Secretin and Insulin: Response to Intraduodenal Acid

F. A. O'Connor, K. D. Buchanan, J. J. Connon, and M. Shahidullah

Depts. of Medicine and Surgery, The Queen's Univ. of Belfast, Belfast, Northern Ireland

Summary. Immunoreactive secretin (IRS) and immunoreactive insulin (IRI) levels were measured in humans and dogs following the intraduodenal instillation of hydrochloric acid. IRS levels rose after acid in both instances, but a concomitant rise in peripheral IRI levels was not noted. Premedication of the humans with Scopolamine prevented a rise of IRS in the human subjects. It is concluded that the endogenous release of IRS alone does not result in increased IRI levels in peripheral blood and that IRS release may be under vagal control.

Key words: Insulin, secretin, intraduodenal acid, enteroinsular axis, vagus, pancreas.

Secretin is a polypeptide hormone secreted by S-cells of the APUD series which, though present throughout the small intestine, are most densely aggregated in the duodenum [1]. It stimulates the flow of pancreatic juice rich in water and electrolytes and assays of secretin based on this action indicate that the main stimulus for its release is duodenal acidification [2, 3]. In addition

to its effect on the exocrine pancreas some workers have suggested that secretin also stimulates the pancreatic B-cells to release insulin [4, 5].

Using immunoassay rather than bioassay techniques we have investigated the effect of perfusing the duodenum with hydrochloric acid on release of secretin and its effect on insulin secretion. The effect of vagolytic compounds on secretin release was also observed.

Material and Methods

Twelve patients after an overnight fast had 100 ml 100 mM HCl infused over a 15-min period into the duodenum and peripheral venous blood samples for secretin and insulin determination were taken at -5, 0, 5, 10, 20, 25 and 30 min following the commencement of the acid infusion. All patients had given informed consent. The patients were divided into two groups of six. Group A contained patients being investigated by jejunal biopsy for possible malabsorption and this group received no medication during the procedure. Table 1 lists the actual diagnosis and jejunal histology in this group. Group B contained patients being investigated for dyspepsia by upper gastrointestinal endoscopy. These six patients had histories very suggestive of duodenal ulceration. Evidence of duodenal ulceration was found in four, the other two patients having normal appearance at endoscopy and barium meal. Group B had two extra venous samples taken at -30 and -15 min before the acid infusion began. Immediately after the -30 min sample these patients were given 13.3 mg Papaveretum and 0.27 mg Scopolamine Hydrobromide by deep intramuscular injection as a premedication to endoscopy. At -10 min 10 mg Diazepam was given intravenously. Care was taken in both groups to ensure that the infusion

Table 1. Jejunal histology and diagnosis of patients in group A

Patient	Jejunal Histology	Diagnosis of Group A Patients
1	Mild Villous Blunting	Crohn's Disease (no upper small bowel involvement)
2	Normal	Functional Bowel Disease
3	Normal	Uraemia, unexplained Diarrhoea
4	Normal	Functional Bowel Disease
5	Normal	Jejunal Diverticulae
6	Normal	Functional Bowel Disease

was delivered into the upper portion of the duodenum. In Group A this was done by determining the positions of the Crosby capsule radiologically and in Group B by passing a catheter under direct vision through the endoscope into the duodenum.

Duodenal fistulae were produced in greyhound dogs (average weight – 20 kilos) by inserting a Gregory cannula into the proximal half of the first part of the duodenum. The dogs were studied at least three weeks after the operative procedure. After an overnight fast, 100 ml of 250 mM HCl was infused over 30 min into the duodenal fistula in seven dogs and peripheral venous blood removed at 0, 5, 10, 15, 30, 45 and 60 min after acid for secretin and insulin determinations. The dogs were fully conscious throughout this procedure and had been fasted overnight.

Blood was collected into a heparinised tube and plunged into an ice bath and plasma separated at 4°C. Plasma for secretin was immediately extracted by ethanol [6] and that for insulin frozen (–20°C) until assayed.

Plasma secretin (SG 18733, Batch EX XXI–14A, kindly donated by Dr. Miguel Ondetti, Squibb Institute for Medical Research) was used for standards and labelling. Secretin was labelled with I^{125} (Radiochemical Centre, Amersham) by the method of Holohan [9] and antibodies were raised to both synthetic secretin and pork natural secretin [10] (kindly donated by Dr. V. Mutt). Two antibodies were used in the studies BB33 raised against synthetic secretin (final titre 1:20,000 to 36,000); and BB101 raised against natural secretin (final titre 1:36,000). The antibodies appeared specific for secretin in that no cross-reaction was noted with pancreatic glucagon, large gut glucagon-like immunoreactivity [11] (GLI), human insulin (Medical Research Council), gastric inhibitory polypeptide and motilin (both kindly donated by Dr. J. C. Brown), 90 per cent pure cholecystokinin-pancreozymin (kindly donated by Dr. V. Mutt), human synthetic gastrin (Imperial Chemical Industries) and vasoactive intestinal polypeptide (kindly donated by Dr. V. Mutt).

Extracts of human jejunum and dog duodenum cross-reacted in the assay in an identical manner to the standards. The patients' samples were assayed using I^{125} secretin purified by ion exchange (R. W. J. Flanagan and K. D. Buchanan, unpublished observations) which resulted in a sensitivity of 6 pg/ml and the dog samples in a system detecting changes of 25–40 pg/ml with 95 per cent confidence. Plasma insulin was measured by radioimmunoassay utilising antibodies against pork insulin. The assay showed identical cross-reaction with human insulin (Medical Research Council) which was used as standards in the human experiments. Canine insulin showed poorer cross-reactivity

in the assay than human insulin and was used as the standard in the dog experiments. The insulin assay could detect 1 μ U/ml of human insulin and 2 μ U/ml of canine insulin. The statistical significance of differences was estimated using the "paired T" test.

Results

The fasting immunoreactive secretin (IRS) levels in the human studies are quite constant, all being less than 30 pg/ml with the exception of one which was 40 pg/ml. In Group A (Fig. 1) at the beginning of acid infusion there was a prompt rise in IRS levels which fell rapidly after termination of the infusion. The rise achieved statistical significance at 5, 10 and 15 min, ($p < 0.025$, < 0.005 and < 0.0125 respectively). In Group B (Fig. 2) there is no such rise.

In the dog experiments (Fig. 3) there was also a prompt rise in IRS after acid infusion. The rise achieved statistical significance at 5 and 10 min ($p < 0.05$ and < 0.01 respectively). There was no accompanying rise of immunoreactive insulin (IRI) in response to the acid in any of the studies.

Discussion

Our results clearly indicate that duodenal acidification is a potent stimulus for releasing secretin in dogs and in patients in Group A since there was a prompt increase in IRS concentration in the peripheral blood following the onset of acid perfusion. This is in agreement with the work of other investigators who found that the presence of acid in the duodenum caused increased secretion of bicarbonate by the pancreas and inferred that this was the result of secretin release. Although the patients in Group A had many different diseases (Table 1) it is justifiable to consider them together as their fasting and stimulated secretin levels were similar and there was no significant abnormality in the appearances of their jejunal mucosa. There are a number of possible explanations for the failure of IRS to respond in Group B. It may be that patients with the duodenal ulcer diathesis have a defective response, or maybe the physical changes brought about by duodenoscopy interfere with the response. However, Bloom and Ward have shown that patients with duodenal ulcer do have a secretin response to acid, although it is less than in normals [12]. The medications given to this group are obviously important, i. e. Papaveretum, Scopolamine and Diazepam. Clearly further experiments would be required to elucidate the separate roles of the drugs on the secretin response. However, it seems likely that Sco-

polamine is the most important. This would suggest that cholinergic mechanisms facilitate the response of the 'S' cells to hydrochloric acid. This concurs with the findings of earlier workers using secretion of pancreatic fluid and bicarbonate as evidence of secretin release [13, 14, 15]. There are three types of mechanism possible: (i) the vagus nerve; (ii) local cholinergic reflexes independent of the vagus and (iii) both (i) and (ii) together. The role of the vagus is undecided. Moreland and Johnson found in dogs a decrease in output of pancreatic fluid and bicarbonate in response to duodenal acidification following vagotomy [16]. Further studies measuring secretin levels in response to duodenal acidification are needed as Moreland and Johnson's results could be due to decreased sensitivity of the pancreas to secretin following vagotomy. Measurement of secretin after vagal stimulation would also be informative. The role of local reflexes is also clouded. Shapiro and Woodward found decreased output of pancreatic fluid and bicarbonate in dogs in response to duodenal acidification following application of topical anaesthetics to the mucosa, but damage to the mucosa by the anaesthetics was not excluded [17].

There are three reports of IRS levels after intraduodenal acid. Bloom et al and Chisholm et al showed, in man, significant rises of IRS after intraduodenal acid and the latter showed an accompanying rise in IRI [12, 18]. Boden et al have shown rises in IRS and portal IRI levels after intraduodenal acid in dogs, although peripheral IRI levels did not change [19]. Our results in man agree with those of Bloom et al., who found peripheral fasting levels of IRS to be below 50 pg/ml and after acid a rapid increase of about 100 per cent [12]. Our fasting levels are lower, but we have a similar percentage increase in response to acid and the duration and timing of our response is similar. The studies of Chisholm et al in man achieved different results in that the secretin levels were slow to achieve a peak (15 min) and a rather massive rise in IRS levels was noted [18]. In addition, they showed a rise in IRI levels which we did not. Our dog results also agree with those of Boden et al., who found peripheral circulating levels of IRS in the dog to be close to 50 pg/ml although, after acid, we noted only a 50 per cent rise in IRS, whereas Boden et al showed approximately a 300 per cent increase [19]. The differences in absolute IRS levels between these studies can be explained by variations in sensitivities of the individual assays, in antibody specificity and in the difficulty in obtaining suitable uniform standards. In this instance, we used an assay capable of detecting a 6 pg/ml change in IRS levels compared with that of Bloom's 25 pg/ml and Boden's 50–100 pg/ml [12, 19]. There is lack of agreement as to whether en-

Fig. 1. Plasma immunoreactive insulin (IRI) and immunoreactive secretin (IRS) levels after instillation of 100 ml 100 mM HCl into the duodenum in six human subjects (Group A). Figures are shown mean $\pm$ standard error of the mean ($M \pm SEM$). Significant rises in IRS were noted throughout the infusion ($p < 0.025$, < 0.005 , and < 0.0125 at 5, 10 and 15 min respectively). No change in IRI levels was noted

Fig. 2. Plasma IRI and IRS levels after instillation of intraduodenal acid in six human subjects (Group B). Neither IRS or IRI changed significantly throughout the infusion

Fig. 3. Plasma IRI and IRS levels after instillation of 100 ml 250 mM HCl intraduodenally in seven dogs. IRS significantly rose at 5' and 10' ($p < 0.05$ and < 0.01 respectively), but IRI was unchanged

ogenous secretin stimulates insulin release. Several workers have been unable to show this [20–25]. However, in patients with portocaval shunts, rises in peripheral IRI levels after acid can be detected [26] suggesting that the hepatic clearance of insulin may mask peripheral changes.

Our study shows that endogenous IRS release does not result in an increase of peripheral IRI levels. However, this does not exclude a rise of IRI in the portal blood and some workers have indicated that intraduodenal acid augments the insulin response to intravenous glucose [27, 28]. Our results do not exclude a secretin induced augmentation of IRI response to rises in blood sugar. It would thus be necessary to compare IRI response to intravenous glucose with and without stimulation of IRS before definite conclusions about the role of secretin in the entero-insular axis could be drawn.

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F. A. O'Connor, M. D.
Dept. of Medicine and Surgery
The Queen's Univ. of Belfast
Grosvenor Road
Belfast
Ireland