21. For light stimulation,
individually were transferred from their own cages to a white chamber (diameter 11 cm, height 6 cm) inside a photic stimulation device. The dose and the timing of injections were chosen on the basis of the results of a previous study (4).

To evaluate serum glucose in the present conditions before a light pulse, the experiment was repeated after 3 wk in constant darkness, each animal receiving the same treatment (insulin or saline, around CT14.5 or CT20.5) as previously described. Thirty minutes later, all mice were deeply anesthetized with methoxyflurane (Mallinckrodt Veterinary, Mundelein, IL) under dim red light, and blood was collected by intracardiac puncture.

Glucose assay. After blood centrifugation, serum glucose was measured with an automatic analyzer (model 23A, Yellow Springs Instruments, Yellow Springs, OH) with a coefficient of variation of <2%.

Data analysis. Data for 10 consecutive cycles were divided into 10-min bins of time. The onset of the nocturnal wheel-running activity was defined as the first 10-min bin when 20% of maximal intensity for that cycle was followed by that level of activity in three out of the next six bins. The circadian onsets of the nocturnal locomotor activity were calculated over the last 8 days before the day of treatment (injection and light pulse) and from the 3rd to the 10th day after the treatment. The data for the first 2 days after the treatment were discarded from the analysis due to possible transients.

To assess the light-induced phase shifts of the circadian rhythm of locomotor activity, linear regression analysis of the onsets of locomotor activity was performed with TableCurve (Jandel Scientific, San Rafael, CA). Data were fitted to the following equation

\[ y = [if \ t \leq 8, (A \times t) + B; (C \times t) + D] \]

where \( t \) was the number of days, \( A \) was the slope and \( B \) the initial phase of the line fitted to the 8 days before the day of treatment, and \( C \) was the slope and \( D \) the initial phase of the line fitted to 8 days (i.e., from the 3rd to the 10th day) consecutive to the treatment. The magnitude of the phase shift was calculated on the day of treatment as the difference between these two lines. The circadian period (\( \tau \)) was assessed by the \( x^2 \) periodogram (Chronobiology Kit software) over the 10 days before and after the treatment.

Statistical analysis. Values are means \( \pm \) SE. Data were analyzed by ANOVA followed by the Student-Newman-Keuls post hoc test (Number Cruncher Statistical System, Kaysville, UT).

RESULTS

Circadian responses to light and endogenous period. Representative actograms show that treatment with STZ did not affect markedly free-running rhythm of wheel-running activity (Fig. 1). A three-way ANOVA was used to analyze the effects of the treatment with STZ or saline, the circadian time (CT15 vs. CT21), and the injection (saline or insulin) on light-induced phase shifts. In keeping with the phase-response curve to light in C57BL/6j mice, the magnitude and direction of the phase shifts were significantly modified by the circadian time when light was given [\( F(1,48) = 29.2, P < 0.001 \)]. Treatment with STZ induced a significant increase in the circadian responses to light [\( F(1,48) = 5.8, P < 0.05 \)], with a more marked effect for phase delays. Light-induced phase delays were greater in diabetic mice compared with those in control mice (41 \( \pm \) 10 min vs. 44 \( \pm \) 16 min, \( P > 0.05 \); Fig. 2). In addition, an insulin injection before a light pulse induced a significant decrease in the circadian responses to light [\( F(1,48) = 29.2, P = 0.01 \)]. Light-induced phase delays after insulin treatment were similar in diabetic and control mice (72 \( \pm \) 19 vs.

Fig. 1. Daily wheel-running activity of 4 mice kept in constant darkness and receiving a light pulse (50 lux of white light lasting 10 min) at circadian time 15. Left panels: control mice receiving an sc injection of either saline (A) or insulin (C) 30 min before a light pulse. Right panels: mice previously treated with streptozotocin (STZ) and receiving an sc injection of either saline (B) or insulin (D) 30 min before a light pulse. Arrows, day of treatment; white stars, time of injection.
-61 ± 14 min, P > 0.05; Fig. 2). Light-induced phase advances after insulin treatment were similarly reduced in diabetic and control mice (8 ± 20 vs. 9 ± 5 min, P > 0.05; Fig. 2).

By means of a four-way ANOVA with repeated measures, τ was found not to be significantly changed by the treatment [STZ vs. saline; F(1,32) = 0.2, P > 0.1], the circadian time [CT15 vs. CT21; F(1,32) = 0.1, P > 0.1], the injection [saline or insulin; F(1,32) = 0.1, P > 0.1], or the 10-day period [before vs. after the injection and light pulse; F(1,32) = 4.0, P > 0.05]. τ was close to 23.7 h in all experimental groups of mice (data not shown).

Changes in body mass and serum glucose. To evaluate possible changes in body condition after STZ treatment, body mass was analyzed by a two-way ANOVA with repeated measures between the treatment groups (STZ or saline) and the period (1st day of STZ treatment under light-dark cycle vs. day of light pulse after 10 days in constant darkness). Body mass was significantly modified by the period [F(1,46) = 161.0, P < 0.01] but not by the STZ treatment [F(1,46) = 0.7, P > 0.1]. There was, however, a significant treatment × time interaction [F(1,46) = 7.6, P < 0.01], indicating that the rate of body mass gain during the 2 wk studied was significantly lower in STZ-treated individuals (23.6 ± 0.4 vs. 24.8 ± 0.3 g) compared with control mice (23.7 ± 0.3 vs. 25.6 ± 0.3 g; data not shown). These data show that STZ-treated mice were studied before the onset of significant loss of body mass.

To evaluate glucose availability at the circadian times when the animals received a light pulse, serum glucose was quantified in the experimental conditions before a light pulse (Fig. 3). With the use of a three-way ANOVA, serum glucose was analyzed between the treatment groups (STZ or saline), the circadian time (CT15 or CT21), and the injection (saline or insulin). Serum glucose was significantly increased in STZ-treated animals compared with control animals [241 ± 11 vs. 190 ± 10 mg/dl; F(1,32) = 13.5, P < 0.001]. Moreover, serum glucose was markedly reduced after injections of insulin [F(1,32) = 348.2, P < 0.001]. The interaction between the STZ treatment and insulin injection was significant [F(1,32) = 14.7, P < 0.001], the degree of insulin-induced hypoglycemia being similar (∆-90 mg glucose/dl) in STZ-treated and control animals (Fig. 3). There was no significant effect of the circadian time on serum glucose [F(1,32) = 1.2, P > 0.1]. These results show that multiple low doses of STZ, with subtoxic effects on pancreatic β-cells (e.g., Ref. 30), induced mild insulin-dependent diabetes mellitus in mice, therefore limiting deleterious dysfunctions associated with severe experimental diabetes.

**DISCUSSION**

The findings reported here indicate that 1) STZ-induced chronic hyperglycemia (i.e., serum glucose >200 mg/dl) in mice can potentiate light-induced phase delays but not advances; and 2) acute insulin-induced hypoglycemia attenuates both light-induced phase advances and delays in mice, diabetic or not.

The present study confirms that diabetes affects photic regulation of the circadian light-entrainable pacemaker (31). In contrast with our results, however, these authors found decreased photic responses in the phase-delay region of diabetic rats by using light-induced Fos expression in the suprachiasmatic nuclei (31), which is considered to be correlated with light-induced phase shifting (12). This discrepancy may be related to species-specific differences or to the intensity and duration of light exposure. We also found that STZ-induced diabetes does not modify the value of τ. Because τ in C57BL/6j mice is <24 h, light-induced phase delays are critical for photic synchronization. Therefore, the increased magnitude of light-induced phase delays in diabetic mice exposed to light-dark cycles would be expected to lead to delayed activity onset relative to light offset. Interestingly, such a...
change in the phase angle of photic entrainment is observed in old mice (27), and senescence, even in lean mice, is usually associated with mild diabetes (16).

Phase resetting of the circadian pacemaker to light is dependent on photic cues received by the retina (14). Long-term diabetes mellitus can induce cataract, which is unlikely to have occurred after 4 wk of diabetes. Retinal rhodopsin regeneration, which may affect visual sensitivity, is decreased in diabetic mice after 3–5 wk of streptozotocin treatment (20). Nevertheless, if diabetes-induced pathological changes in the eyes were critical for circadian responses to light, one would expect light-induced phase shifts to be decreased in diabetic mice. This was not the case. It is also unlikely that diabetes-induced hyperglycemia shifts the phase-response curve to light in C57BL/6j mice. Indeed, we found a 40% increase in light-induced phase delays in STZ-treated mice at CT15, that is, when similar light exposure already induces maximal phase delays in control mice (4). Therefore, diabetes may alter the shape of the phase-response curve to light.

Increased serum glucose and hyperinsulinemia are common physiological features after STZ-induced damage of pancreatic β-cells. Because of its anti-lipolytic effects, insulin affects plasma nonesterified fatty acids, and, consequently, their plasma concentration increases during insulin-dependent diabetes (9). Diabetes is also associated with increase in plasma ketone bodies (18). These fuels derived from adipose tissues are known to be mobilized acutely in fasted hyperinsulinemic rodents (5). Whereas light-induced phase delays are reduced in fasted mice (4), we show here increased responses to light in diabetic mice. These data suggest that changes in plasma nonesterified fatty acids and ketone bodies do not play a key role in the modulation of photic phase resetting, which could be attributed to changes in glucose availability per se.

Hyperglycemia can, in part, explain potentiation of the light-induced phase delays in STZ-treated mice despite the finding that hyperglycemia in mice pre-treated with 2-deoxy-D-glucose leads to an opposite effect (i.e., reduced light-induced phase delays). Indeed, this effect of 2-deoxy-D-glucose has been attributed to a decrease in brain glucose availability after blockade of glucose utilization (4). Because neurons do not require insulin for glucose transport, increased concentration of extracellular glucose after STZ treatment would induce increases in both intracellular glucose concentration and rate of metabolic activity. Moreover, the phase of the rhythmic firing rate in a slice of suprachiasmatic nuclei can be altered (i.e., phase advanced) temporarily by increasing the availability of glucose in the bathing solution (8). Thus one possibility to explain the present results is that cell metabolic activity of the suprachiasmatic circadian pacemaker is enhanced in hyperglycemic STZ-treated mice. To some extent, this interpretation is mitigated by the normal light-induced phase advances observed in diabetic mice. Otherwise, such an unaltered circadian response in STZ-treated mice for phase advances only may reflect a phase of sensitivity of the circadian pacemaker to increased cerebral glucose availability.

Although all neurons utilize glucose as an energy source (23), only a few have their firing rate specifically modified by either reduced or increased availability of extracellular glucose (17). These glucose-receptive neurons, mainly located in the ventromedial hypothalamus (17), are also found in the nuclei of the solitary tract (15), whereas most suprachiasmatic cells do not show alterations of firing rate when extracellular glucose is changed (8). In keeping with these findings, perfusion of the forebrain with glucose increases Fos expression in ventromedial and paraventricular hypothalamic nuclei but not in the suprachiasmatic nuclei (7). Chemical damage of glucose-receptive neurons by gold thioglycolate does not affect photic synchronization in mice fed ad libitum but prevents altered circadian responses to light during chronic hypoglycemia associated with caloric restriction (3). Interestingly, gold thioglycollate-induced ventromedial hypothalamic lesions reverse hyperglycemia after STZ treatment (11). Furthermore, sulfonylurea receptors, expressed on glucose-receptive neurons and involved in the glucose-sensing system, are upregulated selectively in the ventromedial hypothalamus of STZ-induced diabetic rats (13). Considering that ventromedial hypothalamic lesions could integrate glucose availability signals during both hypoglycemia and hyperglycemia, it may well be that the ventromedial hypothalamic mediates glucose modulation of light-induced phase shifts of circadian rhythms. Therefore, further studies are needed to test the hypothesis that alteration of circadian responses to light in diabetic animals can be inhibited by ventromedial hypothalamic lesions.

The present findings show that the overall responses to light of the circadian pacemaker were reduced by insulin-induced hypoglycemia. Photically induced phase advances (i.e., after light exposure at CT21) were markedly reduced in insulin-injected mice, diabetic or not. Insulin treatment, however, lowered (normalized) light-induced phase delays in diabetic mice, without marked changes in control mice exposed to light at CT15. A similar injection of insulin before a light pulse given at CT18 (i.e., in the late phase delay region in C57BL/6j) reduces phase delays (4). Thus there seems to be a phase dependence in the effects of insulin on photic phase resetting, circadian responses to light being essentially unaffected by insulin around CT15 and reduced from CT18 to CT21.

During insulin-induced decrease in serum glucose, the brains in normoglycemic mice also experience decreased glucose availability (25). As mentioned previously, increased circadian responses to light in diabetic mice are correlated with an increase in cerebral glucose availability. Also, reduced responses to light might be explained by a decrease in cerebral glucose availability (4). It appears, however, that changes in light-induced phase shifts do not always match with changes in serum glucose. In particular, light-induced phase delays at CT15 were not significantly reduced after insulin treatment despite hypoglycemia, and light-
induced phase advances at CT21 were not significantly greater in diabetic mice despite hyperglycemia. On the one hand, the fact that cerebral glucose availability is altered in parallel with changes in serum glucose does not imply that extracellular glucose in the brain is a single pool in strict equilibrium with plasma glucose. In this context, microdialysis should be used to determine local concentrations of brain glucose in the present conditions. On the other hand, the observed glucose-related effects on photic phase resetting may result from complex multifactorial mechanisms.

With respect to the effects of insulin, for instance, it is possible that insulin-induced hypoglycemia selectively influences the ventromedial hypothalamus that would, in turn, modify the function of the suprachiasmatic nuclei. Concurrently, because insulin can enter the brain across the blood-brain barrier and insulin receptors are present in the suprachiasmatic nuclei (26), insulin may act on the circadian pacemaker to modulate the processing of photic cues at specific circadian times only. Moreover, insulin per se can modulate the firing rate of ventromedial hypothalamic cells (17). Insulin applied in vitro during the subjective day inhibits firing rate of the suprachiasmatic cells (21), and a 4-day hyperinsulinenic-euglycemic clamp in freely moving rats induces a decrease in glucose utilization in the suprachiasmatic nuclei (6). Little, however, is known about possible phase-shifting effects of the locomotor activity rhythm by insulin alone in constant darkness. Further studies are needed to determine whether insulin is involved in the metabolic modulation to light only indirectly (i.e., via its hypoglycemic effect) or whether the effects of insulin also are mediated by direct modulation of the function of suprachiasmatic and/or ventromedial hypothalamic cells.

Perspectives. Previous studies have shown that the circadian pacemaker may regulate daily rhythms of basal serum glucose and insulin secretion in both humans (28) and rodents (2, 10). The data obtained here during chronic hyperglycemia and in other studies involving reduced glucose availability suggest that changes in brain glucose metabolism could reciprocally influence the circadian light-entrainable pacemaker, leading to a modulation of light-induced phase shifting.

The present study indicates that alterations of daily temporal organization associated with poorly controlled diabetes can, to some extent, result from increased circadian responses to light. These abnormalities in insulin-dependent diabetes are expected to be counteracted by appropriate replacement insulin therapy. Situations in which diabetic patients are exposed to chronobiological challenges (such as scheduled-work rotations, night work, or jet lag) may require specific adjustments. Moreover, non-insulin-dependent diabetes and/or obesity and also aging are frequently associated with abnormalities in daily organization and glucose regulation. These alterations deserve further chronobiological investigations that could lead to significant improvements for restoring normal daily variations and internal synchronization across the 24-h cycle.

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