The Daily Rhythm in Plasma Glucagon Concentrations in the Rat Is Modulated by the Biological Clock and by Feeding Behavior

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Plasma glucose concentrations display a daily rhythm generated by the hypothalamic biological clock, located in the suprachiasmatic nucleus (SCN). How the SCN orchestrates this rhythm is unknown. Because glucagon stimulates hepatic glucose production, we hypothesized that if glucagon has a daily rhythm, then it may be responsible for the glucose rhythm. From hourly blood samples, we determined daily glucagon concentrations for intact and SCN-lesioned rats. Intact ad libitum–fed rats showed a clear daily glucagon rhythm, and fasting resulted in an even more pronounced rhythm. It is interesting that a decrease in glucagon concentrations, instead of the expected increase, occurred already shortly after food removal. Toward the start of the active period, a peak in glucagon levels occurred, with concentrations similar to those measured in ad libitum–fed rats. SCN lesions abolished rhythmicity in plasma glucagon profiles. Scheduled-fed rats showed meal-induced glucagon peaks but also a daily rhythm in basal premeal glucagon concentrations. Plasma glucose concentrations of ad libitum– and scheduled-fed rats, however, were similar. In conclusion, feeding and the biological clock control 24-h plasma glucagon concentrations. In fed rats, glucagon is not responsible for the daily glucose rhythm. During fasting, however, glucagon may contribute to energy mobilization when the activity period starts. *Diabetes* 52:1709–1715, 2003

Mammals adapt their activity pattern to the daily changes in light intensity. This activity rhythm dictates the need for energy at a specific time of day. This includes glucose in particular, because under normal conditions, it is the only fuel that can be metabolized by the brain (1). Circadian rhythms have been shown in mammals (2,3), including humans (4,5). Rat plasma glucose concentrations display a daily rhythm, with peak values at the beginning of the activity period. Studies involving scheduled feeding and thermic lesions of the biological clock, located in the suprachiasmatic nucleus (SCN), have shown that this rhythm is feeding independent and generated by the SCN (6,7), yet it is not clear by which mechanism the SCN creates this glucose rhythm. Studies investigating the role of insulin have shown that insulin concentrations have a daily rhythm but not independent of food intake (6) and therefore cannot be responsible for the glucose rhythm. Corticosterone stimulates gluconeogenesis (8) and also displays a clear daily rhythm (9–12), with peak values occurring just before the onset of the activity period. Despite the hyperglycemic effect of corticosterone and the coincidence of its peak release with the peak in plasma glucose concentrations, blocking corticosterone synthesis does not affect the morning rise of glucose concentrations seen in humans (13) and thus cannot be the main cause of the rhythm in glucose concentrations. Growth hormone, too, is able to stimulate glucose release (8) but in the rat displays an ultradian rhythm rather than a circadian rhythm, which makes it unlikely to control the daily glucose rhythm (14,15). Recent experiments suggest that hepatic glucose output is a major factor in the early morning rise of glucose (16). Pancreatic glucagon is an important factor in the stimulation of hepatic glucose production (17), and there are numerous reports of neural control of glucagon secretion (18–21). Recently, direct neural connections were found between the SCN and the pancreas, by means of the retrograde virus tracing technique (22,23). There are, however, only few reports on daily patterns of glucagon release (7,24–27) and the findings are inconsistent. A possible role of glucagon in the rhythm of glucose concentrations has not been elucidated. We therefore investigated whether glucagon concentrations display a daily rhythm independent of feeding conditions. Our aim was to determine whether a possible 24-h glucagon rhythm could be responsible for the induction of the previously reported rhythm in plasma glucose concentrations (6). We determined the 24-h plasma glucagon profile in intact rats that were fed ad libitum, fasted, or subjected to a scheduled feeding regimen. Furthermore, a 24-h plasma glucagon profile was determined in rats with thermic lesions of the SCN.

RESEARCH DESIGN AND METHODS

Animals. Male Wistar rats (Harlan) were housed in individual cages (25 × 25 × 35 cm in experiments 1, 2, and 4 or 35 × 35 × 40 cm in experiment 3) at a room temperature of 20°C and a light/dark regimen of 12 h:12 h (lights on

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AUC, area under the curve; SCN, suprachiasmatic nucleus; VIP, vasoactive intestinal peptide; VP, vasopressin.

TABLE 1
Rhythm parameters of basal plasma glucose concentrations

	Ad libitum	Fasted 30 h	Fasted 18 h	Scheduled fed	SCNx
n	7	7	7	6	10
r^2*	0.43 ± 0.03	0.35 ± 0.11	0.53 ± 0.05	0.49 ± 0.04	—
F	8.4 ± 1.0	9.0 ± 4.2	6.5 ± 1.1	10.6 ± 1.7	—
M†	6.7 ± 0.2	4.4 ± 0.2	5.9 ± 0.1	6.3 ± 0.1	6.8 ± 0.1
Amplitude (%)	11.8 ± 0.9	8.6 ± 1.6	19.0 ± 2.6	9.2 ± 0.6	—
A (ZT)‡	14.7 ± 1.7	1.0 ± 0.9	19.9 ± 0.6	10.9 ± 0.3	—
F (ANOVA)	2.01	2.86	8.36	7.54	1.43
P (ANOVA)	0.007	<0.0005	<0.0005	<0.0005	0.160

Data are mean ± SE (pg/ml); *goodness of fit; †absolute 24-h mean; ‡acrophase (ZT).

at 0700). The time that lights are turned on is called zeitgeber time 0 (ZT0); lights off is ZT12. The rats were fed a standard rat diet ad libitum, unless stated otherwise. Water was available ad libitum at all times. For blood sampling, a silicon catheter was placed in the right jugular vein under general anesthesia, according to the method of Steffens (28). After surgery, the rats were allowed at least 1 week to recover before the experiments started. One day before sampling, the rats were connected to external catheters fixed to a metal collar that was kept out of reach of the rats by means of a counterbalanced beam. In this way, the rats were able to move freely during the experiments and blood samples could be taken without handling the animals. All experiments were approved by the Animal Care Committee of the Royal Netherlands Academy of Arts and Sciences.

Experiment 1. A 24-h profile of plasma glucagon concentrations was established by taking hourly blood samples (0.2 ml) from ad libitum–fed rats for a total duration of 24 h. The experiment was divided in two 12-h sessions with 1 week in between the sessions to allow recovery. Previous experiments have shown that this frequency of sampling does not decrease the hematocrit level and preserves the corticosterone rhythm (6). Sampling sessions started either at ZT6.5 (in the middle of the light period) or at ZT18.5 (in the middle of the dark period). Another week later, the experiment was repeated in the same animals but now under fasted conditions. Food was removed at the end of the light period (ZT11.5), and sampling started 10 h later (in the dark phase, at ZT21.5) or 19 h later (in the middle of the light phase afterward, at ZT30.5). Rats were refed after the final sample of each session.

Experiment 2. A second group of rats was fasted for a shorter period of time. This time, food was removed at the beginning of the light period (ZT0.5) and hourly blood samples (0.2 ml) were taken, starting from the middle of that same light period (ZT6.5) until the middle of the next dark period (ZT 18.5). Rats were again refed after the final sample.

Experiment 3. A third group of rats were adapted to a scheduled feeding regimen of six meals, equally distributed over the light/dark cycle. During each meal, the rats had access to food in metal food hoppers with sliding doors, for duration of 10 min. In this time, they consumed 3.0–3.5 g of food on average and were able to show a normal growth curve. Meals were offered at ZT2, ZT6, ZT10, ZT14, ZT18, and ZT22. For more details on the six-meals-a-day feeding schedule, see Kalsbeek and Strubbe (29) and La Fleur et al. (6). The 24-h glucagon profile of these animals was determined by taking hourly blood samples (0.2 ml) in two sessions, as described above.

Experiment 4. Bilateral thermic lesions of the SCN were made as described previously (30) to investigate the role of the SCN in the daily variations in glucagon concentrations. A total of 60 male Wistar rats were operated on to obtain a sufficient number of successfully lesioned animals. Initially, quality of the lesions was checked by measuring drinking behavior. SCN lesions were considered successful when an animal drank >30% of its daily water intake during the light period (when intact animals drink 0–5%). By this method, 20 rats were selected as being completely arrhythmic. These rats were fitted with jugular vein catheters as described above and connected to metal collars and blood-sampling catheters 2 days before the experiment. Blood samples (0.3 ml) were taken every other hour. At the hour between two samples, a sham sample was taken to keep blood from clotting in the catheter. The day-night cycle was divided in two sessions in which seven 2-h samples were taken. In this way, there was an overlapping sample at the beginning and end of each session.

Histology. After the experiments, the animals were decapitated and brains were fixed by immersion in a 4% paraformaldehyde solution. Vibratome sections (50 μl) of the hypothalamus were stained for vasoactive intestinal peptide (VIP) and vasopressin (VP). When cell bodies around the lesion area were stained positive for either VIP or VP, the lesion was considered to be incomplete and data were excluded from analysis.

Analysis. Blood samples were chilled immediately at 0°C and centrifuged at 4°C. Plasma aliquots for determination of glucagon were stored at −80°C and aliquots for glucose and corticosterone assays were stored at −20°C until analysis.

Plasma glucose concentrations were determined in triplicate using the GOD-PAP method (Roche Diagnostics, Mannheim, Germany). Plasma glucagon and corticosterone concentrations were measured in duplicate by radioimmunoassay (Linco Research, St. Charles, MO, and ICN Biomedicals, Costa Mesa, CA, respectively). All samples from one animal were included in one assay. For corticosterone, 10-μl plasma aliquots were diluted in 4 ml of assay buffer. The lower limit of the assay was 1 ng/ml, and the coefficient of variation was 7%. The lower limit of the glucagon assay was 20 pg/ml, and the coefficient of variation was 10%.

Statistics. The plasma concentrations of glucagon, corticosterone, and glucose are expressed as mean ± SE. The statistical analysis was conducted using a repeated-measures ANOVA to test for effects of time of day. Meal-induced glucagon peaks at different times of the day were compared, using the areas under the curve (AUCs) of the first 2 h after every meal. The software package TableCurve (Jandel Scientific, Erkrath, Germany) was used for fitting cosine functions to the data of individual animals. For experiments 1–3, only animals that showed significant rhythms in their ad libitum glucose concentrations ($r^2 > 0.30$) were selected for further analysis. See Table 1 for further statistical details. For experiment 4, rats whose glucose and corticosterone data significantly fitted a cosine analysis were considered unsuccessfully lesioned and were not included in the final analysis.

RESULTS

A total of 86 rats were used in these experiments. In experiments 1 and 2, the same rats were used to determine the 24-h plasma glucagon profile during both ad libitum feeding and a 30-h fast. Initially, 18 animals were in this group. A complete 24-h profile could be obtained from nine ad libitum–fed rats. Others missed part of their curve because of catheters that were blocked. Of these nine rats, seven were selected for analysis after curve-fitting analysis. From six of these rats, also a complete curve could be obtained when the animals were fasted. In experiment 3, eight animals were used, of which six full 24-h profiles were obtained. In experiment 4, from the 20 rats operated on, 11 24-h profiles were obtained. Histologic analysis of the brains of these rats revealed no VIP- or VP-stained cells in the SCN area. Ten rats did not produce a significant fit for glucose and corticosterone after curve-fitting analysis.

Ad libitum–fed rats. Glucagon concentrations in rats with ad libitum access to food did not display a significant daily rhythm (Fig. 1), although concentrations tended to increase during the dark phase and decrease at the onset of the light phase. However, ANOVA detected a significant difference between the mean glucagon concentration during the light period (80.1 ± 3.5 pg/ml) and the dark period (87.3 ± 3.4 pg/ml, $F = 14.91$, $P = 0.008$). See Table 2 for

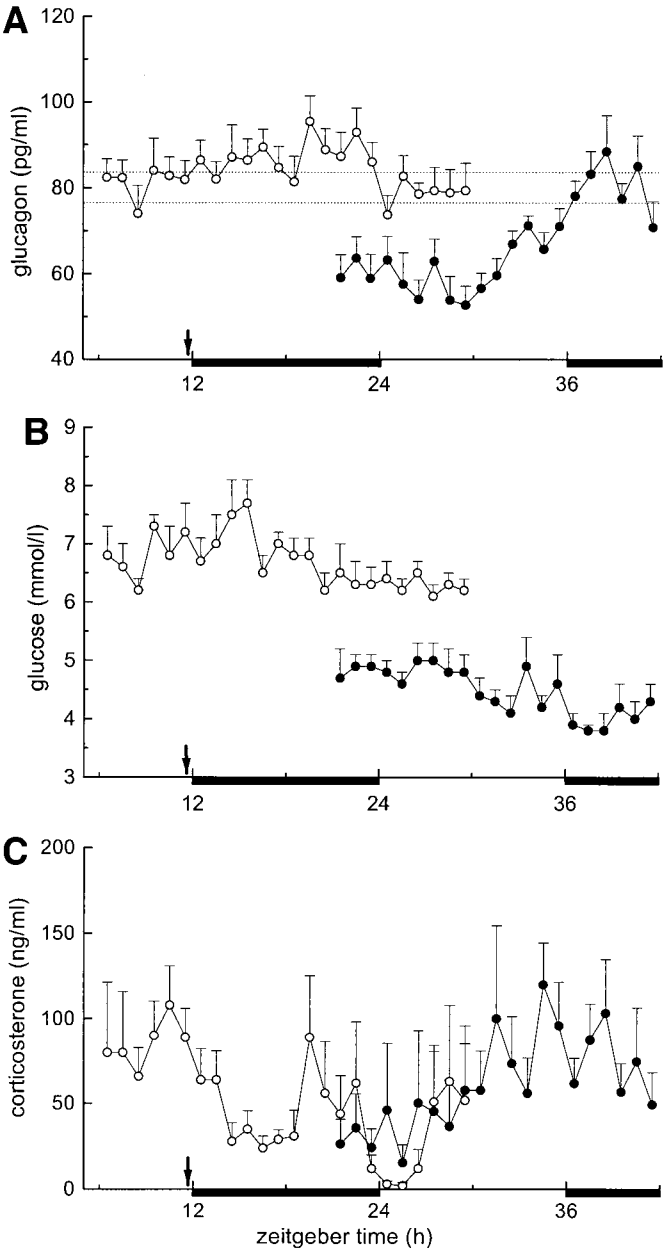

FIG. 1. Plasma concentrations of glucagon (*A*), glucose (*B*), and corticosterone (*C*) in ad libitum–fed (○, $n = 7$) and 30-h fasted (●, $n = 6$) rats. Values are indicated as mean ± SE. Black bars indicate the dark phase, and dotted lines in *A* indicate the mean ± SE glucagon concentration of ad libitum–fed rats during the light period. Arrows indicate the time at which food was removed.

further statistical details. Glucose and corticosterone were similar to previously published data (6).

Fasted rats. In rats that were fasted up to 30 h, glucagon concentrations decreased gradually from 59.2 ± 5.3 at the end of the dark phase to a nadir of 52.8 ± 4.4 pg/ml at ZT6.5 in the following light phase, followed by a sharp increase to a peak of 88.4 ± 8.4 pg/ml at ZT14.5 (Fig. 1). Short–term–fasted rats (up to 18 h) showed a similar glucagon peak at the onset of darkness as compared with long-term–fasted rats. In the middle of the light period (after 9 h of food deprivation), the glucagon concentration was already well below the concentration seen during the light period in ad libitum–fed rats (67.1 ± 3.5) and decreased further to a nadir of 56.7 ± 3.3 pg/ml at ZT9.5. The concentration then increased to a peak of 102.8 ± 9.8 pg/ml at ZT12.5 (Fig. 2).

Glucose concentrations in 18-h–fasted rats decreased compared with ad libitum–fed animals but still showed a significant daily rhythm with peak concentrations at the beginning of the dark period (Fig. 2, Table 1). Rats that were fasted for 30 h had decreased plasma glucose concentrations as well but did not show an increase at the beginning of the dark period (Fig. 2). Fasted animals displayed a significant daily rhythm in plasma corticosterone concentrations, similar to ad libitum–fed animals (Fig. 1*C*, Table 3).

Scheduled feeding conditions. Rats that were subjected to the scheduled feeding regimen displayed increases in glucagon concentrations after each meal, which lasted until 0.5–1.5 h after the meal (Fig. 3). There was no significant difference between the meals with respect to the total amount of glucagon released, as calculated by the AUC of each separate meal (i.e., $t = -30$ to $t = 90$). The $t = -30$ and $t = 30$ concentrations, however, showed significant rhythmicity ($r^2 > 0.30$), with peak levels attained at the end of the dark period (Table 2). Plasma corticosterone (data not shown) and glucose concentrations (Fig. 4) were similar to previous observations (6).

SCN-lesioned rats. Plasma glucagon, glucose, and corticosterone concentrations of SCN-lesioned rats did not display any daily rhythmicity (Fig. 4, Tables 1–3). Glucose and corticosterone data were consistent with previously published data (6). Mean glucagon concentrations in animals with SCN lesions were similar to the mean concentrations in the light period measured in intact rats.

DISCUSSION

The present study provides evidence that plasma glucagon concentrations are modulated by both the hypothalamic

TABLE 2
Rhythm parameters of basal plasma glucagon concentrations

	Ad libitum	Fasted 30 h	Fasted 18 h	Scheduled fed	SCNx
n	7	7	7	6	10
r^2	—	0.49 ± 0.06	0.55 ± 0.04	0.08 ± 0.03	0.15 ± 0.04
F	—	11.8 ± 4.5	6.1 ± 0.9	1.0 ± 0.4	1.0 ± 0.3
M	83.7 ± 3.3	67.2 ± 3.0	75.2 ± 2.7	90.9 ± 2.8	79.1 ± 2.6
Amplitude (%)	—	20.0 ± 1.2	31.8 ± 5.0	—	—
A (ZT)	—	14.9 ± 0.5	19.0 ± 0.8	—	—
F (ANOVA)	1.585	7.315	13.067	3.867	2.527
P (ANOVA)	0.055	<0.0005	<0.0005	<0.0005	0.005

Data are mean ± SE (pg/ml).

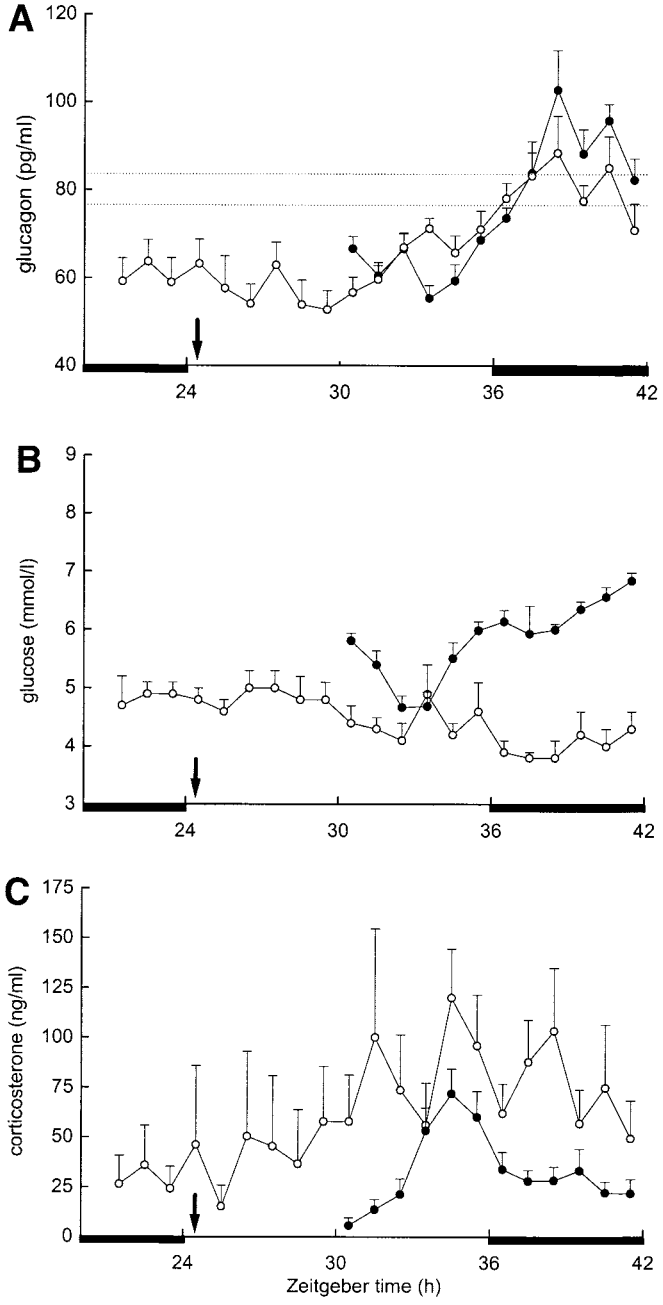

FIG. 2. Plasma concentrations of glucagon (*A*), glucose (*B*), and corticosterone (*C*) in 30-h (○, $n = 6$) and 18-h (●, $n = 7$) fasted rats. Values are indicated as average ± SE. Black bars indicate the dark phase, and dotted lines in *A* indicate the mean ± SE glucagon concentration of ad libitum–fed rats during the light period. Arrows indicate the time at which food was removed for 18-h fasted rats.

biological clock, located in the SCN, and by feeding behavior. A daily rhythm was present in intact rats that were fed ad libitum, fasted, or subjected to a scheduled feeding regimen but was most pronounced in the fasted rats. Thermic lesions of the SCN abolished the rhythm in glucagon concentrations.

Ad libitum–fed rats did not show a clear 24-h glucagon rhythm, but average glucagon concentrations during the dark period were significantly higher than during the light period. This may be related to feeding activity, which occurs almost exclusively during the dark period in rats. In

TABLE 3
Rhythm parameters of basal plasma corticosterone concentrations

	Ad libitum	Fasted 30 h	Fasted 18 h	SCNx
n	7	7	7	10
r^2	0.39 ± 0.09	0.47 ± 0.08	0.27 ± 0.06	—
F	9.5 ± 3.8	9.31 ± 2.1	2.5 ± 1.1	—
M	51.5 ± 17.3	67.7 ± 14.7	37.2 ± 2.2	88.3 ± 12.7
Amplitude (%)	78.3 ± 14.5	90.4 ± 10.1	46.7 ± 7.0	—
A (ZT)	13.9 ± 2.4	12.5 ± 0.9	12.2 ± 0.7	—
F (ANOVA)	2.53	3.25	6.93	0.76
P (ANOVA)	<0.0005	<0.0005	<0.0005	0.703

Data are mean ± SE (pg/ml).

the absence of food, however, rats also showed a pronounced glucagon rhythm, with peak concentrations shortly before dark onset. Two groups of rats were fasted up to either 18 or 30 h. Despite the clear difference in hypoglycemia in the two groups of fasted animals, peak glucagon concentrations occurred at dark onset in both groups, indicating control by the endogenous clock. The classic function of glucagon is to increase hepatic glucose output when glucose concentrations decline during fasting. This is supported by studies in humans (31), and to our knowledge, Mlekusch et al. (32) performed the only study testing this hypothesis in rats. Indeed, glucagon in their animals increased, reaching a plateau after 2 days of fasting. However, their ad libitum–fed animals showed a glucagon rhythm inverted to ours. Furthermore, corticosterone in their fasted animals decreased, whereas starvation is known to increase corticosterone secretion (33). In contrast to the results of Mlekusch et al., our animals, which started fasting at the onset of activity, had decreased plasma glucagon concentrations at the end of the dark period (after 9 h of fasting), instead of the expected increase. In the present study, plasma glucagon levels decreased even further in the first half of the ensuing light period, after which a steep rise occurred at the end of the light period. Although in meal-fed rats, glucagon increases accompany decreasing glucose concentrations (at ZT14 and ZT18), the opposite also occurs (ZT2 and ZT6). Therefore, it seems that during ad libitum and fasting conditions, plasma glucagon levels are not solely controlled by the level of hypoglycemia. Fasting gradually decreases the glucose concentration, whereas insulin or 2-deoxyglucose induces acute hypoglycemia (34,35). Gradual decrease of plasma glucose concentrations may be less effective in evoking a glucagon response than acute hypoglycemia. Indeed, Matschinsky et al. (36) showed that the response of α-cells in the isolated pancreas from 1-, 2-, or 3-day–fasted rats does not differ from those of fed rats.

Further evidence for a role of both the SCN and feeding in the daily modulation of plasma glucagon was gained from animals entrained to a scheduled feeding regimen. These animals received six 10-min meals equally distributed over 24 h. This feeding regimen abolished their natural feeding rhythm but left other rhythms (e.g., corticosterone, glucose, and locomotor activity [6]) intact. Each meal induced a rise in plasma glucagon, which agrees with previous reports of a stimulatory effect of feeding on glucagon secretion. Increased amino acid con-

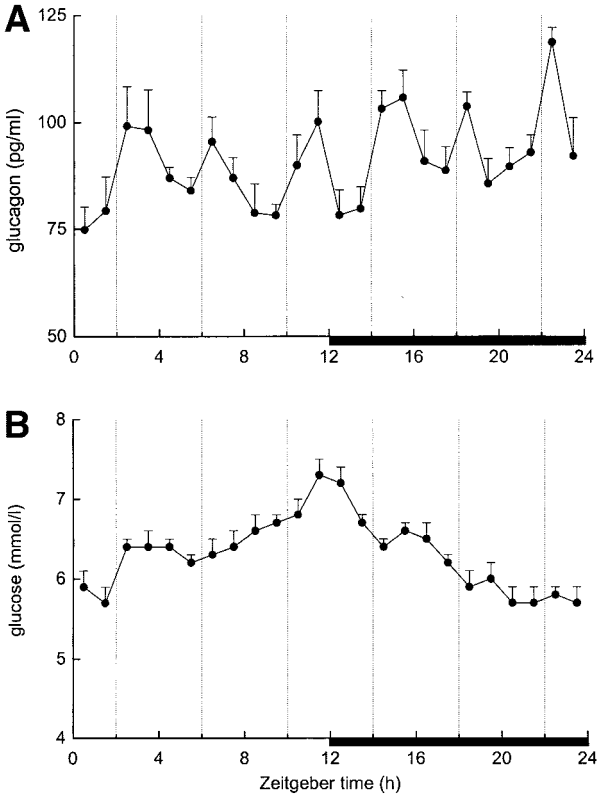

FIG. 3. Plasma concentration of glucagon (*A*) and glucose (*B*) in rats fed six equal meals a day. Values are indicated as mean ± SE. Vertical dotted lines indicate 10-min meals. Black bars indicate the dark period.

centrations after protein intake stimulate α-cells to release glucagon (37). Furthermore, in the first few minutes after the start of feeding, glucagon (and insulin) is released, which is called cephalic-phase release (38,39). This type of glucagon (and insulin) release does not persist >10 min, whereas peaks in our animals persisted 30 or even 90 min after the start of the meals.

Although total plasma glucagon increments (AUC) were comparable for each moment of the light-dark cycle, a daily rhythm was present in the basal (premeal) and peak (postmeal) glucagon levels of these rats. This indicates control by the SCN as well as by feeding. Animals with thermic lesions of the SCN displayed no daily plasma glucagon rhythm, which supports a role for the SCN besides the effect of feeding on the control of plasma glucagon concentrations. Previous results of Yamamoto et al. (7) support this finding. Although the lack of a rhythm in feeding in SCNx animals (they eat small amounts throughout the day-night cycle) may contribute to the absence of a glucagon rhythm, the data of the fasted animals show that the feeding activity is not the only determinant.

Constant food intake in SCNx rats may influence the glucagon level, and on the basis of the meal-induced glucagon peaks, we expected higher glucagon levels in these animals. Instead, we found levels slightly below those of intact rats in the light period. Yamamoto et al. (7) found concentrations even lower than ours. Apparently, the constant intake of small amounts of nutrients by SCNx rats does not stimulate glucagon release as much as the

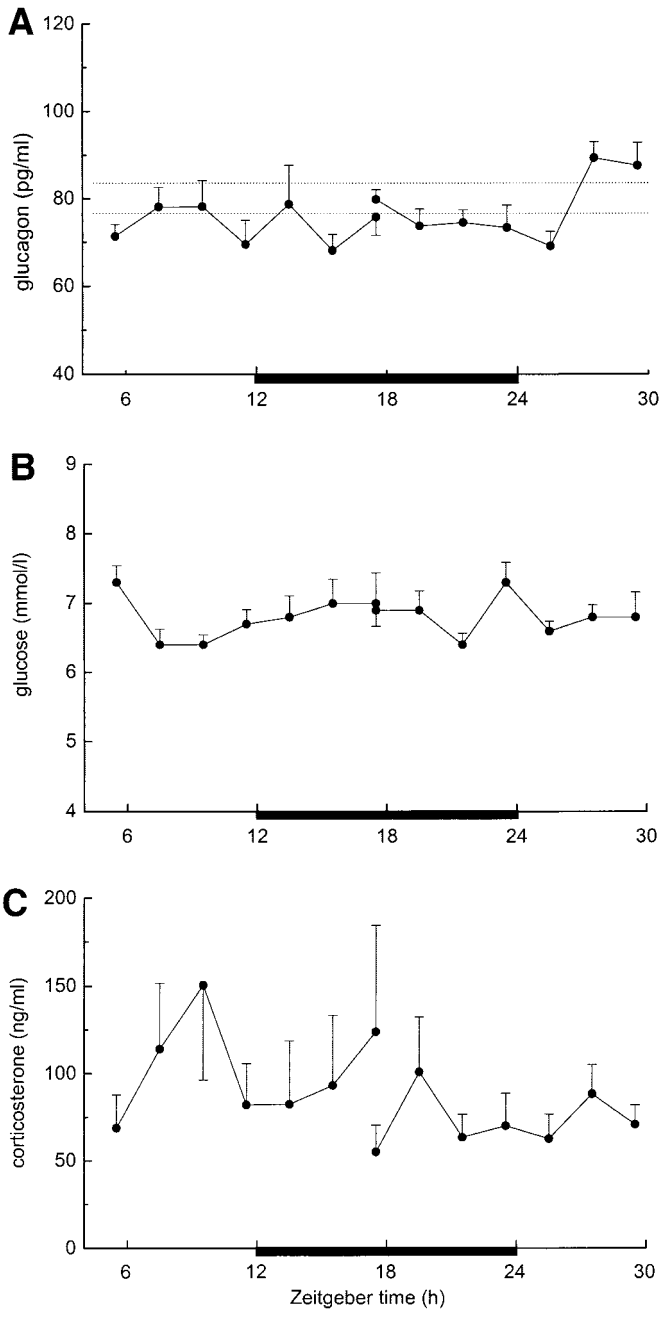

FIG. 4. Plasma concentrations of glucagon (*A*), glucose (*B*), and corticosterone (*C*) in ad libitum–fed rats with thermic lesions of the SCN. Values are indicated as mean ± SE. Black bars indicate the dark period. Dotted lines in *A* indicate the mean ± SE glucagon concentration of ad libitum–fed rats during the light period.

normal meals of ad libitum–fed rats or 10-min meals of intact meal-fed rats.

A second aim of this study was to investigate a possible role of daily glucagon secretion in the modulation of the daily glucose rhythm that has previously been reported (6). The present results provide little evidence for such a modulatory role of glucagon. The glucose and glucagon patterns correlate in some but not all cases. This is clear when comparing ad libitum– and meal-fed animals in which the glucagon profiles are very different, whereas plasma glucose in both conditions is almost identical. Furthermore, the glucagon peaks in both groups of fasted

rats (short and longer duration) are identical, whereas here, the glucose patterns differ.

Insulin is very important in glucose homeostasis, on its own but also in relation to glucagon. The insulin/glucagon ratio determines whether glucose uptake or output occurs (27,40). Although insulin was not assessed in our study, La Fleur et al. (6) previously investigated the role of insulin in experiments very similar to ours. When comparing insulin and glucagon in the meal-fed animals, the patterns are strikingly similar. We cannot calculate a ratio on data from different animals, but it can be assumed that the ratio would be relatively constant throughout the light-dark cycle. Keeping the glucose rhythm of these animals in mind (similar in both studies), we conclude that neither the pattern of glucagon and insulin nor their ratio is directly responsible for the daily glucose rhythm. In fasted animals, however, glucagon may have a role in the mobilization of glucose stores at the onset of the dark period. We have hypothesized that the SCN controls the daily rhythm in basal glucose concentrations via its control on the autonomic inputs to the liver (41,42). Glucagon stimulates both glycogenolysis and gluconeogenesis (43), but the fact that after an overnight fast the glycogen stores are largely depleted (1,44,45) may explain the absence of a rise in plasma glucose in the 30-h–fasted rats. Therefore, increased glucose output as a result of gluconeogenesis alone may not be sufficient to surpass glucose utilization.

The available information on circadian variations in plasma glucagon concentrations is little and inconsistent. In two studies in humans, no daily rhythm in plasma glucagon concentrations was found (25,26). Studies done in mice and rats did show a daily rhythm similar to the one that we show, with peak levels at the end of the dark period (24,27). Although a daily glucagon rhythm was found in two other rat studies, here peak concentrations were measured in the light period and a nadir in the dark period (7,39). We cannot explain these discrepancies. Possibly, methodological differences play a role, e.g., the plasma glucagon measurement recently has become much more standardized than before.

When comparing the 24-h glucagon patterns found in fed and fasted rats with those of body temperature (46; F.A. Scheer, C.V.H., R.M.B., unpublished observations), we observed a striking similarity. Both plasma glucagon and body temperature decreased in fasted rats during the light period and returned to levels measured in fed rats at the onset of the activity period. This suggests that glucagon and thermoregulation are related, which is indeed supported by several studies. Davidson et al. (47) first showed a thermogenic effect of glucagon in rats. Later, several other studies suggested an influence of glucagon on metabolic rate and thermogenesis also in humans, birds, and pigs (40,48–50). Many of these studies show an effect of glucagon in supraphysiological concentrations, but the role of glucagon in physiological conditions is unclear. The similarity of glucagon and temperature in fasted rats, however, supports the idea that glucagon and metabolic rate are functionally related.

On the basis of the present observations, we propose another role for glucagon in energy metabolism besides stimulating hepatic glucose output. Organisms experience a relatively constant routine in their daily activities, dictated by the presence of light. The daily pattern of energy expenditure is therefore relatively constant as well, besides acute changes necessary for, e.g., fight or flight. Thus, a mechanism that prepares the body for everyday variations in energy requirement (besides the mechanisms necessary for quick energy mobilization) may be very useful. The daily rhythms in many hormones, body temperature, and also glucose are well known examples of this daily control of the internal milieu.

When food availability is low, an organism should minimize unnecessary energy expenditure. It can do so by lowering metabolic rate during inactivity, while keeping it unchanged when really needed, i.e., at the onset of activity. This leads to an increase in the amplitude of daily rhythms, as has been shown earlier (46) as well as in our current fasting glucagon pattern. Peak glucagon concentrations in these animals occurred at the onset of the activity period, at which time they were similar to glucagon concentrations in ad libitum–fed animals. In the light period, the glucagon concentrations were low compared with fed rats. This suggests the presence of a mechanism that keeps glucagon stable at the beginning of the activity period and lowers the glucagon concentration during inactivity. Because of the similarity to the pattern in body temperature in fasted rats, we suggest that, at least in conditions of famine, glucagon has a role not in glucose homeostasis alone but also in energy metabolism in general.

In conclusion, a clear day/night rhythm exists in the plasma glucagon concentrations of both fed and fasted rats. In a situation without rhythmicity of feeding, i.e., when animals are fasted or meal-fed, a rhythm was still observed, suggesting endogenous regulation of glucagon release. This is supported by the absence of any rhythmicity in SCNx rats. The pattern of the rhythm, however, differs between fed and fasted rats, indicating a strong influence of food intake as well. The rhythm seen in the glucagon concentration of meal-fed rats does not correlate with the glucose rhythm. We conclude, therefore, that in the fed state, glucagon does not contribute in a major way to the feeding-independent daily glucose rhythm. It may, however, have a role in stimulating hepatic glucose output in fasted rats, besides a possible role in energy metabolism in general. This suggests that glucagon has different roles in different energetic states.

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