Circadian pancreatic enzyme pattern and relationship between secretory and motor activity in fasting humans

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Keller, Jutta, and Peter Layer. Circadian pancreatic enzyme pattern and relationship between secretory and motor activity in fasting humans. J Appl Physiol 93: 592–600, 2002. First published April 19, 2002; 10.1152/japplphysiol.00807.2001.—It is unknown whether nonparallel pancreatic enzyme output occurs under basal conditions in humans. We aimed to determine whether the circadian or wake-sleep cycle influences the relationship among pancreatic enzymes or between pancreatic secretory and jejunal motor activity. Using orojejunal multilumen intubation, we measured enzyme outputs and proximal jejunal motility index during consecutive daytime and nighttime periods in each of seven fasting, healthy volunteers. Enzyme outputs were correlated tightly during daytime phases of wakefulness and nighttime phases of sleep (r > 0.72, P < 0.001). During nocturnal phases of wakefulness, output of proteases (r = 0.84, P < 0.001), but not of amylase and trypsin (r = 0.12), remained associated. Nocturnally, particularly during sleep, pancreatic secretory activity was directly correlated with jejunal motility index (r > 0.50, P < 0.001). In conclusion, parallel secretion of pancreatic enzymes dominates throughout the circadian cycle. Nonparallel secretion during nocturnal phases of wakefulness may be due to merely circadian effects or to the coupling of the wake-sleep and the circadian cycle. The association between fluctuations of secretory and motor activity appears to be particularly tight during the night.

By contrast, there are no data available on physiological effects of the circadian or the wake-sleep cycle on pancreatic exocrine function in humans apart from our laboratory’s previous study that showed a trend toward increased nocturnal enzyme secretion (42). Potential effects of the circadian or the wake-sleep cycle on pancreatic enzyme pattern as observed in animals (27, 38, 53, 54, 57) have not been investigated in humans, so far. This may at least partly be due to the fact that Babkin’s (4) hypothesis of parallel secretion of pancreatic enzymes is still accepted by many investigators, although there are numerous studies in animals as well as humans demonstrating short-term nonparallel enzyme secretion in response to endogenously released or exogenously applied stimulatory and inhibitory mediators (9, 11–15, 24, 27, 29, 38–40, 54–56, 58, 65, 70, 78).

These findings question the concept of strictly parallel secretion of pancreatic enzymes and emphasize the possibility that, not only in animals but also in humans, the ratios among individual pancreatic hydrolases might be modulated by circadian rhythm and/or effects of the wake-sleep cycle.

In the gastrointestinal tract, nutrients play the most important role for regulation of secretory and motor activities. Minor effects induced by environmental factors such as the circadian rhythm can only be studied if subjects are constantly fed or constantly fasting. Therefore, in the present study, one of our aims was to analyze the ratios among individual pancreatic enzymes during 24 h of fasting in healthy volunteers.

For interdigestive motility, a circadian rhythm has been demonstrated with reduced nocturnal motor activity (16, 30, 42, 45). Pancreatic exocrine secretion parallels cyclic changes of interdigestive intestinal motor activity during daytime with minimal enzyme output during phase I and maximal enzyme secretion immediately before onset of or during phase III motility (20). During phase II, enzyme secretion fluctuates in concert with irregular antral motor activity (47).

As our laboratory has shown previously, the cyclic coupling between fasting motility and pancreatic secretion is preserved throughout the night, despite an overall decrease in motor activity (42, 47). It remains unclear whether there are circadian alterations of the

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association between fluctuations of pancreatic exocrine secretion and intestinal motor activity.

Consequently, the second aim of this study was to analyze the association between fluctuations of pancreatic secretory and intestinal motor activity during phases I and II throughout the circadian cycle in fasting healthy volunteers.

METHODS

Human subjects. The study protocol was approved by the local ethical committee. After giving informed written consent, seven healthy volunteers (age: range 20–27 yr) participated in the study.

Tubes and motility recordings. Subjects fasted for 10–12 h; afterward, they were intubated with an orojejunal multilumen tube and an orogastric tube for gastric and duodenal perfusion of marker substances, gastric and duodenal aspiration, and antrroduodenojejunal manometry. Four subjects were intubated in the morning and three in the evening to correct for effects of prolonged intubation on pancreatic secretion and intestinal motility. All subjects intubated in the morning were instructed to take a light dinner (2,000 kJ) at 8:00 PM the evening before and those intubated in the evening to take a breakfast with similar caloric content and nutrient composition at 7:00 AM. The tip of the orojejunal tube was placed in the proximal jejunum with pressure-recording ports in the antrum, proximal, and distal duodenum and jejunum. Polyethylene glycol (15 g/l, 3 ml/min) was constantly perfused at the papilla of Vater, and samples of duodenal chyme were aspirated just proximal to the ligament of Treitz. The gastric aspiration port was placed in the antrum, and phenolsulfophthalein (250 mg/l, 1 ml/min) was constantly perfused via a perfusion port located 10 cm more proximally. 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 (3). The voltage output of each calibrated pressure transducer was preamplified and recorded by an eight-channel recorder (Sensormedics, Essen, Germany).

Experimental procedures. Throughout the study, subjects remained fasting, but they received slow intravenous infusion (83 ml/h) of a glucose solution (5%) for water supply and to prevent hypoglycemia. Complete collection of gastric juice and aspiration of aliquots of duodenal chyme into vials immersed in ice at 10-min intervals was started after 1 h of equilibration. Gastric juice was aspirated as thoroughly as possible and was discharged to prevent acidic inactivation of pancreatic enzyme activity (21) and to exclude salivary amylase. Salivary amylase may otherwise account for ~15% of duodenal amylase activity (75). In both groups of volunteers, in those intubated in the morning as well as in the evening, experimental procedures, including juice sampling, were carried out over 24 consecutive h. During nighttime, the room was darkened, and the investigators avoided all unnecessary movements and noises. Additionally, periods of resting and sleep were recorded for each subject. During the 24 h of experimental procedures, subjects slept for a mean duration of 7.0 ± 0.8 h.

Prevention of artifacts. We assumed that the sheer duration of the study for a full circadian cycle might cause 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 been begun 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, and, subsequently, pancreatic exocrine and intestinal motor functions were studied for 24 h in each volunteer. As our laboratory has shown previously, under these conditions, the duration of the study does not influence pancreatic or motor functions (42).

Distal intestinal, in particular ileal and cecal, nutrients inhibit gastrointestinal functions postprandially, during endogenous stimulation and in the interdigestive state (8, 43, 48–51, 63, 69, 79). Pancreatic enzyme secretion appears to be more susceptible to inhibitory ileal mediators compared with intestinal motility (41, 43, 44, 49). This means that prolonged fasting and disappearance of residual nutrients from the distal intestine might disinhibit pancreatic enzyme secretion while having no effect on intestinal motility. However, as our laboratory has shown previously (42), similar enzyme outputs are observed for the periods 12–24 h and 24–36 h postprandially, provided that the last meal is of equal composition.

Chemical, motility, and statistical analyses. Amylase, trypsin, and chymotrypsin activities in duodenal chyme were measured by routine enzymatic methods (6, 34). Polyethylene glycol concentrations were measured and used to calculate duodenal volume flow rates (28). Phenolsulfophthalein concentrations were measured to ensure that gastric juice was collected almost completely (2, 72). Motility recordings were analyzed visually to identify the phases of interdigestive motility. Additionally, phase I and II intestinal motility recordings of the proximal jejunum were graded at 5-min intervals. Subsequently, the frequency (F) and mean amplitude (A) of contractions were determined for calculation of a motility index [MI = ln(F ÷ A + 1)] as described earlier (49). For comparison with enzyme outputs, mean MI of the two respective 5-min intervals was calculated. Because interindividual variability of proximal jejunal motor activity is particularly small, this segment was chosen for detailed analysis (36). Linear regression analyses were used to evaluate the association between outputs of individual enzymes.

Amylase outputs were not distributed normally but rather in a skewed fashion. Therefore, logarithmic transformation was used to achieve a normal distribution as described previously (47). Negative values resulting from low enzyme output were substituted by 0. Subsequently, the association between mean amylase output and mean jejunal motility index during phases I and II was evaluated by linear regression analysis for the full circadian cycle and for daytime and nighttime periods. To investigate potential effects of the wake-sleep cycle sleep, only periods with data from at least four subjects were taken into account. Because during daytime only few subjects slept at varying points of time, the effect of sleep on secretory motor coupling could only be evaluated for the nighttime period. Data are expressed as means ± SE unless indicated otherwise.

Definitions. 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. Therefore, the daytime period was defined as time between 8:30 AM and 8:30 PM, whereas the nocturnal period started at 8:30 PM and ended at 8:30 AM.

Part of the data on overall circadian pancreatic secretion and motility have been shown before (42).

RESULTS

Circadian pancreatic enzyme pattern. Compared with daytime enzyme outputs, mean amylase output...
significantly increased during the night ($P = 0.03$; Fig. 1). For trypsin and chymotrypsin, there was only a trend toward increased nocturnal outputs ($P = 0.08$ and $P = 0.12$, respectively; Fig. 1). Mean amylase output was correlated with mean trypsin and chymotrypsin outputs during overall daytime and nighttime periods (Fig. 2; daytime: $r = 0.73$, $P < 0.001$ and $r = 0.865$, $P < 0.001$, respectively; nighttime: $r = 0.755$, $P < 0.001$ and $r = 0.850$, $P < 0.001$, respectively). Similarly, there was always a tight correlation between mean trypsin and chymotrypsin outputs (daytime: $r = 0.855$, $P < 0.001$; nighttime: $r = 0.908$, $P < 0.001$). When diurnal and nocturnal phases of vigilance and sleep were taken together, the correlations between outputs of individual enzymes appeared to be unaffected by the wake-sleep cycle (amylase-trypsin: $r = 0.61$, $P < 0.01$, amylase-chymotrypsin: $r = 0.76$, $P < 0.001$; trypsin-chymotrypsin: $r = 0.86$, $P < 0.001$). However, separate analysis of daytime and nighttime phases of vigilance revealed that, only during daytime ($r = 0.73$, $P < 0.001$) but not during nighttime ($r = 0.14$, $P =$ not significant (NS)), there was a significant association between amylase and trypsin outputs. The association between amylase and chymotrypsin outputs was only weak during nocturnal phases of wakefulness ($r = 0.37$, $P < 0.05$), whereas trypsin and chymotrypsin outputs remained tightly correlated ($r = 0.84$, $P < 0.001$; Fig. 3). When subjects were asleep, outputs of all enzymes investigated were tightly correlated ($r > 0.86$, $P < 0.001$; Fig. 3). Pancreatic enzyme pattern during sleep was only evaluated for the nighttime period because during daytime only a few of the subjects slept for short and varying periods of time.

**Fig. 1.** Circadian outputs of amylase (A), chymotrypsin (B), and trypsin (C) in healthy subjects. Values are means ± SE for 7 subjects. Mean amylase output (dashed lines) increased significantly during nighttime compared with daytime ($P = 0.03$), and there was a trend toward increased nocturnal chymotrypsin ($P = 0.12$) and trypsin ($P = 0.08$) outputs.

**Fig. 2.** Association between outputs of individual enzymes in healthy humans during 24 h of fasting. Throughout the circadian cycle, there was a direct correlation between outputs of trypsin and amylase (A), chymotrypsin and amylase (B), and chymotrypsin and trypsin (C). Values are mean data of 7 subjects. ○, Daytime values; ●, nighttime values.
However, there was a direct correlation between amylase output and jejunal motility index (Fig. 4). This was also true for the total circadian cycle ($r = 0.384, P < 0.001$); by contrast, when the daytime period was analyzed separately, the association did not reach statistical significance ($r = 0.081$). Similarly, there was no significant association between amylase output and jejunal motor activity during phases of wakefulness (overall: $r = 0.112, P = NS$), neither during daytime ($r = 0.161, P = NS$) nor during nighttime ($r = 107, P = NS$). By contrast, amylase output and jejunal motility index were clearly associated during sleep ($r = 0.60, P < 0.001$). Because during daytime only a few of the subjects slept at varying points of time, these data were all derived from the nighttime period and the effect of sleep on pancreatic-motor coupling could not be analyzed for the daytime period.

**DISCUSSION**

Our findings can be summarized as follows. In healthy humans fasting for 24 h, outputs of individual enzymes generally occur in parallel. However, the coupling of the wake-sleep cycle with the day-night cycle appears to modulate pancreatic enzyme pattern. The correlation between amylase secretion and secretion of either protease is tight during daytime when subjects are awake and during nighttime when subjects are sleeping. By contrast, secretion of trypsin and chymotrypsin, but not of amylase and trypsin, is correlated during nocturnal phases of wakefulness.

Nocturnally, intestinal motor activity decreases, whereas pancreatic enzyme output tends to increase; still, pancreatic secretory activity fluctuates in concert with irregular intestinal motor activity during nighttime and during sleep; during daytime and when subjects are awake, this association is not significant.

**Circadian pancreatic enzyme pattern.** Many physiological functions, including gastrointestinal secretion and motility, are modulated by circadian rhythm and/or effects of the wake-sleep cycle (16, 20, 30, 42, 45, 74). For the exocrine pancreas, 24-h variations of morphometric and functional parameters (85–87) as well...
of individual pancreatic hydrolases dominates throughout the circadian cycle in fasting healthy humans. However, nonparallel secretion of amylase and proteases occurs during nocturnal phases of wakefulness. Accordingly, interdigestive secretion of individual enzymes or enzyme families may be regulated by different mechanisms or may have variable sensitivities toward influences of the day-night and wake-sleep cycle on regulatory mechanisms.

Our findings pose at least two major questions: 1) how do they fit with the fact that parallel secretion of pancreatic enzymes has been generally accepted for nearly a century by now (4), and 2) why does nonparallel enzyme secretion occur solely during nighttime periods of vigilance?

First, within short periods of time, i.e., minutes to hours, pancreatic enzyme pattern is determined by the presence of large numbers of preformed zymogen granules containing stable proportions of individual enzymes (73). The random selection of these granules during stimulation and the mixing of secretory products derived from different regions of the pancreas explain why, under most conditions, pancreatic enzymes are released in parallel in experimental models (73) as well as in healthy humans (10, 21, 25, 26, 32). For these reasons, many investigators deny the existence of short-term alterations of the pancreatic enzyme pattern.

Nevertheless, under certain conditions, short-term nonparallel secretion of pancreatic enzymes has been observed. Many studies suggest short-term adaptation of the secretory pattern of the rat pancreas to single meals or other experimental conditions (1, 11–13, 17, 18, 24, 27, 38, 53–57, 67, 68, 70, 77, 84, 89); there is also evidence of short-term nonparallel pancreatic enzyme secretion in healthy humans. We observed that pancreatic amylase was less susceptible to low-dose duodenal (40) and ileal (39) nutrient perfusion compared with trypsin and lipase. This lead to alterations of amylase-to-protease and amylase-to-lipase ratios that may occur physiologically at the very beginning and at the end of the digestive period. Similarly, Sommer et al. (78) demonstrated that, in response to graded exogenous stimulation, the relative proportions of pancreatic enzymes change continuously in favor of lipase greater than chymotrypsin and greater than amylase. Several other studies also suggest nonparallel pancreatic enzyme secretion in response to stimulatory or inhibitory mediators in humans (9, 15, 29, 58, 65).

These findings question the concept of strictly parallel secretion of pancreatic enzymes in humans, although the mechanisms underlying differential release of individual enzymes remain largely unknown. Potential mechanisms include selective changes of enzyme synthesis, selective intracellular transport and storage (17, 18, 69), and selective exocytosis or chemical modification of individual enzymes (89). A recent report supports the existence of different types of granules loaded with different proportions of enzymes. Large granules containing a high proportion of trypsinogen and less amylase were selectively released in response
to short-term cholecystokinin stimulation (18). Alternatively, differences in enzyme content among different compartments, i.e., peri- and teleinsular cells (52, 76), and varying sensitivities of these compartments to stimulatory and inhibitory mediators (31) might explain nonparallel pancreatic enzyme secretion.

In any case, so far it appeared that nonparallel enzyme secretion may occur only in response to stimulatory or inhibitory mediators involved in the regulation of the pancreatic exocrine response to nutrients. Our present study is the first to demonstrate that, even in the interdigestive state, human pancreatic enzyme pattern varies in association with the circadian and the wake-sleep cycle.

The second question is why nonparallel enzyme secretion occurs solely during nocturnal phases of wakefulness. During experimental procedures, most subjects were awake until about midnight and woke up at about 6:00 AM. According to our definition of the nighttime period (8:30 PM to 8:30 AM), the nocturnal phases of wakefulness comprise falling asleep and arousal, which may have special impact on the regulation of pancreatic enzyme secretion. On the other hand, we cannot exclude the possibility that the modulation of pancreatic secretory pattern is a merely circadian effect with little influence on the wake-sleep cycle. Nonparallel secretion of trypsin and amylase might occur during the evening and early night hours, which in our study usually comprised the nocturnal phases of wakefulness, as discussed above. The exact mechanisms explaining circadian variations of pancreatic enzyme pattern remain to be elucidated because studies designed to investigate circadian regulation of human pancreatic exocrine secretion are lacking, apart from our laboratory’s previous study (42). However, important regulatory mediators with impact on pancreatic exocrine secretion such as cholinergic tone (7) and release of pancreatic hormones (46, 59, 66, 83, 88) follow a circadian rhythm. Hypothetically, subtle circadian alterations of pancreatic endocrine secretion might predominantly influence enzyme release from peri-insular acini via the enteroinsular axis (31) and thereby induce nonparallel enzyme release as discussed above.

Circadian association between pancreatic enzyme secretion and jejunal motor activity. During the fasting state, interdigestive motility follows a circadian rhythm with decreased motor activity during the night (16, 20, 30, 42, 45). Sleep is another major determinant of intestinal motility (16, 30, 42) and is associated with diminished motor activity throughout the circadian cycle (42). As our laboratory has shown previously, the intimate association between phases I to III of interdigestive motility and pancreatic exocrine secretion is preserved throughout the circadian cycle: amylase output is always minimal during phase I and significantly higher during phases II and III (42).

In awake healthy subjects, there is an intimate association between interdigestive pancreatic exocrine and antral motor activity even within phase II (47): enzyme output fluctuates in concert with irregular antral motor activity. Thus low motor activity during antral phase II is associated with low enzyme output; during periods with high motor activity significantly higher enzyme secretion rates are observed. It is unclear whether physiologically pancreatic enzyme output is also associated with jejunal motor activity and whether this putative association is modulated by circadian rhythm or effects of the wake-sleep cycle. Our data show that, during the full circadian cycle, there is a direct correlation between amylase output and jejunal motor activity. By contrast, when daytime and nighttime periods are analyzed separately, this is only true for the nocturnal period, whereas there is no statistically significant association between daytime amylase output and jejunal motility index (Fig. 4). Moreover, pancreatic secretory and intestinal motor activity are correlated during sleep but not during phases of wakefulness.

It has been shown before that changes in antral but not duodenal motility coincide with changes in pancreatic enzyme output, e.g., peak interdigestive enzyme output occurs during antral phase III but precedes duodenal phase III (37). Moreover, Dominguez-Munoz et al. (23) observed a close correlation between antral but not duodenal motility and fluctuations of pancreatic secretion in awake healthy humans during daytime. Our present results are in agreement with these findings and suggest that pancreatic enzyme secretion is more closely linked to antral motility than to intestinal motility. Moreover, we conclude from our data that the regulation of pancreatic enzyme secretion and jejunal motility is not totally independent. By contrast, common regulatory mechanisms may explain parallel changes of pancreatic secretory and intestinal motor activity during nighttime and during sleep. The reduction of psychological influences on gastrointestinal functions (22, 33, 60, 62, 80) during the night and particularly during sleep may unmask such common regulatory effects.

Conclusions. Merely circadian effects or the coupling of the day-night with the wake-sleep cycle may modulate interdigestive pancreatic enzyme pattern, i.e., the ratios among individual pancreatic hydrolases. The correlation between secretion of amylase and proteases is tight during daytime when subjects are awake and during nighttime when subjects are sleeping. By contrast, there is uncoupling of amylase and trypsin secretion during nocturnal phases of wakefulness. These findings suggest that interdigestive secretion of individual enzymes or enzyme families may be regulated by different mechanisms or may have variable sensitivities toward influences of the day-night and wake-sleep cycle on regulatory mechanisms.

During the night and when subjects are sleeping, not only is the association between individual phases of interdigestive motor and secretory activity preserved (42), but also there is a direct correlation between fluctuations of secretory and motor activity in contrast to daytime functions. This may be due to unmasking of common regulatory mechanisms by reduction of psy-
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