Plasma secretin and pancreatic bicarbonate response
to exogenous secretin in man

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SUMMARY  The dose response of duodenal bicarbonate production during synthetic porcine secretin
infusions was studied in six healthy volunteers and related to plasma secretin immunoreactivity.
Secretin was infused in each individual at four different doses from 0-1 to 2-7 CU/kg/h, each infusion
lasting for 60 minutes. Mean maximal bicarbonate secretion was 33 ± 4 mEq/h. The secretin plasma
level for half maximal bicarbonate response was estimated to be 22 pmol/l. As this level is reported
to be achieved by intraduodenal acidification in man, it is concluded that secretin may well play a
part in the control of duodenal pH.

Secretin, the first substance to be called a hormone, is generally assumed to be the physiological mes-
senger controlling the duodenal neutralisation of gastric acid by stimulation of pancreatic and biliary
alkaline secretion (Hubel, 1972). This is supported by more recent findings of rises in plasma secretin
measured by radioimmunoassay after intraduodenal acid in man (Ward and Bloom, 1974, 1975; Bloom
and Ward, 1975) and in dogs (Boden et al., 1974). In the dog a secretin infusion of 0-4 CU/kg/h produced
an pancreatic bicarbonate response equal to that of a natural meal when the pH was held constant at 5-0
(Grossman and Konturek, 1974). However, the release of secretin from the duodenum is said to
require an intraluminal pH of less than 4-5 and this is achieved in the duodenum for only limited periods
after a meal as there is considerable alkaline secre-
tion. Plasma secretin in man has not been shown to
increase after a meal (Bloom, 1975) and a rise in
plasma secretin has been reported in man only after
acidification of the distal duodenum with pure 0-1
molar hydrochloric acid, which is certainly not a
physiological stimulus. There has been considerable
debate, therefore, concerning the role of secretin—for
example, in ‘Is secretin secreted?’ (Wormsley,
1973). The present study using exogenous infusions
of pure synthetic secretin was undertaken to assess
the plasma level associated with the half maximal
pancreatic response to help clarify the physiological
role of secretin.

Methods

Six male subjects (mean age 36-5 years, range 23-42
years) in good health and free of any evidence of
gastrointestinal disease participated as volunteers
in this study. Each was studied after overnight fast in
the recumbent position. Continuous duodenal drainage was via a modified Lagerlof tube and
recovery of duodenal fluid corrected by continuous
addition of an inert marker as previously described
(Tymnper et al., 1974). Secretin was synthesised and
purified as earlier described (Wünsch et al., 1972),
1 μg synthetic secretin corresponding to 3-9 CU
natural secretin (Lehner et al., 1973).

After a basal period of 30 minutes four consecutive
continuous infusions of synthetic secretin were
started, each lasting 60 minutes. The dose of secretin
was triplicated from one infusion to the next, going
from 0-1 CU/kg/h to 2-7 CU/kg/h. Blood was with-
drawn from an antecubital vein into a heparinised
tube with 1000 KIU aprotinin (Trasylol) added per
1 ml blood, then rapidly centrifuged and the plasma
frozen at −20°C. Samples were taken at 10 minute
intervals before and after the infusions of secretin
and at five minute intervals during the infusions.

Duodenal bicarbonate concentration was mea-
sured by titration in an atmosphere of N₂ and bicarbonate secretin determined as earlier described (Tympner et al., 1974).

Plasma secretin immunoreactivity was determined by radioimmunoassay. Antisera were raised in rabbits to pure porcine secretin coupled by carbodiimide condensation to bovine serum albumin (molar ratio 2:1). Subcutaneous injections of 25 µg secretin per rabbit in complete Freund’s adjuvant were given at three monthly intervals and antisera harvested at one year. The antisera (SC10) of highest affinity reacted most avidly with whole secretin but also showed some partial reaction with C terminal, though not N terminal, secretin fragments. Chromatography of crude extracts of human jejunum demonstrated only a single peak of secretin immunoreactivity eluting in identical position to pure porcine secretin. No reaction was noted with 10 nmol/l gastric inhibitory polypeptide, vasoactive intestinal peptide or glucagon, three hormonal peptides with sequence similarities to porcine secretin. Duplicate assay tubes were set up with 160 µl plasma and 640 µl 0.05 M acetate buffer, pH 5.0, containing antiserum (final dilution 1:300,000), radioactive secretin label (1 fmol/tube) and 500 KIU aprotinin (Trasylol). After six days incubation at 4°C separation of antibody bound from free label was achieved by addition of 16 mg charcoal suspension, in 0.5 ml, to each assay tube followed by centrifugation and separation of supernatant. Secretin antibodies, covalently coupled to sepharose beads by the cyanogen bromide technique (Pharmacia Ltd) were used as a specific immunoabsorbent to produce a pooled secretin-free reference plasma. Known amounts of pure secretin were then reintroduced to this plasma to produce the assay standards. The secretin ¹²⁵I label was prepared from pure synthetic secretin by the lactoperoxidase technique (Holohan et al., 1974). Trace iodination of the secretin (about 5%) minimised peptide damage and pure moniodinated secretin was then separated from non-iodinated secretin by high resolution ion exchange chromatography on a 40 ml SP Sephadex T25 column run over 48 hours in 0.25 M pH 5.0 acetate buffer. The secretin ¹²⁵I was very stable and could be used for assay purposes for up to six months. The secretin content of unknown plasma samples was similarly stable (stored below −20°C) and assayable secretin content was found little changed after two years storage. The assay could detect changes in plasma secretin of 1.5 pmol/l with 95% confidence and addition of plasma containing 7.5 pmol/l secretin caused a drop in the percent label bound from 50% (zero point) of at least 10% (Fig. 1). Intra-assay variation was 4.5% and inter-assay variation 10%.

**Fig. 1** Radioimmunoassay standard curve. Each point is a mean of two duplicates.

**Results**

Figure 2 shows plasma concentrations of secretin immunoreactivity during the study. The ratio of the mean incremental plasma levels at the different infusion rates were 1 to 2.8 to 10.0 to 30.5 reflecting very closely the grading of the infusion secretin content. At the end of the last infusion there was a rapid fall in the plasma secretin to only 8% of the maximal increment after 10 minutes. Although there is not sufficient data for accurate calculation of the half life of secretin, an approximate value of about 2.8 minutes can be estimated.

Recovery of duodenal bicarbonate increased stepwise during the infusions to a maximum of 33 ± 4 mEq/h secreted in a volume of 363 ± 80.8 ml/h (Table). There was only a small further increment of both bicarbonate output and volume between the 0.9 and 2.7 CU/kg/h infusions. The semilogarithmic plot of secretin plasma immunoreactivity and duodenal bicarbonate secretion is shown in Fig. 3. It can be seen that there is a marked increase of bicarbonate output at very low plasma secretin levels. When each bicarbonate output is expressed in percent of the individual maximal response (Fig. 4) half maximal bicarbonate output is observed at a plasma secretin level of 22 pmol/l.

**Discussion**

To our knowledge the present study gives for the
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SECRETIN INFUSION CU/kg/h

0.1 0.3 0.9 2.7

Fig. 2 Plasma secretin in six volunteers during infusion of synthetic secretin (bar = SEM).

Table  Bicarbonate concentrations, bicarbonate output, and volumes of duodenal aspirates during secretin infusions of 0.1, 0.3, 0.9 and 2.7 CU/kg/h

<table>
<thead>
<tr>
<th>Secretin infusion (CU/kg/h)</th>
<th>n = 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>Bicarbonate concentration (mEq/l)</td>
<td>56.9</td>
</tr>
<tr>
<td>± SEM</td>
<td>11.1</td>
</tr>
<tr>
<td>Bicarbonate output (mEq/h)</td>
<td>6.3</td>
</tr>
<tr>
<td>± SEM</td>
<td>1.6</td>
</tr>
<tr>
<td>Volume (ml/h)</td>
<td>106</td>
</tr>
<tr>
<td>± SEM</td>
<td>17.4</td>
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</tbody>
</table>

The present study was conducted with porcine secretin in man. The amino acid sequence of human secretin is not known and may be different. There is some evidence, however, that little species differences occur. Firstly porcine secretin acts fairly uniformly from species to species always giving rise to a similar first time the correlation between exocrine pancreatic function and plasma secretin and also an estimate of the plasma secretin concentration necessary for half maximal stimulation of bicarbonate output. These results indicate that a small increment of plasma secretin is capable on its own of inducing a pancreatic bicarbonate secretion and the rise in concentration required is similar to that produced endogenously in man by acidification of the distal duodenum when measured with an identical assay system (Bloom, 1975).

This type of study has often been suggested but no method previously available was sufficiently sensitive to assay the low levels of secretin present in human plasma. The development of a high specificity radioimmunoassay capable of measuring normal fasting levels of secretin in man has now allowed these experiments to be performed.

Fig. 3 Dose response curve of duodenal bicarbonate output and plasma secretin immunoreactivity, as Fig. 2. The vertical line through each point indicates the SEM of bicarbonate output and the horizontal line the SEM of plasma secretin.
bicarbonate juice production (Hubel, 1972). Secondly, the S-cells of the duodenum and upper jejunum in man can be detected immunocytochemically and thus must contain a substance reacting with an antibody raised against porcine secretin (Polak et al., 1971; Robinson et al., 1975). Thirdly, we have observed that extracts of primate duodenum contain immunoreactive material which chromatographically moves identically to porcine secretin (Bryant and Bloom, 1975). Finally, immunoreactive secretin levels in the pig measured with our assay do not differ greatly from those in man (Bloom et al., unpublished observation).

The maximal bicarbonate secretion observed in this study is of the same order of magnitude as has been reported by others using highly purified natural secretin as well as synthetic secretin (Wormsley, 1968; Petersen, 1970; Konturek, 1970; Berstad et al., 1974; Gutierrez and Baron, 1973; Farooq et al., 1974; Vaysse et al., 1974). It can, therefore, be assumed that our higher dose of 2.7 CU/kg/h was sufficiently large to give virtually maximal response in the subjects studied. Two of the six had already achieved maximal secretion at the third dose level (0.9 CU/kg/h). The use of synthetic secretin assures absence of contamination with other hormones and response may therefore be attributed to the infused secretin. The plasma levels achieved during each infusion rose closely parallel with the infusion rates. This suggests that in man secretin clearance follows first order kinetics within the observed dose range. The roughly estimated half life for secretin of 2.8 minutes may therefore be close to the true value. This is supported by similar data obtained from dogs (Boden et al., 1974; Lehner et al., 1974).

The plasma secretin level of 22 pmol/l for half maximal pancreas stimulation in this study compares closely with previously reported endogenous plasma secretin concentrations during duodenal acidification of 18.5 pmol/l (Ward and Bloom, 1975) and after an oral lemon drink of 16.1 pmol/l (Häcki et al., 1976). This suggests that the rise in endogenous circulating secretin levels may have a significant influence in increasing pancreatic secretion in these circumstances. By contrast, after a meal no statistically significant rise in secretin has been reported. This may be because the release of secretin is phasic and, with the short half life, concentrations rapidly return to basal. As the timing of secretion release may vary from individual to individual, a very large number of subjects may be required for statistical significance. Further, the sensitivity of the present secretin assays may still not be adequate to detect small rises. In its natural physiological setting, however, secretin may be far more effective than in the fasting state. It has convincingly been shown that cholecystokinin at least doubles the effectiveness of secretin at lower dose levels (Vaysse et al., 1974) and cholecystokinin is probably significantly raised during duodenal acidification (Konturek et al., 1974). Thus a smaller secretin increment than seen after intraduodenal acid may be an equally effective bicarbonate stimulus in the postprandial state.

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References


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