A Daily Rhythm in Glucose Tolerance

A Role for the Suprachiasmatic Nucleus

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The suprachiasmatic nucleus (SCN), the biological clock, is responsible for a 24-h rhythm in plasma glucose concentrations, with the highest concentrations toward the beginning of the activity period. To investigate whether the SCN is also responsible for daily fluctuations in glucose uptake and to examine how these fluctuations relate to the rhythm in plasma glucose concentrations, SCN-intact rats and SCN-lesioned rats were injected intravenously with a glucose bolus at different time points. We found an increase in glucose uptake toward the beginning of the activity period, followed by a gradual reduction in glucose uptake toward the end of the activity period. The daily variation in glucose tolerance seemed not to be caused by fluctuations in insulin responses of the pancreas but by a daily variation in insulin sensitivity. Lesioning the SCN resulted in the disappearance of the daily fluctuation in glucose uptake and insulin sensitivity. Interestingly, SCN-lesioned rats showed an enhancement in glucose tolerance that could not be explained by higher insulin responses or enhanced insulin sensitivity. Therefore, these findings suggest a role for the SCN in insulin-independent glucose uptake. The present results further show that the daily rhythm in glucose tolerance follows the same pattern as the daily rhythm in plasma glucose concentrations. We hypothesized that the biological clock prepares the individual for the upcoming activity period by two separate mechanisms: increasing plasma glucose concentrations and making tissue more tolerant to glucose. *Diabetes* 50:1237–1243, 2001

Maintaining glucose homeostasis is essential for daily functioning. Disturbances in glucose regulation and insulin action may have severe consequences and may even lead to disease, including type 2 diabetes and obesity. Plasma glucose concentrations fluctuate daily between strict boundaries. Recently, we showed that the suprachiasmatic nucleus (SCN) of the hypothalamus, the biological clock, is responsible for this 24-h rhythm in plasma glucose concentrations in the rat. Plasma glucose concentrations increase toward the end of the light period, just before the onset of activity (1). This phenomenon has also been described in humans as the dawn phenomenon, i.e., higher glucose output and insulin requirements during the hours just before the onset of activity (2,3). Apart from regulating plasma glucose concentrations, our data (1) suggested a role for the SCN in glucose tolerance or insulin sensitivity. Compared with SCN-intact rats, we observed a decrease in meal-induced insulin release in SCN-lesioned rats, whereas glucose concentrations before and after a meal did not differ significantly in these two groups of rats (1).

In humans, glucose tolerance seems to vary during the day; more glucose is taken up early in the morning than in the afternoon and evening (4–6). Therefore, when plasma glucose concentrations are high before the onset of activity, humans seem to be more tolerant to glucose. The mechanism for this phenomenon is not well understood; therefore, we investigated, in rats, the hypothesis that the SCN is responsible for daily changes in glucose tolerance.

To do this, a glucose bolus was injected intravenously in both SCN-intact and SCN-lesioned rats at different time points during the light/dark cycle. To delineate whether the observed daily variation in glucose uptake is due to daily variation in insulin sensitivity, we also injected, at the same time points, an insulin bolus in the circulation in both SCN-intact and SCN-lesioned rats.

RESEARCH DESIGN AND METHODS

Animals. Male Wistar rats (Harlan) were kept at a room temperature of 20°C, 4–6 animals per cage, with a 12-h light/dark cycle (lights on at 0700 or 2300, depending on the experiment). Animals were allowed to adapt to the lighting schedule for several weeks before the procedure. For the experiments, animals were transferred to individual cages (25 × 25 × 35 cm). Before and between experiments, food and water were available ad libitum. All experiments were conducted under the approval of the Local Animal Care Committee.

SCN-lesioning. A total of 44 animals (180–200 g) were anesthetized with an intramuscular injection of 0.6 ml/kg Hypnorm (Duphar, Weesp, the Netherlands), and bilateral thermic lesions of the SCN were made. Lesion electrodes (0.2-mm diameter) were lowered into the brain at an angle of 6° (coordinates lateral 1.0 mm and anterior + 1.4 mm from bregma and −8.3 mm from dura, with the incisor bar at +5 mm) and heated to 80°C for 1 min. After a recovery period of 2 weeks, the effectiveness of the lesions was assessed by measuring water intake during the middle 8 h of the light period (0900–1700) for a total of 2 weeks. When the relative consumption during those 8 h exceeded 30% of the 24-h consumption, animals were considered arrhythmic until final histological screening and were used for additional experiments.

An intra-atrial silicone catheter was implanted through the right jugular vein, according to the method of Steffens (7), in both arrhythmic SCN-lesioned rats and SCN-intact rats when body weight reached 300 g. After surgery, rats were allowed to recover for 2 weeks. During the experiments, the animals were permanently connected to the blood-sampling catheter, which was attached to a metal collar and kept out of reach of the rats by means of a counterbalanced beam. This allowed all manipulations to be performed outside the cages without handling the animals.

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ANOVA, analysis of variance; AUC, area under the curve; IVGTT, intravenous glucose tolerance test; IVITT, intravenous insulin tolerance test ; SCN, suprachiasmatic nucleus; VIP, vasoactive intestinal peptide; VP, vasopressin; ZT, Zeitgeber Time.

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Experiment 1. We submitted three groups of SCN-intact rats and one group of SCN-lesioned rats to an intravenous glucose tolerance test (IVGTT) at different times during the light/dark cycle (see below for description of IVGTT). Glucose infusions were administered to the first group of SCN-intact rats ($n = 9$) at Zeitgeber Time 2 (ZT 2) (ZT 0 is defined as the onset of the light period), ZT 8, and ZT 14. Glucose infusions were administered to the second group of SCN-intact rats ($n = 7$) at ZT 11 and ZT 22. In the third group of SCN-intact rats ($n = 5$), glucose infusions were administered at ZT 18; two rats also received a glucose bolus at ZT 22. The group of SCN-lesioned rats ($n = 7$) was submitted to an IVGTT at ZT 2 and ZT 14. After each experiment, rats were allowed 1 week to rest before the next experiment was performed. To avoid disturbances in the room in which the different groups of rats were kept, only one experiment was performed per day.

IVGTT. On the day of an experiment, food was withdrawn 1 h before the glucose infusion. A glucose solution (0.5 ml, 500 mg/kg body wt) was injected as a bolus via the jugular vein catheter. First, a blood sample (0.2 ml) was collected (t = 0), immediately followed by the glucose injection. Subsequently, blood samples (0.2 ml) were taken at $t = 5$, 10, 20, 30, and 60 min. Samples were used to determine plasma concentrations of glucose and insulin at these time points. Plasma concentrations of glucose and insulin were plotted as line graphs for every ZT. The total amount of insulin released after a glucose bolus injection at different ZTs was calculated from the area under the curve (AUC) of every individual rat and averaged for the experimental group.

Experiment 2. A total of 25 SCN-intact rats and 6 SCN-lesioned rats were subjected to an intravenous insulin tolerance test (IVITT) at different time points over the light/dark cycle. A total of 6 SCN-intact rats were injected with insulin at ZT 2, ZT 8, and ZT 14. Another group of rats ($n = 5$) was injected at ZT 11 and ZT 18. An additional 4 rats were injected at ZT 18 and ZT 22 and, to complete the groups, individual rats were injected at different time points, i.e., at ZT 8 ($n = 3$), ZT 14 ($n = 1$), ZT 18 ($n = 4$), and ZT 22 ($n = 2$). The group of SCN-lesioned rats ($n = 6$) was injected with insulin at ZT 2 and ZT 14.

IVITT. On the day of an experiment, food was withdrawn 2 h before the insulin infusion. An insulin solution (0.5 ml, 0.5 IU/kg body wt) was injected as a bolus via the jugular catheter. First, a blood sample (0.2 ml) was collected ($t = 0$), immediately followed by the insulin injection, and subsequent blood samples (0.2 ml) were collected at $t = 5$, 10, 20, 30, and 60 min. Samples were used to determine plasma glucose concentrations at these time points. Plasma glucose concentrations were plotted as line graphs for every ZT. Insulin sensitivity was estimated from the AUC of the decrease in glucose during the first 10 min after the insulin injection. To measure the AUC, the decreases in plasma glucose concentration in the individual rats were plotted as positive concentrations on a line graph.

Experiment 3. Because the feeding history, which is different for every ZT, may influence the daily rhythm in glucose uptake, we also examined the glucose uptake without the influence of the SCN on feeding behavior. We calculated the glucose disappearance rate after a 15-min glucose infusion in rats that were subjected to a scheduled feeding regimen. Rats were entrained to a feeding schedule of six 10-min meals spaced equally over the light/dark cycle. They had access to the food at ZT 2, ZT 6, ZT 10, ZT 14, ZT 18, and ZT 22. Rats were allowed to adapt to this feeding schedule for 2 weeks. Adaptation was considered complete when rats had learned to consume ±3.5 g of food at each meal. Six rats adapted readily to the feeding schedule, resumed growth (2.7 g/day), and underwent insertion of a jugular vein catheter as described above.

Intravenous glucose infusions. The experiments were started after 2 weeks of recovery. Rats received a 15-min intravenous glucose infusion (0.1 ml/min; 7%) at random order during the six periods when food intake normally occurred (i.e., at ZT 2, etc.). To avoid conditioning influences, the infusion was postponed until 15 min after the ordinary meal onset (no food was offered). Blood samples were taken at −10, −1, 1, 2, 3, 5, 10, 20, 35, and 45 min after the start of the infusions. After an experiment, rats were allowed to rest for 4 days before the next experiment was begun.

Calculations. Calculations were performed on data obtained from SCN-intact rats ($n = 6$) from a previous experiment (8). Glucose uptake was estimated from the first-order constant for the disappearance rate of plasma glucose after the cessation of the glucose infusion. A logarithmic plot of absolute glucose concentrations against time between 15 and 35 min (after the start of the glucose infusion) was drawn, and the glucose uptake was calculated from the slope of regression (9).

Activity measures. The locomotor activity recordings were performed using an analog piezoelectric stabilimeter, the signals of which were transmitted to a computer-based analog interface (CED 1401; Cambridge Electronic Design). The rat cages were placed on a baseplate, which was then placed on four parallel-connected piezoelectronic sensors (27-mm round disks; Murata). The voltage output of the sensors is proportional to relative changes in pressure, i.e., activity of the rat. The activity signals were transmitted directly to the analog inputs of the interface and, with the help of relevant software, were transformed into absolute values, i.e., activity bouts. They were summed over a period of 5 min and stored in text files for later analysis. To measure the activity levels of SCN-intact rats during a glucose injection at the different ZTs, the activity bouts per individual rat (measured every 5 min) were plotted against time, and the AUC was calculated.

Histology. After completion of the experiments, the rats were decapitated, and their brains were fixated by immersion in a 4% paraformaldehyde solution at 4°C for 2 weeks. The hypothalamus was sectioned using a vibratome, and the 50-μm-thick sections were stained for vasopressin (VP) and vasoactive intestinal peptide (VIP) (one of five sections). Brain sections were rinsed extensively in Tris-buffered saline (pH 7.4) and then incubated overnight with either rabbit anti-VIP (Viper 1:2,000; Netherlands Institute for Brain Research, Amsterdam, the Netherlands) or anti-VP (Truus 1:2,000; Netherlands Institute for Brain Research) according to a procedure described previously (1). If rats had cell bodies that stained positively for either VP or VIP in the region of the SCN or around the border of the lesion, they were considered to have a partial SCN lesion and were excluded from data analysis.

Analytical methods. Blood samples were immediately chilled on ice and centrifuged at 4°C. The plasma was then stored at −20°C until further analysis. Plasma glucose concentrations were determined using a Glucose/GOD-Perid method (Boehringer Mannheim, Mannheim, Germany). Plasma immunoreactive insulin concentrations were determined using a radioimmunoassay kit (Linco Research); samples were assayed in duplicate. Amounts of sample, standards, label, antibody, and precipitating reagent as described in the procedures of the assay were divided by 4. The lower limit of the assay was 0.1 ng/ml, and the coefficient of variation of the immunoassay was <5%.

Statistical analysis. The plasma concentrations of insulin and glucose are expressed as the mean ± SE. Because the plasma concentrations varied over the light/dark cycle, we expressed the observed responses in glucose and insulin as the difference compared with the respective $t = 0$ values. The statistical analysis was conducted using a repeated-measures analysis of variance (ANOVA) to test for effects of injection, interaction, and the ZT at which the injection was given. If ANOVA detected a significant effect of interaction or ZT, Student's t test was used for post hoc analysis; each ZT experiment was considered a different (independent) group. Activity measures and AUCs are expressed as means ± SE. For the ANOVA and t tests, $P < 0.05$ was considered a significant difference.

RESULTS

Histology. Microscopical inspection for the presence of VP and VIP positive cells or fibers in the brain sections containing the lesion site revealed that 13 of the 18 rats selected with the drinking test before the experiment had a complete lesion of the SCN. Data obtained from these 13 rats were therefore used for further analysis.

Experiment 1. In SCN-intact rats, injection of the glucose bolus resulted in an immediate and pronounced increase in plasma concentrations of both glucose and insulin (Fig. 1). The highest glucose concentrations were seen 5 min after injection, directly followed by a rapid decrease. Within 20 min after injection, the glucose concentrations had returned to preinfusion concentrations. ANOVA showed a significant effect of injection [$F(5,210) = 134$; $P < 0.001$] and of interaction [$F(25,210) = 31$, $P < 0.001$], whereas the effect of ZT showed a trend [$F(5,42) = 25$; $P = 0.067$]. Further analysis revealed that the maximum glucose increment at the beginning of the dark period (ZT 14) were lower than those at ZT 2, ZT 8, ZT 11, ZT 18, and ZT 22 ($P < 0.003$, $P < 0.001$, $P < 0.035$, $P < 0.002$, and $P < 0.002$, respectively). This indicates that the differences in injection-induced increases in glucose concentrations depend on the time of the day.

In parallel with the rapid rise of plasma glucose concentrations, plasma insulin concentrations in SCN-intact rats increased during the first 5 min, returning to preinfusion values at $t = 20$ min. ANOVA detected an effect of injection [$F(5,210) = 172.8$; $P < 0.001$] and of interaction

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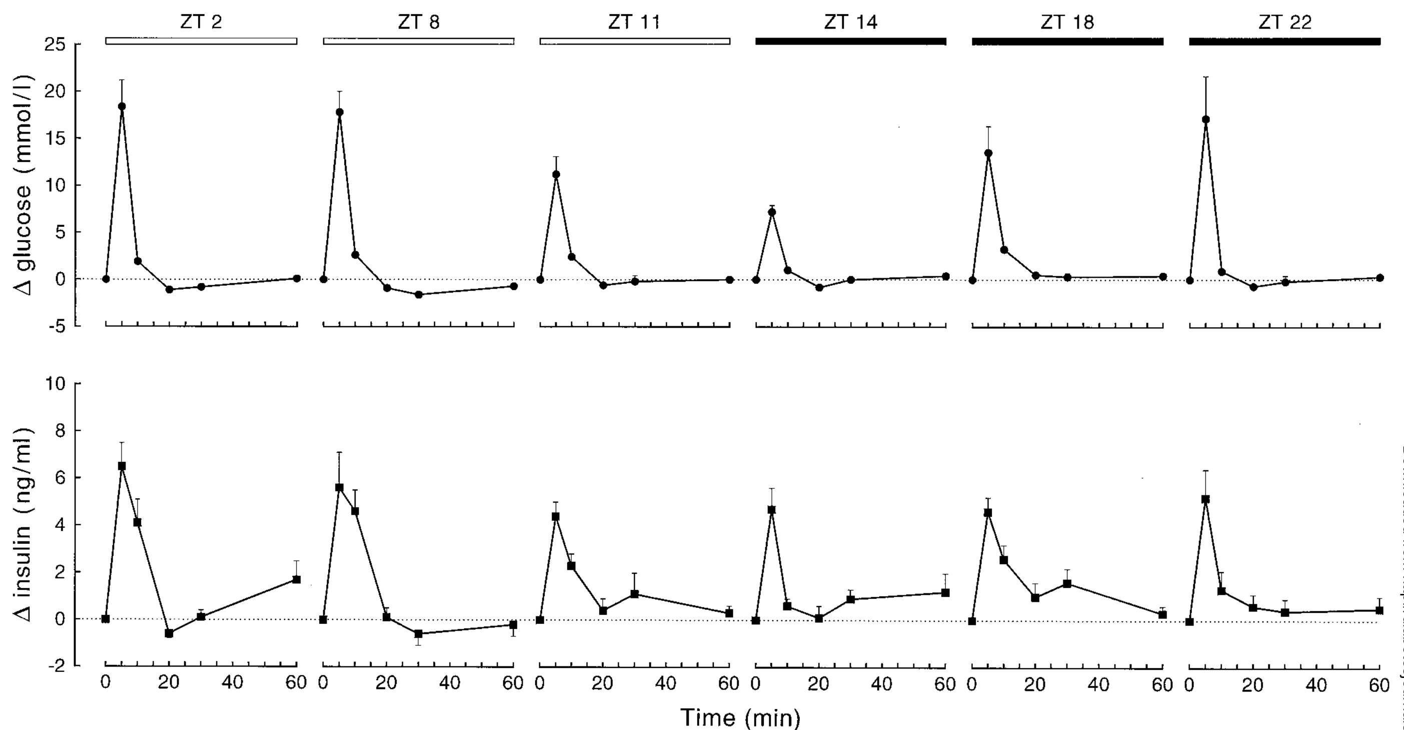

FIG. 1. Plasma glucose and insulin responses after injections of a glucose bolus in SCN-intact rats (n = 5–9) during the light periods (open bars) and dark periods (solid bars). Responses are expressed as the difference from the respective t = 0 values. Absolute values at t = 0 are listed in Table 2.

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[F(25,210) = 8.6; $P < 0.001$], but no significant effect of ZT (F[5,42] = 0.9; P = 0.5) was found. Post hoc analysis revealed significant differences between the insulin concentrations at different ZTs, especially at t = 20 min. However, the height of the insulin response at the different ZTs was not significantly different. Analysis of AUCs indicated that the total amount of insulin released after a glucose injection at different ZTs did not differ either (Table 1). This indicated that the time course of the insulin response differed but that similar amounts of insulin were released after a glucose injection at different ZTs. We calculated the ratio of ΔI_{5-0} to ΔG_{5-0} ($\Delta I_{5-0}/\Delta G_{5-0}$) as an index for the ability of the β-cells to respond to a glucose challenge (Table 1). The difference between the insulin concentrations at 5 and 0 min (ΔI_{5-0}) was divided by the difference between the glucose concentrations at the same time (ΔG_{5-0}). Comparisons of the ratio of ΔI_{5-0} to ΔG_{5-0} ($\Delta I_{5-0}/\Delta G_{5-0}$) indicated that the ability of the endocrine pancreas to respond to the glucose challenge is the same at the different time points of the light/dark cycle.

In SCN-lesioned rats, injection of the glucose bolus also caused an immediate and pronounced increase in plasma glucose concentrations. Insulin responses, however, were clearly reduced (Fig. 2). The highest glucose concentrations were seen 5 min after injection, directly followed by a rapid decrease (ANOVA detected a significant effect of injection [F(5,25) = 67, $P < 0.001$]). Within 20 min after injection, the glucose concentrations had returned to preinfusion concentrations. Plasma glucose responses were similar at ZT 2 and ZT 14 (Fig. 2). There was no significant effect of ZT (F[1,5] = 0.2; P = 0.67) and no significant effect of interaction (F[1,25] = 0.14; P = 0.98). Injection of the glucose bolus in SCN-lesioned rats caused a plasma glucose response that was similar to that in SCN-intact rats at ZT 14 and a lower response compared with that in SCN-intact rats at ZT 2 (ANOVA: interaction, F[5,65] = 5.4, $P < 0.001$; lesion, F[1,13] = 6.8, $P < 0.05$).

In parallel with the rapid rise in plasma glucose concentrations, plasma insulin concentrations in SCN-lesioned rats increased markedly during the first 5 min and de-

TABLE 1
Ratios of ΔI_{5-0} to ΔG_{5-0} (experiment 1) and the AUCs for insulin responses (experiment 1) and for insulin-induced decrease in glucose (experiment 2) at different-time points

	SCN-intact rats						SCN-lesioned rats	
	ZT 2	ZT 8	ZT 11	ZT 14	ZT 18	ZT 22	ZT 2	ZT 14
Experiment 1								
Insulin response	84 ± 17	50 ± 24	69 ± 22	66 ± 24	93 ± 22	59 ± 20	18 ± 4*	28 ± 9*
ΔI_{5-0} ΔG_{5-0} ratio	0.5 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.7 ± 0.2	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
Experiment 2								
First-10-min decrease in glucose	15.9 ± 1.6†	14.8 ± 0.8†	20.1 ± 2.8	21.8 ± 0.9	11.6 ± 2.4†‡	11.9 ± 0.6†	17.4 ± 1.1	17.6 ± 1.9

Data are means ± SE. *Significant difference compared with SCN-intact rats at the same time; †significant difference compared with ZT 14; ‡significant difference compared with ZT 11.

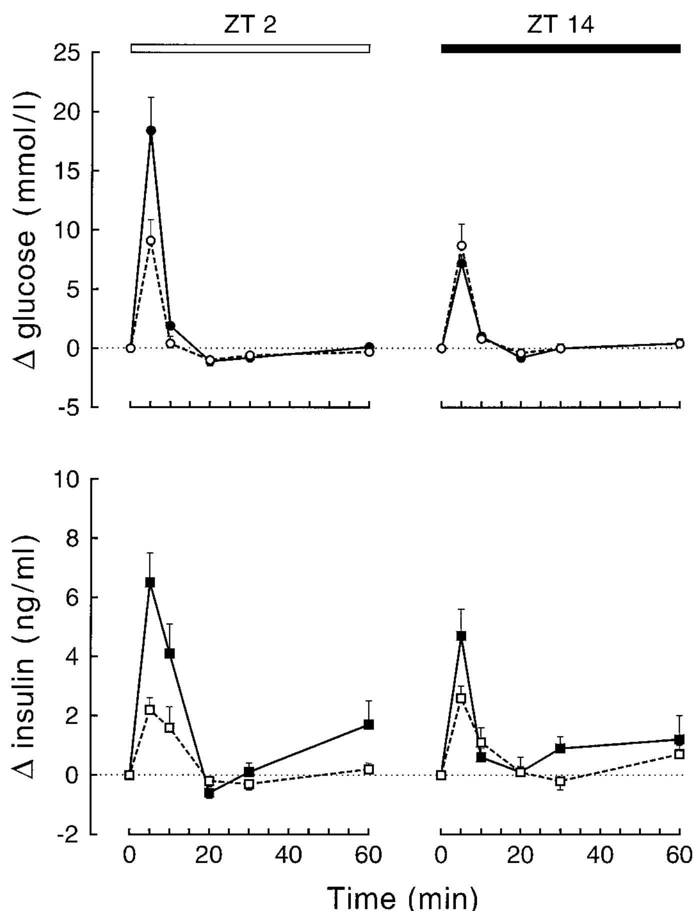

FIG. 2. Plasma glucose and insulin responses after injections of a glucose bolus in SCN-lesioned rats (○) and SCN-intact rats (●) at ZT 2 (light period, open bars) and ZT 14 (dark period, solid bars). Concentrations are expressed as the difference from the respective t = 0 values. Absolute values at t = 0 are displayed in Table 2.

creased thereafter, reaching preinfusion values at $t = 20$. The plasma insulin response at ZT 2 was similar to that at ZT 14. ANOVA detected an effect of injection [F(5,25) = 13, $P < 0.001$] but not of ZT [F(1,25) = 2.0, $P = 0.21$] or of interaction [insulin response: F(5,25) = 1.0, $P = 0.43$]. The plasma insulin response in SCN-lesioned rats at ZT 2 was significantly different from that in SCN-intact rats at ZT 2 (Fig. 2*B*); ANOVA detected a significant effect of interaction [F(5,65) = 4.2, $P < 0.002$] and of lesion [F(1,13) = 12.4, $P < 0.004$]. Plasma insulin responses at ZT 14 in SCN-lesioned rats and in SCN-intact rats were not significantly different [interaction (F[5,65] = 1.9, $P < 0.1$) and lesion [F(1,13) = 1.7, $P = 0.22$]. Also, the ratios of ΔI_{5-0} to ΔG_{5-0} of SCN-intact and SCN-lesioned rats did not differ significantly at ZT 2 and ZT 14 (Table 1). Analysis of AUCs indicated, however, that the total amount of insulin released after a glucose injection was significantly lower in SCN-lesioned rats than in SCN-intact rats ($P < 0.05$) (Table 1).

Experiment 2. Insulin-induced reductions in plasma glucose concentrations in SCN-intact rats at different ZTs are shown in Fig. 3. Plasma concentrations before the injection are shown in Table 2. Because of daily variation in plasma concentrations, we expressed the concentrations of glucose at t = 5, 10, 20, 30, and 60 min as their difference from the respective $t = 0$ values (Table 2). Glucose concentrations decreased rapidly, reaching the lowest concentrations at 10 min postinjection. ANOVA detected significant effects of injection [F(5,180) = 195, $P < 0.001$], interaction [F(25,180) = 2.6, $P < 0.001$], and ZT [F(5,36) = 2.6, $P < 0.04$]. Further analysis of AUCs revealed that the glucose uptake during the first 10 min postinjection at ZT 14 was higher compared with those at ZT 2, ZT 8, ZT 18, and ZT 22 (Student's t test, $P < 0.006$, $P < 0.001$, $P < 0.02$, and $P < 0.001$, respectively), and glucose uptake during the first 10 min at ZT 11 was higher than at ZT 18 ($P < 0.05$) (Table 1).

SCN-lesioned rats did not show significant differences between the decrease in glucose concentrations after an insulin injection at ZT 2 and ZT 14 (ANOVA only detected an effect of injection [F(5,25) = 48, $P < 0.001$], not of interaction [F(5,25) = 0.565, $P = 0.73$] or ZT [F(1,5) = 0.09, $P = 0.78$]. SCN-lesioned rats showed a similar reduction in glucose concentrations after an insulin injection, as seen in SCN-intact rats at ZT 2 (Fig. 4). The decrease in glucose after the insulin injection at ZT 14 in SCN-lesioned rats tended to be smaller than in SCN-intact rats at ZT 14 ($P = 0.05$) (Table 1).

Experiment 3 (calculations). Plasma concentrations of glucose and insulin at the moment glucose infusion was stopped are shown in Table 3. Glucose disappearance rates at the different ZTs are shown in Table 3. The disappearance rate at ZT 14 was higher than at ZT 2 ($P < 0.02$) and ZT 10 ($P < 0.05$). The glucose disappearance rate at ZT 18

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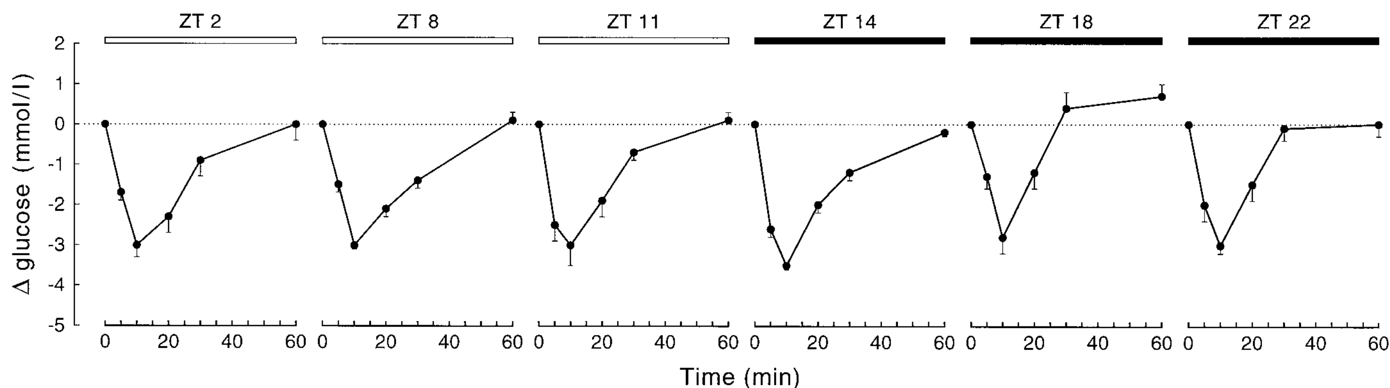

FIG. 3. Disappearance and recovery of plasma glucose concentrations after injections of insulin in SCN-intact rats (n = 6–9) during the light periods (open bars) and dark periods (solid bars). Responses are expressed as the difference from the respective t = 0 values. Absolute values at t = 0 are displayed in Table 2.

TABLE 2
Absolute plasma levels of glucose and insulin in SCN-intact rats at $t = 0$ for experiments 1 and 2

	SCN-intact rats						SCN-lesioned rats	
	ZT 2	ZT 8	ZT 11	ZT 14	ZT 18	ZT 22	ZT 2	ZT 14
Experiment 1 (1-h fasting before the experiment)								
Glucose (mmol/l)	7.3 ± 0.1	7.5 ± 0.1	7.0 ± 0.1*	7.5 ± 0.2	7.0 ± 0.2*	7.1 ± 0.1*	7.3 ± 0.3	6.6 ± 0.3
Insulin (ng/ml)	3.6 ± 0.2†	3.4 ± 0.1†	2.5 ± 0.4	3.6 ± 0.5	2.3 ± 0.3	3.3 ± 0.4	1.9 ± 0.3‡	2.1 ± 0.2‡
Experiment 2 (2-h fasting before the experiment)								
Glucose (mmol/l)	6.7 ± 0.2	6.5 ± 0.1	7.0 ± 0.2	6.9 ± 0.2	7.1 ± 0.2	7.1 ± 0.3	6.4 ± 0.2	6.4 ± 0.3

Data are means ± SE. *Significant difference compared with ZT 8; †significant difference compared with ZT 18; ‡significant difference between SCN-lesioned and SCN-intact rats.

was lower than that measured at ZT 10 ($P < 0.05$) and ZT 14 ($P < 0.02$).

Locomotor activity levels. Locomotor activity levels of SCN-intact rats during the experiments at the six ZTs are shown in Fig. 5. Activity levels showed daily fluctuations; the highest activity levels were recorded during the dark period. Significant differences were found between activity levels measured at the beginning of the light period (ZT 2–3, ZT 8–9), at the end of the light period (ZT 11–12), and in the dark period (ZT 14–15, ZT 18–19, ZT 22–23) ($P < 0.001$). Activity levels between ZT 11 and ZT 12 were also significantly different from those measured at ZT 18−19 in the dark period ($P < 0.01$). The activity levels recorded at different time points in the dark period were not significantly different.

DISCUSSION

Glucose uptake showed a clear 24-h rhythm irrespective of whether rats were fasted 1 h before the experiment or subjected to a scheduled feeding regimen. The 24-h rhythm in glucose uptake showed a peak at the beginning of the dark period and a trough at the beginning of the light period. The 24-h rhythm in glucose uptake correlates strongly with the 24-h rhythm in plasma glucose concentrations (1), i.e., glucose uptake is high at the moment plasma glucose concentrations are high. A higher glucose uptake together with high plasma glucose concentrations at the end of the light period may only occur when endogenous glucose production exceeds glucose uptake. A similar situation takes place in humans; before the beginning of the activity period, glucose production and glucose concentrations are increased, while at the same time, glucose utilization is high (2,10). Consequently, the increase in plasma glucose concentrations before the onset of activity is due to increased glucose production and is not the result of decreased glucose use.

The 24-h rhythm in glucose uptake, like that in plasma glucose concentrations, was eliminated after SCN lesioning, and glucose uptake was comparable to the high glucose uptake at ZT 14 in SCN-intact rats, suggesting that SCN lesioning enhances glucose tolerance. This is in agreement with data from Yamamoto et al. (11), who showed enhanced glucose tolerance after an oral glucose tolerance test in SCN-lesioned rats. Therefore, the SCN may regulate the rhythm of glucose uptake and plasma glucose concentrations via separate mechanisms.

Daily variation in glucose uptake may be explained by differences in insulin release from the β-cells of the pancreas evoked by glucose or by daily variation in sensitivity of the tissue to insulin. The amount of insulin released after a glucose bolus did not depend on the time of the day it was given, but we did observe a clear daily variation in insulin sensitivity, as indicated by the daily variation in insulin-induced hypoglycemia. This suggests that a 24-h rhythm in insulin sensitivity contributed to differences in glucose uptake over the light/dark cycle because at ZT 14, when the highest glucose uptake was observed, the highest insulin sensitivity was also found. Lesioning the SCN eliminated the rhythm in tissue sensitivity to insulin; insulin-induced hypoglycemia in SCN-lesioned rats at ZT 2 and ZT14 was comparable to that in intact rats at ZT 2. The findings that SCN lesioning increased glucose tolerance and that the insulin responses of SCN-lesioned rats at ZT 2 and ZT 14 were comparable to those of intact rats at ZT 14 suggest that the higher glucose tolerance observed in SCN-lesioned rats is not due to higher sensitivity of tissue to insulin. A higher glucose tolerance in SCN-lesioned rats as compared with SCN-intact rats was also observed in our previous study (1). Considered together, these results suggest an increased insulin-independent glucose uptake in SCN-lesioned rats as compared with SCN-intact rats. Interestingly, these data suggest that the SCN normally inhibits insulin-independent glucose uptake at the end of the dark period and the beginning of the light period and increases insulin sensitivity 12 h later.

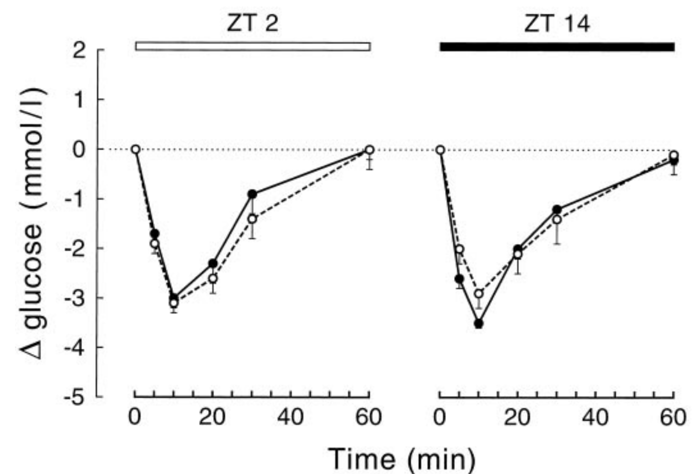

FIG. 4. Disappearance and recovery of plasma glucose concentrations after injections of insulin in SCN-lesioned rats (○) and SCN-intact rats (●) at ZT 2 (light period, open bar) and at ZT 14 (dark period, solid bar). Concentrations are expressed as the difference from the respective $t = 0$ values. Absolute values at $t = 0$ are displayed in Table 2.

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TABLE 3
Glucose disappearance rate calculations on data from Kalsbeek and Strubbe (8)

	SCN-intact rats					
	ZT 2	ZT 6	ZT 10	ZT 14	ZT 18	ZT 22
Experiment 3 (concentrations of glucose and insulin at moment infusion stopped)						
Glucose (mmol/l)	8.4 ± 0.9	9.4 ± 0.6*	9.8 ± 0.2*†	9.6 ± 0.3*†	8.2 ± 0.2	8.9 ± 0.4
Insulin (ng/ml)	4.3 ± 0.4	4.1 ± 0.2	4.5 ± 0.2*‡	3.6 ± 0.4	3.6 ± 0.7	3.4 ± 0.3
Glucose disappearance rate	42 ± 6	52 ± 5	58 ± 4*†	63 ± 4*†	40 ± 6	56 ± 7

Data are means ± SE. *Significant difference compared with ZT 2; †significant difference compared with ZT 18; ‡significant difference compared with ZT 22.

Conducting experiments at six different time points over the light/dark cycle enabled us to provide a more detailed description of daily changes in glucose uptake as compared with other studies (9,11). Our results are also consistent with data obtained from human studies, showing lower glucose uptake in the afternoon and in the evening compared with glucose uptake in the morning in response to an intravenous injection of glucose (4–6). It has been suggested, however, that in humans, fluctuations in insulin sensitivity (12,13) as well as pancreatic β-cell sensitivity to glucose contribute to daily fluctuations observed in glucose uptake (4–6). In contrast to our data, insulin responses to an intravenous glucose load in humans show clear daily fluctuations, with a more intense insulin response in the morning hours, when glucose uptake is higher in the evening (4–6).

Before the injection of the glucose bolus, the rats in our study were fasted. The prevailing feeding conditions, however, which are also under the influence of the SCN, could have influenced glucose uptake differently at the six time points. Therefore, we calculated glucose disappearance rates from rats that were subjected to a scheduled feeding regimen of six 10-min meals equally distributed over the light/dark cycle (8). This scheduled feeding regimen eliminated the influences of the daily feeding pattern on plasma glucose concentrations. The scheduled feeding regimen appeared to disturb only the feeding pattern of the rat. Other overt daily rhythms of the rat, e.g., locomotor activity (8) and corticosterone release (1), were not affected. As observed in experiment 1, rats under a scheduled feeding regimen showed a similar daily fluctuation in glucose uptake, indicating that the diurnal rhythm in glucose uptake is independent of the feeding pattern. In addition to effects of feeding, locomotor activity might also influence glucose uptake differently at various time points during the light/dark cycle. Measurements of locomotor activity levels at the time points when the infusions were given showed that the higher glucose uptake at ZT 11 and ZT 14 is paralleled by an increased locomotor activity at these ZTs, whereas changes in glucose uptake during the dark period did not correlate with changes in locomotor activity. Therefore, changes in the activity levels are not likely to account for the changes observed in glucose uptake.

There are several mechanisms via which the SCN may generate daily fluctuations in glucose tolerance and insulin sensitivity. Hormones such as corticosterone, catecholamines, glucagon, and growth hormone all have been suggested to play a role in glucose metabolism and to relate to rhythms in glucose metabolism. Corticosterone is known to decrease insulin sensitivity and is able to increase glucose production from the liver. The rhythm in plasma corticosterone concentrations, however, is not likely to be involved in the rhythms observed in insulin sensitivity and glucose uptake because corticosterone concentrations are at peak levels when both insulin sensitivity and glucose uptake are high as well. Growth hormone levels may increase glucose production by inducing hepatic resistance to insulin (14) and may play a role in the dawn phenomenon in humans (3). In contrast to growth hormone concentrations in humans, which show a circadian rhythm, growth hormone concentrations in rats show a strong 3-h ultradian rhythm (15,16). Consequently, this does not suggest that release of growth hormone is responsible for the 24-h rhythms in glucose uptake, insulin sensitivity, or plasma glucose concentrations. Approximately 10 min after the administration of insulin, plasma glucose concentrations returned more rapidly to preinfusion concentrations at ZT 18 and ZT 22 as compared with other time points, indicating a daily variation in the recovery from hypoglycemia. Glucagon and adrenaline are released after hypoglycemia and increase the rate of endogenous glucose production (17). The SCN may regulate the daily variation in recovery from hypoglycemia via differential signals over the light/dark cycle, ranging from the α-cells of the pancreas that synthesize glucagon, to the adrenal gland, where adrenaline and corticosterone are produced. The anatomical network for such interactions is in place (18,19). Further studies are needed to determine whether these connections are functioning under such conditions.

In summary, we have observed a clear SCN-generated

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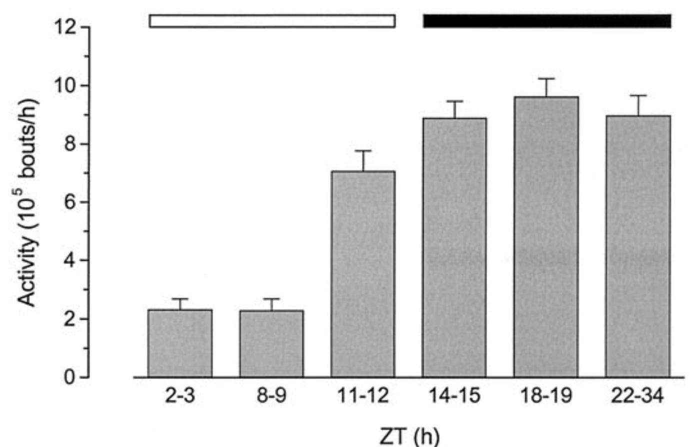

FIG. 5. Average activity level values per hour in SCN-intact rats after injection of a glucose bolus during the light periods (open bar) and dark periods (solid bar).

daily rhythm in glucose uptake, with a rise toward the beginning of the activity period. This daily rhythm in glucose uptake coincides with a rhythm in plasma glucose concentrations (1). Therefore, at the end of the inactive period, the SCN stimulates endogenous glucose production, thereby increasing glucose concentrations and compensating at the same time for the high glucose uptake (2,10). In addition, we observed an enhancement in glucose tolerance in SCN-lesioned rats that could not be explained by higher insulin responses or enhanced insulin sensitivity, suggesting a possible role of the SCN in insulin-independent glucose uptake.

The picture that emerges is that the biological clock prepares the individual for the upcoming activity period by two separate mechanisms, i.e., by increasing plasma glucose concentrations and by making the tissue more tolerant to glucose.

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