Reduced glucose availability attenuates circadian responses to light in mice

ETIENNE CHALLET, SUSAN LOSEE-OLSON, AND FRED W. TUREK
Center for Circadian Biology and Medicine, Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois 60208

Challet, Etienne, Susan Losee-Olson, and Fred W. Turek. Reduced glucose availability attenuates circadian responses to light in mice. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1063–R1070, 1999.—To test whether circadian responses to light are modulated by decreased glucose availability, we analyzed photic phase resetting of the circadian rhythm of locomotor activity in mice exposed to four metabolic challenges: 1) blockade of glucose utilization induced by 2-deoxy-D-glucose (2-DG), 2) fasting (food was removed for 30 h), 3) insulin administration, and 4) insulin treatment after fasting. In mice housed in constant darkness, light pulses applied during early subjective night induced phase delays of the rhythm of locomotor activity, whereas light pulses applied during late subjective night caused phase advances. There was an overall reduction of light-induced phase shifts, with a more pronounced effect for delays, in mice pretreated with 500 mg/kg ip 2-DG compared with mice injected with saline. Administration of glucose with 2-DG prevented the reduction of light-induced phase delays. Furthermore, phase delays were reduced in fed mice pretreated with 5 IU/kg sc insulin and in fasted mice injected with saline or insulin compared with control fed mice. These results show that circadian responses to light are reduced when brain glucose availability is decreased, suggesting a metabolic modulation of light-induced phase shifts.

suprachiasmatic nucleus; circadian rhythm; 2-deoxy-D-glucose; glucose utilization; insulin; fasting; hypoglycemia

ENDOGENOUS CIRCADIAN rhythmicity is principally synchronized by the daily variation in ambient light. Recent findings suggest that the daily synchronization to light can be altered by metabolic challenges (6). Therefore, the present study was designed to test whether the circadian responses to light are modified when glucose availability is decreased.

In mammals, the suprachiasmatic nuclei (SCN) contain the light-entrainable pacemaker, which is sensitive to photic cues during the subjective night (14). In addition to the light-dark cycle, a number of behavioral and pharmacological stimuli are also capable of resetting the phase of the circadian pacemaker under constant lighting conditions. These different nonphotic stimuli share the ability to shift the phase of the SCN during the subjective day when light has no direct resetting effect on the circadian pacemaker. Because nonphotic stimuli are usually associated with acute increases in locomotor activity and arousal, changes in the activity-rest state or some correlates thereof are thought to play a crucial role in nonphotic phase shifting (17, 30, 33).

Periodic feeding, without undernutrition, affects the circadian system differently than other nonphotic stimuli. In animals maintained under constant lighting conditions, schedules of restricted feeding (i.e., food supplied for a limited daily period) usually synchronize a bout of locomotor activity anticipating the time of feeding, whereas the other circadian components of locomotor activity continue to free run (see Ref. 16 for review). The food-anticipatory activity is considered to be driven by a food-entrainable pacemaker outside the SCN (16). The circadian phase, assessed by outputs of the SCN other than locomotor activity (e.g., the daily rhythm of vasopressin release from the SCN and that of neuronal firing in the SCN), is unchanged by restricted feeding schedules in rats kept under light-dark cycles (13, 16). Taken together, these data suggest that the phase of the SCN pacemaker is not markedly modulated by the time of feeding.

Periodic feeding coupled with caloric restriction, however, can phase shift circadian rhythms and modify the phase angle of photic entrainment (5, 6). The mechanisms involved in these altered circadian responses to light are not well understood, but appear to be independent of locomotor activity feedback and may be related to metabolic cues (6) such as decreased serum glucose (10). Therefore, the present study investigated the phase-resetting properties of light in several experimental situations involving decreased glucose availability.

Blockade of glucose utilization (“glucoprivation”) can be achieved with 2-deoxy-D-glucose (2-DG), a competitive inhibitor of glucose transport and phosphorylation that interferes with glycolysis at the phosphohexoisomerase step. I injected systemically, 2-DG has been shown to act mainly on the brain (27). As a result of cerebral glucoprivation, compensatory physiological mechanisms lead to marked hyperglycemia without causing an increase in plasma insulin (18, 19). In the first part of the present study, we tested the hypothesis that injections of 2-DG before a light pulse can affect the phase-shifting effects of light during the subjective night. Because an effect of 2-DG on the circadian system might be secondary to the hyperglycemia induced by decreased central glucose utilization, we investigated whether the responses of the pacemaker to light could be altered by acute hyperglycemia. In the second part of this study, we tested the hypothesis that the phase-shifting effects of light can be altered in two other experimental situations associated with reduced glucose availability: fasting- and insulin-induced hypoglycemia.
MATERIALS AND METHODS

Animals and laboratory conditions. Eight- to ten-week-old male C57BL/6j mice (The Jackson Laboratory, Bar Harbor, ME) were initially maintained in a temperature-controlled room with a 12:12-h light-dark cycle. During daytime, light intensity was ~300 lx 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 air flow and background noise. After at least 2 wk of exposure to this light-dark cycle, animals were then transferred to constant darkness. Animal maintenance was performed in constant darkness with the aid of an infrared viewer (Find-R-Scope; FJ Optical System, Palatine, IL). Mice were housed individually in cages equipped with a running wheel (11-cm diameter). Each revolution of the wheel activated a microswitch. Continuous wheel running activity was acquired and analyzed using the Chronobiology Kit (Stanford Software Systems, Stanford, CA).

Experimental design. Experiment 1 was designed to test whether blockade of glucose utilization affects the phase-resetting response to a light pulse. In 60 mice, we analyzed the effects of intraperitoneal injections of either 500 mg/kg body mass of 2-DG (Sigma Chemical, St. Louis, MO) or saline at circadian time (CT) 12 (defined as the onset of locomotor activity), CT15, CT18, CT21, or CT24, 60 min before a subsaturating 10-min light pulse (50 lx of white light; n = 6 mice per group). For light stimulation, individual mice were transferred from their own cages to a white chamber (11-cm diameter, 6-cm height) inside a photic stimulation device. We determined light intensity using a digital photometer. By convention, one circadian hour lasts 24 h. The dose and time of 2-DG injections were chosen based on the time course of hyperglycemic and feeding responses to 2-DG (3, 28), such that maximum glucoprivation would occur at about the time of the light pulse. Thereafter, we investigated whether the effect of 2-DG could be mimicked by acute hyperglycemia or antagonized by a concomitant injection of glucose. Twelve mice were injected intraperitoneally with either 2 g/kg of d-glucose (see Ref. 3 for explanation of dosage; Sigma) or an equimolar amount (0.4 M) of 2-DG and glucose (500 and 550 mg/kg, respectively) 30 min before a 10-min light pulse started at CT18 (n = 6 mice per treatment). The timing of glucose injections was selected based on previous observations (26).

We quantified serum glucose during the middle of the night in a second group of mice kept under a 12:12-h light-dark cycle. Animals received an injection of either 2-DG or saline intraperitoneally at zeitgeber time (ZT) 17 (5 h after lights off, defined as ZT12), or they received an injection of glucose alone or 2-DG plus glucose intraperitoneally at ZT17.5 (n = 12 mice per treatment). The doses of drugs were the same as those described above. Thereafter, all mice were deeply anesthetized with methoxyflurane (Mallinkrodt Veterinary, Mundelein, IL) and blood was collected by intracardiac puncture. For a given treatment (n = 12 mice), half the mice were anesthetized at ZT18 under dim red light and the other half were anesthetized after a 10-min light pulse given at ZT18.

Experiment 2 was designed to test whether insulin- and/or fasting-induced hypoglycemia affect the phase-resetting properties of light. The day on which a light pulse was administered is denoted as day 0. Twenty-eight mice were divided into four groups based on nutritional state, with or without insulin treatment. Half of the mice were fed ad libitum whereas the other half were fasted by food removal for 30 h (from CT12 on day –1 to CT18 on day 0), a duration of food deprivation that has been shown to induce sustained hypoglycemia in mice (21). For each nutritional state, mice received an injection of either insulin (5 IU/kg; Sigma) or saline subcutaneously. Injections were administered 30 min before a 10-min light pulse given at CT18 (n = 7 mice per treatment). The dose and time of insulin injections were selected based on the results of a previous study (32), so that hypoglycemia occurred at the time of the light pulse.

For quantification of serum glucose during the middle of the night, we kept a second group of mice under a 12:12-h light-dark cycle. Mice were either fed or fasted and given an injection of either insulin or saline subcutaneously, as described above. For a given treatment (n = 12 mice), mice were anesthetized either at ZT18 under dim red light or after a 10-min light pulse given at ZT18, and blood was sampled as described in experiment 1.

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

Data analysis. To quantify the light pulse-induced phase shifts, a line was fitted by eye to the onsets of locomotor activity for the first 10 days after the light pulse. This line was projected to the day of the light pulse. Similarly, a line was fitted to the onsets of activity for the 10 days after the photic pulse. That line was retroprojected to the day of the pulse. The magnitude of the phase shifts was calculated as the difference between these two lines. Daily activity was defined as the total wheel revolutions per cycle beginning at CT12. The circadian period (T) was assessed by the χ² periodogram (Chronobiology Kit software) over the 10 days before each treatment to determine the appropriate time of injection.

Statistical analysis. Values are means ± SE. Unless otherwise stated, data were analyzed by ANOVA followed by a Scheffé’s multiple-comparison test (Number Cruncher Statistical System, Kaysville, UT).

RESULTS

Experiment 1. Representative actograms for the effects of 2-DG injected before a light pulse are shown in Fig. 1. A two-way ANOVA was used to compare the effects of CT (CT12, CT15, CT18, CT21, and CT24) and the treatment (2-DG or saline). In accordance with the mouse phase-response curve to light, the amplitude and direction of the phase shifts differed according to the time of the night when the light was applied (F[4,50] = 40.1, P < 0.001; Fig. 2). A 2-DG injection administered 1 h before a light pulse induced a significant decrease in the circadian responses to light (F[1,50] = 8.7, P < 0.01; Figs. 1 and 2). The magnitude of this effect varied according to the time of the night (F[4,50] = 3.7, P = 0.01), with the reduction of the light-induced phase shifts being more marked during the phase delay region (Fig. 2). Light-induced phase delays were not significantly different after injections of glucose or a mixture of glucose and 2-DG before a light pulse during the mid-subjective night (t[10] = –1.1, P > 0.1; Fig. 3).

By means of a two-way ANOVA with repeated measures, we analyzed the total number of wheel revolutions performed by treatment (2-DG, saline, glucose, or a mixture of glucose and 2-DG) and day (day 0 vs. day –1). Only the main effect of day was significant.
indicating that the daily activity was decreased the day of the light pulse, regardless of the chemical injected (Table 1).

Serum glucose was quantified in the middle of the night, with or without the application of a light pulse, in mice previously treated with 2-DG, saline, glucose, or a mixture of glucose and 2-DG (Table 2). As expected, serum glucose, regardless of the lighting conditions, was modified significantly by the treatment \(F(3,40) = 5.3, P < 0.05\); Table 2.

**Experiment 2.** We used a two-way ANOVA to compare the effects of the nutritional state (fed or fasted) and the treatment with insulin or saline on phase shifts. The phase delays induced by a light pulse at CT18 were modified significantly by the nutritional state \(F(1,24) = 15.5, P < 0.001\), being reduced in fasted mice (Figs. 4 and 5). In addition, an injection of insulin 30 min before a light pulse induced a significant decrease in the light-induced phase delays \(F(1,24) = 23.7, P < 0.001;\) Figs. 4 and 5). In fasted mice that received an injection of insulin, the phase-delaying effect of light at CT18 was completely blocked (Fig. 5).

Using a two-way ANOVA with repeated measures for each nutritional state (fed or fasted), we analyzed the total number of wheel revolutions between the treatment groups (insulin or saline) over 3 days (day 0, day –1 with or without fasting, and day –2, when all the mice were fed ad libitum). In mice fed ad libitum over the course of the experiment, only the main effect of day was significant: 6.9, \(P < 0.001\), being higher after injections of 2-DG and glucose plus 2-DG than after saline injections (221 ± 9 and 242 ± 8 vs. 194 ± 6 mg/dl, respectively; \(P < 0.05\)). Serum glucose, regardless of the lighting conditions, was similar after injections of 2-DG or glucose (221 ± 9 vs. 209 ± 6 mg/dL, respectively; \(P > 0.05\)). Moreover, serum glucose was found to be significantly higher after a light pulse \(F(1,40) = 5.3, P < 0.05;\) Table 2.

**Table 1.** Total wheel revolutions performed per cycle beginning at circadian time 12 (experiment 1)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day –1</th>
<th>Day 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>24,650 ± 1,820</td>
<td>22,440 ± 2,120</td>
</tr>
<tr>
<td>2-DG</td>
<td>26,420 ± 1,810</td>
<td>20,210 ± 1,360</td>
</tr>
<tr>
<td>Glucose</td>
<td>26,050 ± 3,720</td>
<td>20,660 ± 2,220</td>
</tr>
<tr>
<td>2-DG + glucose</td>
<td>30,720 ± 6,670</td>
<td>22,630 ± 4,410</td>
</tr>
</tbody>
</table>

Values are means ± SE. 2-DG, 2-deoxy-D-glucose; day –1, 1 cycle before light pulse; day 0, day of light pulse. There was a significant decrease of wheel running the day of light pulse \((P < 0.01)\).
was significant \( F(1,24) = 9.6, P < 0.001 \). As demonstrated earlier, there was a significant decrease of daily activity the day of the light pulse, with or without any further treatment. In fasted mice, we found no significant effect of treatment or day \( F(1,40) = 0.5 \) and \( F(2,24) = 1.8 \), respectively, \( P > 0.05 \); Table 3).

Serum glucose was quantified in the middle of the night in these four groups of mice (Table 4). We used a three-way ANOVA to compare the effects between the nutritional state, the injection, and the light pulse. Serum glucose was modified significantly by the nutritional state \( F(1,40) = 31.1, P < 0.001 \), being larger in fed than in fasted mice. Serum glucose was also modified significantly by the injection \( F(1,40) = 321.2, P < 0.001 \), being reduced after injections of insulin. Contrary to results in experiment 1, serum glucose was not significantly affected by a light pulse \( F(1,40) = 2.1, P > 0.05 \). None of the possible interactions were significant.

**DISCUSSION**

The present findings clearly demonstrate that the photic regulation of circadian rhythmicity can be affected by metabolic challenges. The photic phase resetting of the circadian clock is attenuated when availability of glucose is decreased by blockade of glucose utilization, by fasting, and/or after insulin administration. Previous studies indicate that the SCN may regulate some aspects of the autonomic nervous system (19, 22), in particular those responsible for glucose homeostasis (19) and lipid mobilization (1). Consistent with previous hypotheses (5, 6, 8), the present results indicate that changes in glucose metabolism could reciprocally modulate the SCN function through a metabolic feedback mechanism. This view supports the suggestion that physiological status can affect circadian rhythmicity (30).

The overall responses of the circadian clock to light in mice were reduced by the blockade of glucose utilization during the subjective night, with a more marked effect during the phase delay region. Because 2-DG leads to blockade of glucose utilization and to hyperglycemia, we investigated the effects of injections of glucose and a mixture of 2-DG and glucose. The ability of 2-DG to reduce light-induced phase delays at CT18 was not mimicked by injections of glucose, but was prevented by the injection of equimolar doses of 2-DG and glucose. These data suggest that the effects of 2-DG on the photic regulation of circadian rhythms are due to the blockade of glucose utilization per se. Conditions of glucoprivation after 2-DG treatment attenuate the phase-shifting effects of light. The prediction that glucoprivation shifts the phase-response curve to light in mice would be supported either by greater light-induced phase delays at the beginning of the subjective night (around CT12) in mice treated with 2-DG compared with controls, suggesting an advance in the phase-response curve to light, or by greater light-induced phase advances at the end of the subjective night (around CT24) in mice treated with 2-DG compared with controls, suggesting a delay in the phase-response curve to light. None of these effects was observed.

---

**Table 2. Serum glucose in the middle of the night (experiment 1)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>In darkness</th>
<th>After light pulse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>182 ± 6</td>
<td>207 ± 6</td>
</tr>
<tr>
<td>2-DG</td>
<td>213 ± 10</td>
<td>230 ± 15</td>
</tr>
<tr>
<td>Glucose</td>
<td>197 ± 8</td>
<td>221 ± 14</td>
</tr>
<tr>
<td>2-DG + glucose</td>
<td>240 ± 11</td>
<td>245 ± 11</td>
</tr>
</tbody>
</table>

Values are means ± SE in mg/dl. Effects of both treatment and light conditions were significant \( P<0.001 \) and \( P<0.05 \), respectively. Treatment \( \times \) light interaction was not significant \( P = 0.8 \).
Letters in common are significantly different from one another (P < 0.05).

Treatments that decrease glucose availability, such as experiment 1, we hypothesized that if glucopenia after the reducing phase delays sufficient for photic entrainment. Given that the reducing effect of photic phase resetting was more marked during the phase delay than the phase advance region, it is possible that glucoprivation not only flattens, but also alters the shape of the phase-response curve to light. In a previous study, we hypothesized that the temporal window for light-induced phase delays may have been reduced and/or that the window for phase advances may have been extended during calorie restriction because phase advances of the circadian rhythm of locomotor activity were found in calorie-restricted mice kept under light-dark cycles (6). The circadian period (τ) in C57 mice is <24 h, so that light-induced phase delays are critical for photic entrainment. Given the reduced responses to light after 2-DG administration in the present study, the phase advances seen in calorie-restricted mice might allow more light to fall in their delay region, thus inducing phase delays sufficient for photic entrainment.

Because 2-DG attenuated photic phase shifts in experiment 1, we hypothesized that if glucopenia after 2-DG treatment is the critical parameter involved in the reduced circadian responses to light, then other treatments that decrease glucose availability, such as hypoglycemia, should also attenuate the light-induced phase shifts of the circadian clock. Indeed, the responses of the circadian clock to light were attenuated during hypoglycemia induced by fasting, acute insulin treatment, or the combination of both treatments (experiment 2). Despite the similarity of the circadian responses to light during fasting and insulin-induced hypoglycemia, it cannot be determined whether similar mechanisms are involved in both situations. The more marked effects after combination of the two treatments could indicate additive effects. On the other hand, the transient severe hypoglycemia induced by this combination may have transiently impaired the firing rate of neurons within the SCN, as observed in vitro after a drastic reduction of the concentration of glucose in solutions bathing SCN slices (8).

Changes in the activity-rest state have been implicated as modulators of the circadian clock. Therefore, it is conceivable that the present findings reflect an alteration in the level or temporal pattern of locomotor activity. An acute increase in locomotor activity during the resting phase can induce phase advances in the hamster circadian clock (17, 33). In fasted mice, the level of total activity was not significantly modified over the days analyzed (experiment 2). Fasted mice, however, exhibited an apparent increase of wheel running activity during the 2 h before the usual onset of nocturnal activity (~8 h before the pulse of light). The phase-advancing effects of behavioral activation during the resting phase can be markedly enhanced by a subsequent pulse of light started several hours after the exercise (17). In contrast, the light-induced phase shifts were reduced in fasted mice, with or without insulin injection, relative to fed controls, indicating that the apparent increased activity toward the end of the subjective day in fasted mice is not likely to be the main cause of the attenuation of light-induced phase shifts.

As demonstrated by local cerebral glucose utilization using [14C]DG, 2-DG injected peripherally can enter the brain where it blocks intracellular glycolysis (7). Insulin can also cross the blood-brain barrier via a specialized transport system (23). Brain glucose levels in mice and rats fall during hypoglycemia induced either by fasting (15, 35) or insulin administration (26, 29, 32). Therefore, an effect of reduced cerebral glucose availability provides the most parsimonious explanation for the

### Table 3. Total wheel revolutions performed per cycle beginning at circadian time 12 (experiment 2)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day −2</th>
<th>Day −1</th>
<th>Day 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed, saline</td>
<td>23,950 ± 1,840</td>
<td>27,020 ± 1,490</td>
<td>20,050 ± 2,040</td>
</tr>
<tr>
<td>Fed, insulin</td>
<td>23,530 ± 4,640</td>
<td>24,120 ± 5,040</td>
<td>19,630 ± 4,490</td>
</tr>
<tr>
<td>Fasted, saline</td>
<td>24,890 ± 3,880</td>
<td>25,920 ± 3,950</td>
<td>24,780 ± 3,210</td>
</tr>
<tr>
<td>Fasted, insulin</td>
<td>27,940 ± 5,470</td>
<td>36,650 ± 7,460</td>
<td>24,370 ± 5,150</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 mice per group. Groups with no letters in common are significantly different from one another (P < 0.05).

### Table 4. Serum glucose in the middle of the night (experiment 2)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lighting Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In darkness</td>
</tr>
<tr>
<td>Fed, saline</td>
<td>193 ± 13</td>
</tr>
<tr>
<td>Fed, insulin</td>
<td>61 ± 11</td>
</tr>
<tr>
<td>Fasted, saline</td>
<td>137 ± 15</td>
</tr>
<tr>
<td>Fasted, insulin</td>
<td>29 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SE in mg/dl. Effect of both treatment and nutritional state was significant (P < 0.001). Effect of light conditions was not significant (P = 0.1), nor were any of the possible interactions (P > 0.08).
present results given that different metabolic challenges produce similar effects on the circadian responses to light. It is unlikely that conditions of glucoprivation or hypoglycemia, induced either by fasting or insulin, lead to a general, nonspecific reduction in brain functioning. As revealed by quantitative autoradiography in normal rats in the conscious state (as were the mice we injected with 2-DG), the impact of cerebral glucoprivation varies widely according to the local rates of glucose consumption (7). Moreover, a 4-day hyperinsulinemic euglycemic clamp did not alter the cerebral glucose utilization as a whole in freely moving rats, but altered local glucose utilization in discrete, selective brain regions (7). Therefore, in the present study, one can consider that decreased cerebral glucose availability may directly affect the SCN pacemaker or other brain structures that may have an impact on SCN activity.

The phase of the firing rate peak in an SCN slice preparation can be modified temporarily by decreasing the bathing concentration of glucose, although without permanent resetting of the clock (8). In autoradiography using [14C]DG, a typical feature of the SCN oscillation is an increase in cell metabolic activity during the subjective day and a decrease during the subjective night in vivo, as in vitro (14). If 2-DG has direct effects on SCN function, an injection of 2-DG impairing cellular glucose oxidation would more markedly affect the circadian clock during the subjective day, when cells of the SCN are the most active. We found, however, that injections of 2-DG, without subsequent light pulse, during the mid-subjective night (CT18), but not during the mid-subjective day (CT6), induced phase shifts (advances or delays) with bigger magnitudes than those induced after saline injections (Challet and Turek, unpublished data). This observation does not readily support the hypothesis regarding the direct effects of glucoprivation on the circadian clock.

Insulin receptors are abundant in the SCN (31) and insulin applied during the subjective day inhibits the firing rate of SCN cells in vitro (25). Moreover, insulin injected into the SCN in vivo modifies the sympathetic firing rate (22). These findings raise the possibility that a direct action of insulin on the SCN cells may account for the reduced phase delays observed in mice treated with insulin. This possibility seems unlikely, however, because exogenous glucose, which typically induces an increase in insulin secretion, did not affect the light-induced phase delays in the present study. Moreover, the photic phase shifts were also attenuated after 2-DG treatment and fasting, two situations associated with normo- (18) and hypoinsulinemia (21), respectively. These results suggest that insulin does not play a necessary role in the altered circadian responses to light.

Considering the indirect effects on SCN function, reduced brain glucose has been shown to increase the release of serotonin from the raphe nuclei and of norepinephrine from the locus ceruleus (32). These structures provide inputs to the SCN (12, 14). Because neurotoxic destruction of serotonergic terminals in the SCN region increases light-induced phase delays in mice (4), the reduced light-induced phase delays of hypoglycemic mice might be partly related to an increased serotonergic activity in the vicinity of the SCN. Also, application of norepinephrine can inhibit the firing rate of SCN cells (24). To our knowledge, the effects of noradrenergic activation on the light-induced phase delays have not yet been reported in mice. Although neurotoxic destruction of catecholaminergic terminals in the region of the SCN might be expected to potentiate the light-induced phase shifts, we found no significant effect after such lesions in golden hamsters fed ad libitum (Challet and Turek, unpublished data). Therefore, increased inhibitory noradrenergic inputs might not be critical in the reduction of the circadian responses to light in hypoglycemic mice.

Ibotenic lesions of the ventromedial hypothalamus prevent the phase advances in calorie-restricted rats (5). Reduced availability of glucose activates cerebral sensors of glucopenia, mainly located in the ventromedial hypothalamus (3, 20). In addition to, or independently of, possible brain stem involvement, activation of these hypothalamic neurons may feed back to the SCN clock and alter the circadian responses to light. Indeed, preliminary results indicate that the ventromedial hypothalamus could participate in the metabolic modulation of the photic phase resetting of the circadian rhythm of locomotor activity (E. Challet and D. J. Bernard, unpublished data). The paraventricular nuclei of the hypothalamus may also be involved in the metabolic modulation of photic phase resetting because they participate in the regulation of food intake and energy metabolism (34). As discussed, the primary metabolic consequence shared by glucoprivation and hypoglycemia after fasting or insulin administration is a reduced glucose availability, which is one likely candidate in explaining the similar effects of different metabolic challenges on the circadian responses to light. During fasting, hypoglycemia is associated with acute lipid mobilization leading to an increase in plasma free fatty acids and ketone bodies (15). Free fatty acids or ketone bodies may affect the firing rate of hypothalamic neurons (20). During fasting, therefore, the metabolic modulation of photic phase shifting may be mediated, in part, by these fuels derived from lipid stores.

Although central sites involved in the integration of metabolic and/or photic inputs to the circadian system have yet to be clearly defined, the data suggest that reduced food availability in the field can have an impact on the photic synchronization of mammals. This may partly account for the changes in the daily timing of activity in rodents during winter periods of food shortage (9). Seasonal changes of body lipids in hamsters are under photoperiodic control via the daily rhythm of melatonin produced by the pineal gland (2). In mammals, the light-entrained clock located in the SCN regulates the melatonin rhythm by suppressing melatonin during the day (14). Alteration of the circadian responses to light during reduced metabolic fuel availability may fine tune the photoperiodic responses.
In summary, the present results indicate that reduced glucose availability is associated with attenuation of the light-induced phase resetting of the mouse circadian clock. These data therefore suggest that the photic regulation of circadian rhythmicity in mammals can be modulated by metabolic status.

Perspectives

This study suggests a functional link between changes in glucose homeostasis and circadian rhythmicity. The present results were achieved using animals with reduced glucose availability. Although the underlying mechanisms are not yet fully understood, their potential application to humans may be relevant in the case of voluntary reduced energy intake, such as during the Ramadan month, a Muslim religious tradition, or in the case of diseases leading to reduced glucose availability, including anorexia nervosa or cancer cachexia. (For a summary of the human circadian organization during controlled, normocaloric Ramadan, see Ref. 11.) Other studies in animals are also needed to investigate whether the circadian responses to light are altered during chronic hyperglycemia.

We thank Drs. Sylvie Massenim and J-aquin Redio for helpful discussions and comments on the experimental design. We are grateful to Dr. Terry Horton for helpful assistance. We also thank Drs. Dan Bernard, Kathryn Scarbrough, and Eve Van Cauter for providing constructive comments on the manuscript. This work was supported by National Institutes of Health Grants P01-AG-11412, R01-AG-09297, and HD-09885.

Received 18 June 1998; accepted in final form 10 December 1998.

REFERENCES


