Circadian coupling between pancreatic secretion and intestinal motility in humans

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Keller, Jutta, Gabriele Groger, Leelamma Cherian, Britt Gunther, and Peter Layer. Circadian coupling between pancreatic secretion and intestinal motility in humans. Am J Physiol Gastrointest Liver Physiol 280: G273–G278, 2001.—Human interdigestive pancreatic exocrine secretions follow a circadian rhythm with reduced nocturnal activity, but circadian pancreatic exocrine secretion is unknown. To determine whether circadian changes in interdigestive pancreatic secretion occur and are associated with motor events, pancreatic enzyme outputs, proximal jejunal motility, and plasma pancreatic polypeptide concentrations were measured during consecutive daytime and nighttime periods (12 h each) in seven healthy volunteers using orojejunal multilumen intubation. Studies were randomly started in the morning or evening. Nocturnally, motility decreased (motor quiescence: 67 ± 22 vs. 146 ± 57 min; motility index: 3.59 ± 0.33 vs. 2.78 ± 0.40 mmHg/min; both P < 0.05) but amylase output increased (273 ± 78 vs. 384 ± 100 U/min; P < 0.05) and protease output remained unchanged (P > 0.05); consequently, enzyme/motility ratio increased. Amylase outputs were always lowest during phase I. Motor but not pancreatic circadian activities were associated with sleep. Pancreatic polypeptide plasma concentrations were unchanged. Consequently, intestinal motor and pancreatic exocrine functions may have different circadian rhythms, i.e., decreased motor and stable secretory activity during the night. However, the association between individual phases of interdigestive motor and secretory activity is preserved. The nocturnal increase in enzyme/motility ratio is probably not caused by increased cholinergic tone.

interdigestive; postprandial; enzyme secretion; motor activity; pancreatic polypeptide

Physiologically, gastrointestinal secretory and motor activities are closely interrelated not only postprandially but also in fasting humans. In particular, pancreatic exocrine secretion parallels changes of intestinal motor activity (11, 13). During phase I of the interdigestive motor cycle (migrating motor complex), motor quiescence is associated with minimal enzyme output. Throughout phase II, which comprises ~80% of the total cycle length in awake subjects, pancreatic enzyme secretion fluctuates in concert with irregular motor activity (28). Maximal enzyme secretion is reached immediately before onset of or during phase III motility.

For interdigestive motility, a circadian rhythm with decreased nocturnal motor activity has been established (8, 17, 24, 26, 27). During the night, duration of periods of motor quiescence (phase I motility) increases and may reach 80% of migrating motor complex cycle length, whereas duration of phase II motility decreases in turn. In addition, motor activity during phase II decreases. Because of the intimate association between interdigestive pancreatic exocrine secretion and intestinal motility during daytime, the idea that human pancreatic secretion may follow a circadian rhythm with reduced enzyme secretion during the night is attractive (9, 11), but this hypothesis has not been studied so far.

Changes of cholinergic tone are pivotal for the integration of gastrointestinal motor and secretory activity (21, 28, 29) and are reflected by plasma pancreatic polypeptide (PP) concentrations (21, 23, 28, 39, 40). An increase in cholinergic tone is considered one of the main characteristics of the nocturnal period (6). Consequently, circadian secretory and motor activity may also be modulated by cholinergic tone.

We tested the hypothesis that interdigestive pancreatic secretion parallels intestinal motor activity and follows a circadian rhythm with reduced nocturnal enzyme secretion and that circadian variations are associated with changes in cholinergic tone. We therefore analyzed pancreatic secretory and intestinal motor patterns and PP plasma concentrations during 24 h of fasting in healthy volunteers.

METHODS

Human subjects. The study protocol was approved by the local ethical committee. After giving informed written consent, seven healthy volunteers participated in the study.

Tubes and motility recordings. After at least 10 h without food intake, subjects were intubated with an orojejunal multilumen tube and an orogastric tube for gastric and duodenal perfusion of marker substances, gastric and duodenal aspiration, and antroduodenjejunal manometry. To correct for the effects of prolonged intubation on secretory and motor activities, immediately before onset of or during phase III motility.

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activity, four subjects were intubated in the morning and three in the evening. All subjects intubated in the morning had taken a light dinner (2,000 kJ) at 8:00 PM the evening before, and those intubated in the evening had taken a breakfast with similar caloric content and nutrient composition at 7:00 AM. The tip of the oroejejunal tube was placed in the proximal jejunum so that pressure-recording ports were in the antrum, proximal and distal duodenum, and jejunum. The port for continuous perfusion of polyethylene glycol (15 g/L, 3 mL/min) was at the papilla of Vater, and the duodenal aspiration port was just proximal to the ligament of Treitz. The gastric aspiration port was placed in the antrum, and phenolsulfonphthalein (250 mg/ml, 1 mL/min) was constantly perfused via a perfusion port located 10 cm more proximal. The correct positioning of both tubes was verified fluoroscopically. For intestinal manometry, the pressure-recording ports were connected to a low-compliance perfusion system and were constantly perfused with deionized water (2). The voltage output of each calibrated pressure transducer was preamplified and recorded with an eight-channel recorder (Senomedics, Essen, Germany).

Experimental protocol. Subjects were continuously fasting throughout the study but received slow intravenous infusion (83 mL/h) of a glucose solution (5%) for water supply and to prevent hypoglycemia. After 1 h of equilibration, complete collection of gastric juice and aspiration of aliquots of duodenal juice into vials that were immersed in ice at 10-min intervals were started and continued for 24 h. Gastric juice was aspirated as thoroughly as possible to prevent acidic inactivation of pancreatic enzyme activity (12), to exclude salivary amylase that otherwise may account for ~15% of duodenal amylase activity (42), and because duodenal delivery of gastric acid has regulatory effects on pancreatic secretory functions (1, 38). Venous blood samples were drawn within 3 min after the onset of typical phase I, II, and III motility. During the night, the room was darkened. The investigators avoided all unnecessary movements and noises, and periods of resting and sleep were recorded for each subject.

Prevention of artifacts. The prolonged duration of the study for a full circadian cycle might cause at least three artifacts that could confound our observations because of the duration of intubation, prolonged fasting, and the effects of the wake-sleep cycle. These artifacts were controlled for by random intubation of subjects in the morning or evening, equal composition of, and interval from, the last meal in all subjects, and recording and analysis of phases of vigilance and sleep.

Chemical, motility, and statistical analyses. In duodenal chyme, amylase, trypsin, and chymotrypsin activities were measured by routine enzymatic methods (3, 19, 35). Marker concentrations (polyethylene glycol and phenolsulfonphthalein) were measured and used to calculate recovery of gastric juice and duodenal volume flow rates (15). Motility recordings allowed visual identification of the phases of interdigestive motility. In addition, phase I and II intestinal motility recordings of the proximal jejunum were graded at 5-min intervals, and the frequency (F) and mean amplitude (A) of contractions were determined for calculation of a motility index [MI = ln(F × A)/5 + 1], as described earlier (31). Jejunal motility was chosen for detailed analysis because interindividual variability of proximal jejunal motor activity is smaller compared with other intestinal segments (20). Plasma levels of PP were determined by radioimmunoassay (41) and served as a measure of cholinergic tone (28). Two-tailed Student's t-test for paired data was used for comparison of data obtained during daytime and nighttime periods (5). For evaluation of the association between motility phases on the one hand and enzyme output and PP plasma levels on the other, linear regression analysis was performed for individual data. Forward, whether the slopes of the regression curves were significantly greater than zero was tested by one-tailed Student's t-test. Data are expressed as means ± SE unless otherwise indicated.

Definitions. The daytime period was defined as the time between 8:30 AM and 8:30 PM. The nocturnal period started at 8:30 PM and ended at 8:30 AM. These definitions were chosen because correct tube positioning was achieved at 8:30 AM in most of the subjects intubated in the morning and at 8:30 PM in all subjects intubated in the evening. Mean enzyme output was defined as mean output per minute (U/min). Cumulative enzyme output during a given period of interest (e.g., motility phase) was defined as the area under the output curve, i.e., outputs per minute (U/min) multiplied by the duration of the period of interest (min). Total enzyme output means the sum of enzymatic activities of amylase, trypsin, and chymotrypsin (U/min). The enzyme/motility ratio (mean enzyme output divided by motility index) was calculated as a measure of the relationship between pancreatic secretory and small intestinal motor activity.

RESULTS

Circadian intestinal motility. At the beginning of and throughout the study, all subjects showed cyclical interdigestive motility patterns. Mean duration of a total interdigestive cycle was 109 ± 9 min, and there was no difference between cycle length during the day and the night (Table 1). During daytime, significantly fewer cycles showed phases of motor quiescence (number of motility cycles with phase I motility per 12 h: 2.9 ± 0.8 daytime vs. 4.9 ± 0.7 nighttime; P < 0.001), and duration of phase I motility was lower than during nighttime (Table 1). In contrast, duration of phase II activity in relation to total cycle length was longer during the day than the night (Table 1). Duration of phase III activity was similar throughout the 24-h observation period (Table 1). Contractile activity significantly decreased during the night, as shown by a decreased motility index in a representative individual (Fig. 1, top) and decreased mean motility index (Table 1).

Circadian enzyme secretion. Compared with daytime enzyme outputs, mean (P = 0.030) and cumulative

<table>
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<tr>
<th>Table 1. Circadian variations in intestinal motility</th>
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<td>Total cycle length, min</td>
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<td>Cumulative duration, min</td>
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<td>Phase I</td>
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<td>Phase III</td>
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<td>Jejunal motility index, mmHg/min</td>
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Values are means ± SE. N.S., not significant.
(P = 0.046) amylase output, as well as mean total enzyme output (P = 0.035), increased significantly during the night (Fig. 2). The nocturnal increase in trypsin and chymotrypsin outputs was statistically not significant (Fig. 2).

**Association between secretory and motor activities.**
As a measure of the relationship between secretory and motor activity, the ratio of individual enzyme outputs and motility indices was calculated. Enzyme/motility ratios rose significantly during the night for amylase, trypsin, and chymotrypsin (Fig. 3).

**Association between enzyme outputs and phases of interdigestive motility.** Throughout the circadian cycle, mean amylase output increased during phases II and III compared with phase I (daytime: P = 0.016; nighttime: P = 0.025; Table 2). The nocturnal increase in enzyme/motility ratio could not be attributed to a single phase of the interdigestive motility cycle. There were no statistically significant differences between daytime and nighttime values for either mean or cumulative outputs during phases I–III of the interdigestive motility cycle (Table 2).

**Circadian changes in PP plasma levels.** Throughout the day, PP levels increased during phases II and III compared with phase I (P = 0.004; Table 3). Mean PP plasma levels were similar during the day and the night (23.7 ± 0.3 vs. 21.2 ± 0.2 pmol/l). Moreover, there was no difference between daytime and nighttime PP levels during individual phases of the interdigestive motility cycle (Table 3).

**Effect of the start of the study.** For prevention of artifacts, four subjects were intubated in the morning and three in the evening. Therefore, the first 12-h period following intubation comprised daytime in four and nighttime in three subjects. Conversely, the second 12-h period comprised the nocturnal period in four and the daytime period in three subjects. Overall, enzyme outputs and jejunal motor activity were similar during the first and second 12-h periods following intubation (mean amylase output: 330 ± 99 vs. 292 ± 83 U/min, respectively; P > 0.50; mean motility index: 3.63 ± 0.26 vs. 2.92 ± 0.45 mmHg/min, respectively; P = 0.09).

**Effect of duration of fasting.** When amylase output was analyzed with respect to the intake of the last meal, enzyme outputs were similar for the periods 12–24 h and 24–36 h postprandially (mean amylase output: 329 ± 99 vs. 292 ± 83 U/min; P > 0.40).

**Effect of sleep.** During the 24 h of experimental procedures, subjects slept for a mean duration of 7.0 ± 0.8 h. Motor activity was significantly higher during phases of wakefulness compared with phases of sleep (motility index: 3.46 ± 0.31 vs. 2.54 ± 0.45 mmHg/min; P = 0.009). In contrast, sleep had no effect on mean enzyme output (mean amylase output: 335 ± 84 U/min during wakefulness vs. 305 ± 112 U/min during sleep; P > 0.50).
During the 24-h study period, subjects slept for intestinal motility index nocturnally (Fig. 1 and Table duration of phase II motility as well as a decreased response to equal meals (26) is diminished. Apart from this, duration of the fed motor amplitude of phase II contractions (24, 27) and propagation of phase III complexes (8, 24, 27) are reduced. In addition, the increase in enzyme/motility ratio nocturnally cannot be attributed to a single phase of the interdigestive cycle. Moreover, it is not associated with changes in PP plasma level, which is a marker of cholinergic activity.

Circadian intestinal motility. Many physiological functions such as vigilance, body temperature, blood pressure, and hormone secretion are modulated by circadian rhythms. In the gastrointestinal tract, nutrients play the most important regulatory role for secretory and motor activity, and minor changes induced by circadian rhythms can only be observed if subjects are constantly fed or constantly fasting. So far, no studies with continuous application of nutrients have been published. Investigations of circadian changes of gastrointestinal functions during the fasting state have demonstrated a circadian rhythm for gastrointestinal motility with reduced activity during the night (8, 11, 17, 24, 27). Duration of periods of motor quiescence increases nocturnally and may reach 80% of total cycle length. Duration of phase II motility decreases in turn, but the overall interdigestive cycle length remains unaltered (8, 11, 24, 27). In addition, frequency and amplitude of phase II contractions (24, 27) and propagation velocity of phase III complexes (8, 24, 27) are reduced. Apart from this, duration of the fed motor response to equal meals (26) is diminished.

In agreement with these reports, our findings demonstrate increased duration of phase I and decreased duration of phase II motility as well as a decreased intestinal motility index nocturnally (Fig. 1 and Table 1). During the 24-h study period, subjects slept for h, i.e., a normal period. However, disturbances of sleep by inevitable experimental procedures such as suction of chyme and blood may explain why the nocturnal increase in phase I and decrease in phase II motility observed in our study are not as pronounced as might have been expected from earlier studies (8, 11, 17, 24, 27).

Circadian pancreatic enzyme secretion and association with motility. We tested whether interdigestive pancreatic enzyme secretion follows a circadian rhythm with reduced secretory activity during the night. This hypothesis was based on two observations. On the one hand, interdigestive intestinal motor activity decreases nocturnally, as described above (8, 11, 17, 24, 27). On the other hand, in awake healthy subjects, there is an intimate association between interdigestive pancreatic exocrine and intestinal motor activity. Enzyme output is lowest during phase I; it fluctuates in concert with the phase II motor activity and reaches maximal values during antral phase III and late duodenal phase II (10, 11, 28, 44). However, circadian pancreatic exocrine secretion has only been studied in single subjects (18) or in a few subjects suffering from severe pancreatic disease (4). Accordingly, it is not known whether nocturnal reduction of interdigestive pancreatic secretion occurs in healthy humans.

Our findings show that, nocturnally, interdigestive enzyme outputs remain stable. By contrast, intestinal motor activity decreases during the night. Together, this leads to a significant increase in the ratio between pancreatic secretory and small intestinal motor activity. Hence, interdigestive motility and pancreatic enzyme secretion may have different circadian rhythms with reduced motor activity but unchanged enzyme secretion during the night.

Despite different trends for overall daytime and nighttime secretory and motor activity, the association between single phases of fasting motility and of interdigestive pancreatic exocrine secretion is preserved throughout the circadian cycle. Mean amylase output is always minimal during phase I and significantly higher during phases II and III (Table 2). In addition, the increase in enzyme/motility ratio nocturnally cannot be attributed to a single phase of the interdigestive cycle because daytime and nighttime amylase secretion rates and cumulative amylase outputs during individual phases of the interdigestive cycle are similar (Table 2).

| Table 2. Circadian association between interdigestive motility phases and enzyme output |
|-----------------------------------------------|------------------|
|                                   | Day     | Night    |
| Mean amylase output, U/min            |         |          |
| Phase I                              | 103 ± 51 | 128 ± 43 |
| Phase II                             | 319 ± 69 | 403 ± 104|
| Phase III                            | 549 ± 199| 576 ± 237|
| Cumulative amylase output, kU         |         |          |
| Phase I                              | 13.6 ± 8.2 | 16.9 ± 7.0 |
| Phase II                             | 142.8 ± 28.1 | 199.7 ± 67.4 |
| Phase III                            | 42.3 ± 16.7 | 53.1 ± 16.6 |

Values are means ± SE. There were no significant differences for day vs. night.

DISCUSSION

Our findings can be summarized as follows. In healthy humans fasting for 24 h, intestinal motility follows a circadian rhythm with reduced motor activity during the night. In contrast, pancreatic amylase output increases significantly during the night, whereas protease output remains unchanged, and, consequently, the ratio between secretory and motor activity is about tripled nocturnally compared with daytime. The cyclical coupling between interdigestive motility and pancreatic secretion is preserved throughout the circadian cycle, with lowest enzyme secretion during phase I motility and significantly higher enzyme outputs during phases II and III. The increase in enzyme/motility ratio nocturnally cannot be attributed to a single phase of the interdigestive cycle. Moreover, it is not associated with changes in PP plasma level, which is a marker of cholinergic activity.

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<th>Table 3. Circadian association between interdigestive motility phases and pancreatic polypeptide plasma levels</th>
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<td>Pancreatic polypeptide in plasma, pmol/l</td>
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<td>Phase I</td>
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Values are means ± SE. There were no significant differences for day vs. night.
Prevention of artifacts by the experimental protocol. The sheer duration of the study for a full circadian cycle might have caused artifacts confounding our observations. Conceivably, continued intestinal intubation and aspiration as well as psychological influences (such as boredom or impatience) might affect motor and/or pancreatic functions. In consequence, if all studies had started at the same time, these artifacts might have been misinterpreted as responses to circadian effects. Therefore, subjects were randomly intubated in the morning or in the evening. Enzyme outputs and motility were similar during the first and second 12-h periods following intubation, independently of the time of intubation. This suggests that the duration of the study did not influence pancreatic or motor function.

Distal intestinal, in particular ileal and cecal, nutrients inhibit gastrointestinal functions postprandially during endogenous stimulation and in the interdigestive state (7, 22, 30–33, 37, 43). Pancreatic enzyme secretion is more susceptible to inhibitory ileal mediators than intestinal motility (6, 22, 23, 31). Therefore, prolonged fasting and disappearance of residual nutrients from the distal intestine might disinhibit pancreatic enzyme secretion but have no effect on intestinal motility. Theoretically, this might have explained the nocturnal increase in enzyme/motility ratio if all studies had been started in the morning. However, subjects were randomly intubated either in the morning or in the evening, and the time of intubation did not influence enzyme output (see above). In addition, reanalysis of data with respect to the time of the last meal, which was of equal composition in all subjects, showed similar enzyme outputs for the periods 12–24 h and 24–36 h postprandially. Thus we have found no evidence that artifacts, either due to the prolonged duration of the study or due to decreasing inhibitory tone on enzyme secretion caused by disappearance of nutrients from the distal intestinal tract during prolonged fasting, explain our observations.

To avoid acidic inactivation of enzymes, interference with salivary amylase, and regulatory effects of duodenal acid delivery on pancreatic functions, our protocol included aspiration of gastric juice. Thus whether experiments with a setup allowing delivery of gastric acid to the duodenum would have yielded additional findings remains uncertain.

Effects of sleep. Sleep has been shown to be a major determinant of interdigestive (14, 25) and digestive (16, 26) motility. Sleep is associated with diminished motor activity during the night and the day (25). Our present data demonstrating reduced motor activity when subjects were sleeping throughout the circadian cycle are in line with these findings. By contrast, our data suggest that sleep does not affect pancreatic enzyme secretion. Sleep was interrupted a few times in most volunteers due to inevitable experimental procedures. Nevertheless, mean duration of sleep was normal during the study period. Similar enzyme outputs during phases of vigilance and sleep suggest that changes in the integrated regulation of gastrointestinal motor and secretory functions caused by sleeping do not account for the nocturnal increase in enzyme/motility ratio.

Potential regulatory mechanisms: importance of PP. The regulation of interdigestive secretory and motor activity is interdependent and at least partially exerted by common mediatory mechanisms. Changes of cholinergic tone are pivotal for the integration of gastrointestinal motor and secretory activity (21, 28, 29) and are reflected by plasma PP concentrations (21, 23, 28, 39, 40). An increase in cholinergic tone is considered one of the main characteristics of the nocturnal period (6). Therefore, we hypothesized that circadian secretory and motor activity may also be modulated by cholinergic tone. However, neither mean daytime and nighttime PP plasma levels nor daytime and nighttime hormone levels during individual phases of the interdigestive cycle differed. Consequently, our data do not suggest that the nocturnal increase in enzyme/motility ratio is caused by increased cholinergic tone.

Similar to our data obtained in humans, previous findings in rats treated with atropine and a CCK_A receptor blocker have shown that the circadian secretory pattern persists, with a rise of enzyme output during the dark period and a fall during the light period (34). Thus, at least in rats, the circadian rhythm of pancreatic secretion may be regulated by very basal mechanisms, which are not dependent on CCK or the vagal cholinergic system.

Interestingly, a circadian rhythm of endocrine pancreatic secretion has recently been demonstrated in isolated rat pancreatic islets. The authors (36) concluded that a stable endogenous circadian oscillator is located within the pancreatic islets of the rat and regulates circadian insulin secretion. Provided that an analogous pacemaker exists within human pancreatic islets, circadian regulation of pancreatic enzyme secretion might be at least partly mediated via the insulocinacinar portal system without necessarily causing measurable changes of hormone levels in peripheral blood.

In conclusion, the present study demonstrates that intestinal motility and pancreatic enzyme secretion may have different circadian rhythms, with decreased motor activity and stable enzyme secretion nocturnally compared with daytime functions. The nocturnal increase in enzyme/motility ratio cannot be attributed to a single phase of the interdigestive cycle and is not explained by an increase in cholinergic tone.

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