Rapid Publications

Diurnal Episodic Pattern of Insulin Secretion in the Dog

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SUMMARY
Sampling portal blood every 15 min by means of indwelling cannulae, we have found evidence that basal insulin secretion in nonanesthetized dogs takes place in six or seven major secretory episodes over a 24-h period. Reproducible patterns were obtained in four experiments conducted on three normal animals. When frequency of peaks was plotted against the log of insulin concentration, a normal distribution was obtained (lognormal distribution). The means and variances were nearly the same in the three animals. Low levels of insulin (10-25 μU/ml) alternated with peaks which were occasionally higher than 200 μU/ml. The average duration of a peak was 30 min and seemed to be independent of ambient glucose levels. It is suggested that these peaks are the result of surges in plasma growth hormone concentrations and/or spontaneous repetitive discharges along neuronal pathways functionally related to insulin secretion. DIABETES 29:326-328, April 1980.

MATERIALS AND METHODS
Under general anesthesia (Nembutal, 35 mg/kg) and aseptic conditions, indwelling silastic catheters were placed into the portal vein through the cranial branch of the splenic vein in three young male dogs weighing 13-16 kg. The open end of each catheter was brought out through the skin of the back, out of reach of the dog. The catheters were filled and frequently flushed with heparinized saline (1:40). Two weeks after surgery, when the animals were fully recovered, the actual experiment was performed. Every 15 min over a 24-h period, 2-ml blood samples were withdrawn and immediately replaced by 2 ml of saline. Care was taken to maintain normal routine in that the animals were fed once a day and lights were turned off from 2100 to 800 h. Plasma glucose was determined in fresh samples by the Beckman Analyzer (true glucose) and insulin was determined in stored frozen plasma samples, after thawing, by the double antibody assay of Hales and Randle, using the kit of Amersham Searle Corporation (Chicago, Illinois). Plasma samples giving values in excess of 100 μU/ml were suitably diluted and reassayed. Analyses of auto- and cross-correlations were carried out by standard procedures. A probit analysis was carried out using the natural logarithm of insulin concentration; the probit regression line was calculated in each case. Values obtained for the first 5 h after feeding were excluded from calculations so as to minimize the possibility of equalization by supplying the same type and amounts of nutrients.

RESULTS
The daily pattern of portal insulin and glucose concentrations of three dogs is shown in Figure 1. Dog N606 was studied under identical conditions on two separate occasions 1 wk apart. The cross-correlation analysis showed that insulin concentrations varied independently from glucose levels. With respect to insulin peaks, the analysis of auto-correlation gave no indication of regular cycling. The plot of probit against log concentrations gave an excellent straight line in every case, showing that the log of concentration was normally distributed. The results are comparable in all four experiments, and are shown in Figure 2.
DISCUSSION

The results of our experiments provide, for the first time, evidence that insulin is secreted throughout the day and night in the form of intermittent bursts. The pronounced episodic secretion of insulin is reflected only in the portal vein. Except for a few hours after feeding, the peaks in insulin concentrations were not related to glucose levels. Also, spike concentrations were present during both periods of sleep and wakefulness.

It was reported that in arterial blood of conscious dogs, sampled every 0.1 to 2 min up to 10 h, insulin concentrations showed “no fluctuations or linear trends,” unless the animals were infused with glucose. However, in a subsequent communication from the same laboratory, it was concluded that “oscillations are part of the normal dynamics of the glucose/insulin system.” In fasting normal monkeys (but not in man) regular oscillations of insulin, glucagon, and glucose levels were observed in the vena cava. Samples were taken at 2- or 5-min intervals for 30 min; the cycling period averaged 9 min and the secretion of insulin increased to 120% of the baseline values. Oscillations of glucose preceded those of insulin levels, suggesting a stimulus-secretory relationship. Plasma C-peptide was found to cycle synchronously with insulin. In humans, small oscillations in the plasma insulin concentration were found in peripheral venous blood when sampling was done every minute for 2 h. The length of a cycle was 11 to 15 min, the mean amplitude 1.4 μU/ml, and glucose cycled in advance of insulin.

There are several basic differences between our study and the investigations from other laboratories. We are reporting on irregular episodic bursts of insulin secretion over a period of 24 h, and the surges that we saw are of a variable magnitude up to 20 times the baseline values. This is different from the results of short-term studies, showing oscillations with regular cycling and amplitudes seldom exceeding twice the baseline values. Furthermore, we were sampling “at source,” in the portal vein, i.e., before passage through the liver, while in other studies sampling was done in the peripheral circulation where factors other than the rate of insulin secretion may have appreciably altered ambient hormone concentrations. Finally, the secretory bursts described by us were independent of plasma glucose concentrations, while the peripheral insulin oscillations described by others were related to preceding oscillations in plasma glucose concentrations. Thus, it is unlikely that regular peripheral insulin oscillations and irregular portal insulin surges would be governed by identical control mechanisms.

Since we have not concentrated on studying the relatively small oscillations in plasma insulin concentrations, we cannot exclude the possibility that we may have missed them on occasion by sampling every 15 min. However, it is highly unlikely that we missed a real burst in insulin secretion, because when sampling was done at 2-min intervals.
for 15-min periods at random, the results did not appreciably alter the pattern obtained by 15-min sampling. Thus the connecting of individual points by lines as done in Figure 1 appears to be justified. The rhythmicity of the pattern of secretion shows similarities in all three dogs. The nearly identical slopes of the probit lines (similar variances) are indicative of closely related patterns of basal insulin secretion.

Alterations in blood flow would be expected to produce fluctuations within the range that we have seen for plasma glucose levels, but it is difficult to visualize how blood flow could be responsible for a 20-fold increase in insulin concentration. Moreover, the similarity of insulin profiles, particularly in dog N606 in which almost identical patterns were seen on two separate occasions, argues against alterations in blood flow as a reasonable explanation.

Very high insulin concentrations were observed six to seven times over a 24-h period. The frequency of these peaks is similar to the frequency of surges in plasma growth hormone (GH) secretion observed in man and the rat. We have shown earlier that a sudden increase of the plasma GH concentration in the dog results in an immediate release of insulin and glucagon from the pancreas. Thus, it is attractive to speculate that the peaks in insulin are driven by preceding surges in plasma GH. Since in the present series of experiments we have not determined GH levels, we cannot make further comments at this time, nor exclude the possibility that the insulin surges are the result of a neural or neurohumoral stimulation originating from levels of the brain higher than the pituitary. Enhanced insulin release was observed after electrical stimulation of certain areas of the brain and after injections of hypothalamic extracts.

Assuming that the control of basal insulin secretion is driven by such a central mechanism, the moving force would have to rest in the spontaneous firing of neuronal cells (i.e., in primary neural activation) and the neurohumor would reach the pancreatic beta cell either directly by release from local nerve endings, or indirectly through endocrine or paracrine communications. By considering the pancreatic beta cell as a neuroendocrine cell, spontaneous activity becomes another possibility. Further experiments are in progress to elucidate the mechanism underlying the pulsatile secretion of insulin.

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