Duodenal Contribution to Pancreaticoduodenal Vein Islet Hormones During Stimulation of the Canine Pancreas with Calcium

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SUMMARY

The possible influence of the duodenum on pancreatic hormones in the pancreaticoduodenal venous effluent of the canine pancreas was studied. The magnitude and dynamics of somatostatin, glucagon, and insulin responses during stimulation with 5 mM calcium were identical whether or not the duodenal remnant was excluded from the perfusion by clamping.

The results indicate that during perfusion with calcium the duodenal remnant is without any significant regulatory or contributory role on pancreatic hormone secretion from the isolated canine pancreas preparation. DIABETES *29*:361–364, May 1980.

The development of techniques for perfusing the pancreas initiated an era of increasing knowledge of the secretory mechanisms and dynamics of hormonal release. In most perfusion methods currently used, a portion of the duodenum remains attached.[1–3] The question has often been raised whether the endocrine cells of the duodenum might contribute to or modify the secretion of pancreatic somatostatin, glucagon, and insulin measured in the pancreatico-duodenal efflux.[4–7] This is obviously of considerable importance for the interpretation of many studies.

The present study was designed to determine if the duodenal remnant had any contributory significance for the hormone secretion from the isolated pancreaticoduodenal preparation. The responses of somatostatin, glucagon, and insulin during calcium stimulation were compared before and after exclusion from the perfusion of the duodenal remnant. Calcium was chosen as stimulus since it is a common key substance in the normal secretory process of the D,[3,8,9] A,[10–12] and B cells.[13,14]

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Received for publication 28 September and in revised form 14 December 1979.

MATERIAL AND METHODS

Mongrel dogs, fasted overnight, weighing 18–25 kg, were used as pancreas donors. The techniques for isolation of the canine pancreas and the perfusion system have previously been described in detail.[1] In brief, the preparation consists of the pancreas and the proximal 10 cm of the attached duodenum. A non-recirculating medium of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 40 g/L dextran (mol wt 75,000), 2 g/L bovine albumin, glutamate, fumarate, and pyruvate, each at a concentration of 5 mM, was pumped through the splenic and the celiac arteries, and the total portal effluent was collected every minute. Oxygenation of the Krebs-Ringer bicarbonate buffer was achieved by means of a rotating roller screen in an atmosphere of 94.4% O_2 and 5.6% CO_2.

Two types of experiments were performed—clamping and control experiments. During the entire perfusion in the control experiments and during the first half of the perfusion in the clamping experiments the duodenum was kept intact as previously described.[1] However, after the initial 40 min the duodenum was excluded from the perfusion circuit in the clamping experiments. To localize the outlet of the main pancreatic duct, the duodenum was cut through by a longitudinal incision on the side opposite the attachment of the pancreas. Two clamps were then applied close to each side of the main pancreatic duct, leaving the outlet patent and concomitantly excluding from the perfusion practically all the duodenum. At the end of each experiment, the preparation was perfused with methylene blue which colored the pancreas but not the duodenum. The perfusion flow was constant throughout the experiments (18–20 ml/min), except in two perfusion experiments where the flow rate decreased by about 5% after the exclusion of duodenum. The perfusion pressure (30–40 mm Hg) was kept constant throughout the experiments.

Somatostatin,[3,8] glucagon,[15] and insulin[15] were measured by specific radioimmunoassays, as described in detail previously. In the glucagon assay, an antibody was used which does not distinguish between glucagon-like immunoreactivity from the gut and pancreatic glucagon.

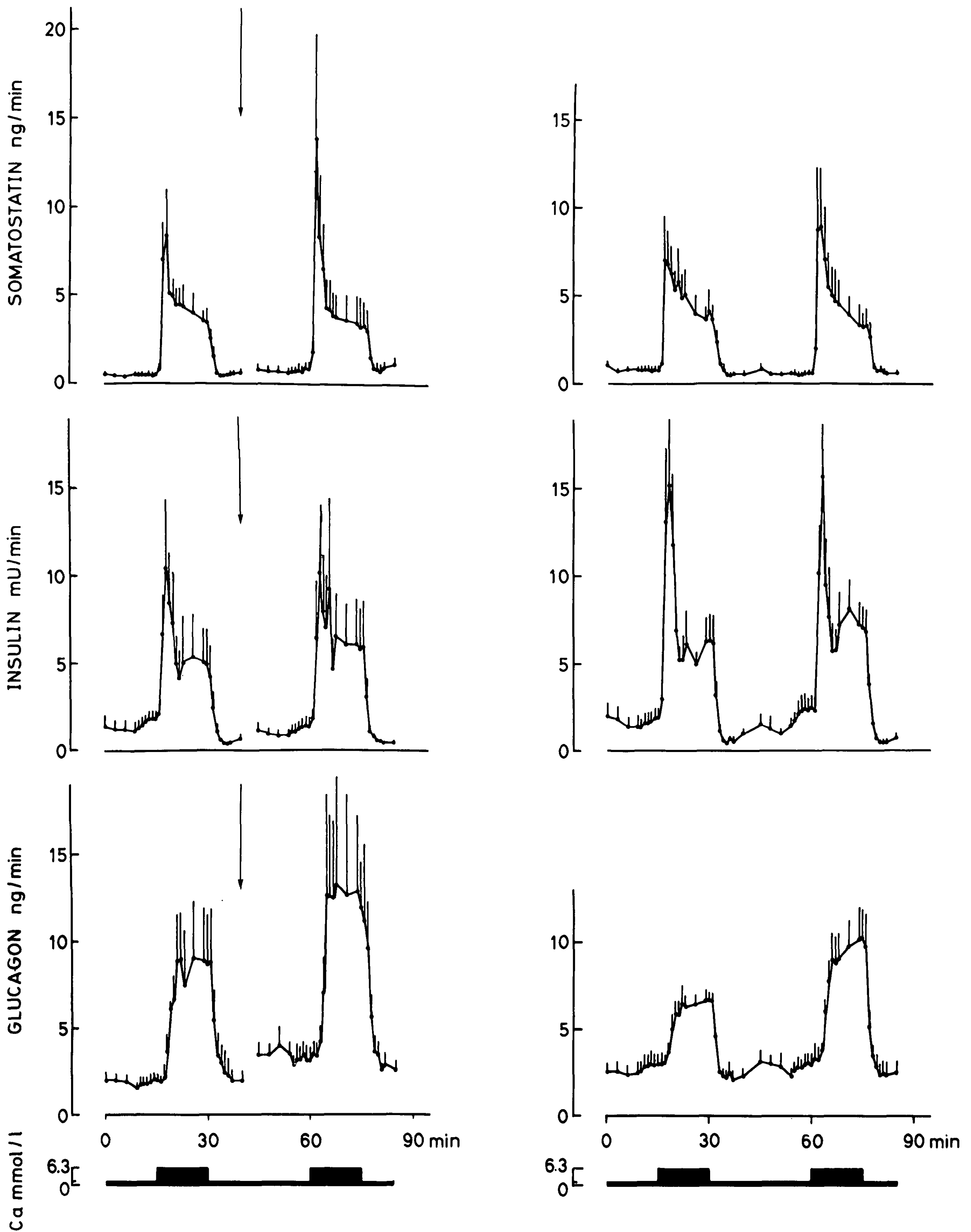

FIGURE 1. ***Left panel:*** **Lack of effect of duodenal exclusion on calcium (5 mmol/L)-stimulated somatostatin, glucagon, and insulin release from the isolated, perfused canine pancreas. The clamping off of the duodenum is indicated by arrows. Mean results ±SEM of four perfusion experiments.** ***Right panel:*** **Effect of 5 mmol/L calcium on somatostatin, glucagon, and insulin secretion in the control experiments with duodenum left intact. Mean results ±SEM of four perfusion experiments.**

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Calculations. The incremental increase in hormone secretion in response to the second calcium stimulation was calculated as the average percentage increase above the basal level (±SEM). The basal hormone level was calculated as the mean of the last five one-minute values preceding the addition of high calcium. When comparing the two basal levels in each of the pancreas perfusions, the second of these was expressed in per cent of the first. Two-tailed Student's *t* tests were used for statistical evaluation of data employing a 5% limit of significance. Nonpaired tests were used for comparisons of results from control experiments with those from clamping experiments; otherwise, paired tests were used.

RESULTS

The effect of calcium on somatostatin, glucagon, and insulin release was tested before and after exclusion of the duodenum. During perfusion with a constant concentration of glucose (100 mg/100 ml perfusate), calcium was increased from 1.3 to 6.3 mM for 15 min. As illustrated in the left panel of Figure 1, this elicited an increase in the secretion of somatostatin, glucagon, and insulin whether or not the duodenum was included in the perfusion circuit. In both situations, somatostatin and insulin responses to an increase in calcium were immediate and biphasic, whereas glucagon responses were uniphasic.

After exclusion of the duodenal remnant, the total hormone output during the 15-min period with high calcium averaged (for somatostatin) 98 ± 25%, (for insulin) 107 ± 9%, and (for glucagon) 156 ± 11% of the total output obtained during the first calcium stimulation ($\bar{x}$ ± SEM, N = 4). For comparison, four control perfusions were performed in which no clamping was applied (Figure 1, right panel). As it can be seen, the patterns of response appeared similar to those obtained in the clamping experiments. Corresponding values in the controls were 98 ± 5% for somatostatin, 109 ± 5% for insulin, and 142 ± 18% for glucagon ($\bar{x}$ ± SEM, N = 4). There was no statistically significant difference between the two groups of experiments for any of the three hormones.

When the increments in hormone release were expressed as per cent above basal level ($\bar{x}$ ± SEM, N = 4), the somatostatin, insulin, and glucagon responses to high calcium following the clamping procedure were 709 ± 165%, 391 ± 103%, and 259 ± 92%, respectively. The analogous results in the control experiments were 886 ± 218% for somatostatin, 441 ± 103% for insulin, and 218 ± 18% for glucagon ($\bar{x}$ ± SEM, N = 4). The incremental increases in hormone output were not significantly different in the two types of experiments.

The basal levels for the three hormones did not change significantly during the perfusions (Figure 1). Glucagon levels tended to increase in both clamped and nonclamped experiments, but the change did not reach statistical significance.

DISCUSSION

Samols et al.[5,6] have repeatedly emphasized that it is important to use a duodenum-free preparation of pancreas in studying pancreatic hormone secretion since the duodenum contains somatostatin[16] and glucagon,[17] both of which, they thought, might contribute to changes in the hormone concentrations estimated in the pancreaticoduodenal efflux. They have accordingly termed their duodenum-free preparation 'the truly isolated pancreas preparation.' On the other hand, Unger et al.,[4] who employ an identical preparation to ours, have the opinion that the duodenal content of somatostatin and glucagon in the dog is so small as to be without significance for hormone release from the pancreaticoduodenal preparation. However, it has often proved fallacious to judge release activity from tissue content of hormones, since the latter reflects a balance between synthesis, storage, and release. We thought it of interest to try to settle the issue by excluding the duodenal remnant from the perfusion by clamping and to compare the magnitude and dynamics of the hormone release before and after exclusion. The results have been compared with those obtained in control experiments, where an exactly identical experimental protocol was followed, except that the clamping off of the duodenum was omitted. The present report shows that identical release patterns of hormones were obtained whether or not the duodenum was excluded both during basal conditions and during stimulation with calcium, which stimulates the A, B, and D cells of the pancreas. It therefore appears that the duodenal remnant does not contribute to or regulate hormone release in the perfused canine pancreas, at least under the circumstances examined in this study.

ACKNOWLEDGMENTS

Cyclic somatostatin for immunization and standards was kindly donated by Norman Grant, Wyeth Laboratories, Philadelphia, Pennsylvania; and Roger Guillemin, Salk Institute, La Jolla, California, generously gave us Tyr[11]-somatostatin for the iodination. Karen Just and Joan Hansen are thanked for expert technical assistance and Anette Larsen for typing the manuscript.

This work was supported by the Danish Medical Research Council, P. Carl Petersen Foundation, and a Novo research grant.

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