Diurnal Variations of Cephalic Exocrine Pancreatic Response in Dogs

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Summary The purpose of this study was to determine effects of oral stimulation of sucrose on the diurnal output of pancreatic secretion in conscious dogs. Male beagle dogs weighing 9–11 kg were prepared with gastric and duodenal fistulae. Once a day at 14:00 the animals were trained to eat a sufficient amount of a commercial stock diet to maintain their body weight. Gustatory receptors were stimulated at 9:00, 13:00, 15:00, and 18:00 for 5 min with 100 ml of 0.5% agar solutions containing 0.3 M sucrose. Pancreatic juice was collected every 5 min before and after stimulation, and volume flow and protein output were measured. As a result, we demonstrated daily fluctuations timed by feeding not only in the pancreatic basal secretions but also in pancreatic responses to the gustatory stimulation. These results suggest the significant role of taste stimuli in the nutrition of, at least digestion in, animals.

Key Words diurnal variations, cephalic phase, pancreatic juice, dogs, taste stimuli

It has been recognized that oral stimulation will modify the secretory process along with the gastrointestinal tract. Best and Taylor (1) indicated the cephalic and gastric phases account for about 45% each of the total secretion of gastric acid, and the intestinal phase for the remaining 10%. But, Guyton (2) reported that the cephalic phase probably accounts for less than one-tenth of the gastric secretion normally associated with eating a meal, while the gastric phase accounts for more than two-thirds of the total gastric secretion.

In a previous paper (3), we reported the existence of a cephalic phase of pancreatic exocrine secretion in conscious dogs, when a single stimulus without any other components of the food was used. Sucrose or monosodium glutamate was
found to produce in dogs a significantly greater secretory response than sodium chloride or water. There is a paucity of information in regard to daily fluctuations in such cephalic pancreatic response. Sarles et al. (4) suggested the importance in the human of conditioning to certain food and found that stimulated pancreatic secretory activity in humans was low in the early morning. Ito et al. (5) observed that the postprandial pancreatic secretory pattern in the conscious dog was biphasic. The first peak, which was rich in enzyme, occurred 2 h after feeding, while the second, which was rich in bicarbonate, occurred 11 h after feeding. These observations suggest that the variations in basal pancreatic secretory activity also result in fluctuations in cephalic pancreatic response. None of these reports, however, examined specific effects of taste stimuli on pancreatic secretion. The present study was undertaken to determine diurnal changes of stimulated pancreatic secretions in conscious dogs.

METHODS

Four adult male beagle dogs weighing 9–11 kg were used in the experiment. They were individually housed in $45 \times 120 \times 100$ cm cages at a temperature of $24 \pm 2^\circ$C with a cycle of 12 h of light and 12 h of darkness. Once a day at 14:00, the dogs were usually fed a sufficient amount of a commercial stock diet (DS, Oriental Yeast, Tokyo) to maintain their body weight. This feeding schedule was continued at least over half a year for each dog. On testing days, however, they were not fed at 14:00 and were fed after finishing the experiment on the same days. Water was available ad libitum. Oral stimulation and collection of pancreatic juice were carried out in an isolated room.

As previously described (6, 7), dogs were anesthetized with sodium pentobarbital (25 mg/kg) and prepared with chronic Thomas gastric and duodenal fistulae (Labtician Products, New York) (8, 9). The duodenal fistula was placed opposite the opening of the major pancreatic duct to allow cannulation. The pancreatic accessory duct was ligated during the operation. The gastric fistula was placed in the lowest part of the greater curvature of the stomach to promote efficient drainage.

The dogs were suspended in a vinyl hose sling during the collection of pancreatic juice. They had been trained to accept this physical restriction through several preliminary trials. During the experiment, the gastric and duodenal fistulae were opened to drain residual contents. The gastric cannula drained the swallowed test substance to avoid the effects of the gastric phase, which appears later (40 min after stimulation) (6, 10). Therefore, any pancreatic response as a result of this procedure can be considered largely cephalic in origin, even if the swallowed test substance was in transient contact with the gastric mucosa. The duodenal cannula permitted intubation of a glass tube to the major pancreatic duct through the method outlined by Thomas (8), enabling pancreatic juice to be collected from the conscious dogs without contamination by the intestinal contents. Pancreatic juice...
was collected every 5 min for 15 min immediately after the dog was suspended in the sling at 9:00, 13:00, 15:00, and 18:00, and then the pancreatic juice was also collected every 5 min for 15 min after oral administration of the taste stimulus mixture, that is, there were three measurements before taste stimulus, one during, and two after taste stimulation. No more than two stimulations a week were carried out for each dog.

The taste stimulus mixture was prepared by mixing 100 ml of the taste stimulus solution with 0.5% agar. The taste stimulus-agar mixtures were boiled until they attained a jelly-like consistency. These mixtures were warmed to 37°C just before the 5-min oral administration by spoon. The taste stimulus solution tested was 0.3 M of sucrose.

Pancreatic secretions were analyzed for protein content by determining absorbance at 280 nm (11). Optical density units were converted to micrograms of protein by adjustment to the nitrogen content determined by Kjeldahl’s method (12).

Data were statistically evaluated by analysis of variance. The level of significance was set at 0.05 (13).

RESULTS

Diurnal variations in pancreatic exocrine response to the 0.3 M sucrose-agar mixture are shown in Figs. 1, 2, and 3. Pancreatic volume and protein output during the first 15 min before oral administration of the 0.3 M sucrose-agar mixture were considered to be basal secretion responses (Fig. 1). Basal secretion rates were

![Graph 1](image)

Fig. 1. Effect of time of feeding on basal pancreatic volume flow and protein output. The dogs were usually fed at 14:00 but on the testing days measurements were taken when dogs were not fed. Each point represents the mean and SE of 4 to 7 experiments using 4 dogs for each time. Points without the same superscript letter are significantly different at 5% level.
Fig. 2. Effect of time of feeding on pancreatic volume flow and protein output after oral stimulation by 0.3 M sucrose. The dogs were usually fed at 14:00 but on the testing days measurements were taken when dogs were not fed. Each point represents the mean and SE of 4 to 7 experiments using 4 dogs for each time.

Very low before the usual feeding time. A volume of 0.05±0.02 ml and protein output of 4.6±1.9 mg/5 min were observed at 9:00; similarly, 0.06±0.05 ml and 5.1±3.7 mg were observed at 13:00. After the expected time of feeding, however, volume and protein output increased even in the fasted state. A volume of 0.11±0.05 ml and protein output of 8.4±3.2 mg/5 min were observed at 15:00, and 0.25±0.11 ml and 16.5±6.3 mg were observed at 18:00.

Following oral administration of the 0.3 M sucrose-agar mixture, pancreatic response clearly increased at 9:00 and 13:00, whereas there was no response to the taste stimuli at 15:00 and 18:00, regardless of the fact that dogs had not been given food and were hungry (Fig. 2). When the dogs had been fed, copious pancreatic secretions were observed before gastrointestinal drainage (data not shown).
Figure 3 shows pre- and post-gustatory stimulation difference in pancreatic volume flow and protein output after oral stimulation with 0.3 M sucrose at different times of the day. The effects of oral stimulation on pancreatic secretion were clearly observed at 9:00 and 13:00, but there was no response to oral stimulation at 15:00 or 18:00.

DISCUSSION

In order to know the importance of the time when animals receive taste stimulation, dogs were trained to take a commercial diet once a day at 14:00, then placed in a sling at 9:00, 13:00, 15:00, and 18:00, and their pancreatic juice was collected right after the dogs were settled. As a result, although pancreatic secretions during starvation proceeded slowly, the basic output (without oral stimulation) was lower before and higher after the scheduled feeding time showing daily fluctuation.

Generally, exocrine pancreatic secretion is affected by feeding (5), elevating in response to a meal and then returning to the basal. Therefore, periprandial times appear more important for measurements of daily fluctuations in cephalic pancreatic response. But, to verify whether a true circadian rhythm exists, additional times of the day (i.e., before 9:00 and after 18:00) may probably be needed.

In regard to circadian rhythmicity in digestive secretions, anticipation response proposed by Saito et al. (14-16) is an example of psychological control of nutritional functions. After rats were fed at a scheduled definite time of day for a couple of weeks and became adapted to a feeding schedule, levels of digestive enzymes on the intestine of the rats were observed to begin to rise one hour earlier before the scheduled feeding time. Similar observations were made also on absorption of L-histidine and of glucose (17).

Sarles et al. (4) reported that time of day is an important factor affecting cephalic pancreatic secretion in humans; secretory activity in humans was observed to be low in the early morning. Preshaw (18), as previously mentioned, measured pancreatic secretions prior to feeding time and found that they were low.

Furthermore, pancreatic response to gustatory stimulation was observed to follow an opposite course. When 0.3 M sucrose was administered to the dogs, pancreatic response was discerned at 9:00 and 13:00, whereas no response was noted at 15:00 and 18:00, that is, taste stimuli did not produce the effect of increased pancreatic secretion after the expected time of feeding when animals were hungry. It seemed very likely that the psychic or cephalic components of the secretory process declines under such a condition.

Steffens (19) observed that the concentration of insulin in rat plasma increased right after the rat took food into the mouth and before the food was swallowed into the stomach. Such an increase of insulin was not detected if the food was injected directly into the stomach of the rat. Louis-Sylvestre (20) reported that the preabsorptive release of insulin facilitates and accelerates the postingestive glucose
uptake in rats. Diamond et al. (21) determined the contribution of the cephalic phase to postprandial thermogenesis.

Considering our present results and the reports by preceding researchers, it may be concluded that taste stimulations, especially those preferred by animals and conveying a chemical message of the presence of useful (macro) nutrients, must produce a good effect on the digestion and/or utilization of the nutrients in the food together with psychological anticipation for ingestion of foods.

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