J. Physiol. (1984), **356**, *pp.* 391–399
With 6 text-figures
Printed in Great Britain

CHARACTERISTICS OF SECRETIN-STIMULATED PANCREATIC SECRETION IN DOGS

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(*Received 6 January 1984*)

SUMMARY

1. The effect of the periodic interdigestive activity of the gut on secretin-stimulated pancreatic secretion was studied in eight conscious dogs each with a Thomas duodenal fistula, a gastric fistula and a Heidenhain pouch.

2. Pancreatic water and bicarbonate responses to a small dose of secretin were greatly augmented in phase with the spontaneous periodic activity of the gut. This augmentation was closely related to pancreatic protein secretion.

3. As the dose of secretin was increased the interval between peaks was prolonged, the peaks became less sharp, the nadirs were raised, and finally the periodic activity was no longer seen.

4. Bilateral cervical vagal blockade with local anaesthetics reduced the secretin-stimulated bicarbonate secretion by 50 % but the augmentation at the peak was not abolished. Atropine abolished the periodic augmentation completely and reduced the bicarbonate response by 80 %.

5. The peak response of volume and bicarbonate to secretin obeyed Michaelis–Menten kinetics. The nadir secretin dose response, however, was a sigmoid curve with a Hill coefficient larger than one. The action of atropine or hexamethonium was to shift the peak response kinetics to the nadir kinetics.

6. It is concluded that the pancreatic response to secretin is greatly modulated by the spontaneous periodic activity of nerves.

INTRODUCTION

The pancreatic response to a continuous intravenous infusion of secretin is believed to be a plateau, based on the assumption that the gland is resting during the interdigestive period. A sudden fall of the secretion during secretin infusion, therefore, has often been ascribed to technical failures such as the occlusion or malposition of the pancreatic cannula. Indeed the secretion usually recovers in half an hour. Contrary to this commonly accepted notion, basal pancreatic secretion waxes and wanes with the periodic interdigestive motility of the upper gastrointestinal tract

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(Magee & Naruse, 1983). Though this phenomenon has been known from the beginning of this century (Boldyreff, 1911), little attention has been paid to its effect on stimulated secretion. We have, therefore, re-evaluated the effect of secretin on the pancreatic secretion in relation to the interdigestive activity of the gut.

METHODS

Each of eight dogs (17–27 kg) was provided with a gastric fistula, a Heidenhain pouch and a Thomas type duodenal fistula under thiopentone and ether anaesthesia. One month was allowed for recovery and each was tested once a week.

After an 18 h fast the animals were restrained in Pavlov stands during the study. Pancreatic secretion was collected every 10 min by cannulation of the duct via the Thomas fistula. The gastric fistula was kept open to drain its secretion.

An intravenous infusion of saline (150 mM-NaCl, 8 mM-KCl) was maintained to replace fluid loss. The infusion rate was adjusted to 1–4 ml/min depending on the pancreatic secretory rate. Motility of the stomach and the duodenum was recorded by the balloon method and pouch motility by the direct method, via pressure transducers, on a polygraph (Narco Bioscience, Houston, TX) (Magee & Naruse, 1983).

Control observations for 6 h were made on each animal with saline infusion alone. Porcine secretin (Karolinska Institutet, Stockholm) was given intravenously at 0·1, 0·2, 0·4, 0·8 and 1·6 clinical units (c.u.)/kg per hour 30 min after the spontaneous peaks when the basal secretion was in its trough. Only one dose was tested on each experiment day. Following the observation of one complete cycle (from the beginning of the trough to the next one) under secretin infusion (0·2 c.u./kg per hour) either atropine sulphate (Lilly, Indianapolis, 25 μg/kg I.V. followed by 25 μg/kg per hour) or bilateral cervical vagal blockade by 2% lidocaine HCl (Astra, Worcester) was given, 30 min after the pancreatic peak, for 100 min (Magee & Naruse, 1983). In another series secretin was given intravenously, doubling the dose from 0·1 to 1·6 c.u./kg per hour every 50 min, with concomitant infusion of atropine (25 μg/kg per hour) or hexamethonium chloride (Mann Research Lab., New York, 2 mg/kg per hour).

To analyse the periodic activity samples were renumbered by choosing either peaks or troughs as origins (Magee & Naruse, 1983). Means and standard errors were calculated for each of the newly numbered samples; n is the number of dogs. Regression analysis was carried out by the method of least squares. Hill's equation (Dixon & Webb, 1979) was applied for the analysis of dose–response curves

$$V = \frac{V_{max}(S)n_H}{K+(S)n_H}, \quad (1)$$

where V is secretory rate; V_{max}, maximal secretory rate; S, dose of secretin; n_H, Hill's coefficient; K, constant. When $n_H = 1$, the equation is equivalent to Michaelis–Menten's equation and $K = K_m$ (Michaelis constant). V_{max} is calculated by the Lineweaver–Burk transformation of Michaelis–Menten's equation. n_H is obtained by the logarithmic transformation of eqn. (1)

$$\log \frac{V}{V_{max}-V} = n_H \log S - \log K. \quad (2)$$

Paired t tests were used for comparison and $P < 0{\cdot}05$ was taken as level of significance.

RESULTS

Basal secretion. Basal pancreatic secretion increased in phase with the motility of the stomach, the duodenum and the Heidenhain pouch. It peaked regularly within the 10 min preceding the duodenal motility peaks. Peak secretions of water, bicarbonate and protein were $2{\cdot}2 \pm 0{\cdot}3$ (mean $\pm$ S.E. of mean, $n = 6$) ml/10 min, $0{\cdot}18 \pm 0{\cdot}04$ mmol/10 min and 201 ± 40 mg/10 min, respectively. The average interval between peaks was 98 ± 8 min.

Secretin-stimulated secretion. Pancreatic water, bicarbonate and protein responses to continuous infusion of lower doses of secretin maintained periodicity in phase with the gut motility. The volume and bicarbonate responses at each lower dose of secretin were greatly augmented by the intrinsic periodic activity (Fig. 1). The augmented secretion (protein volume and bicarbonate) decreased rapidly with the beginning of

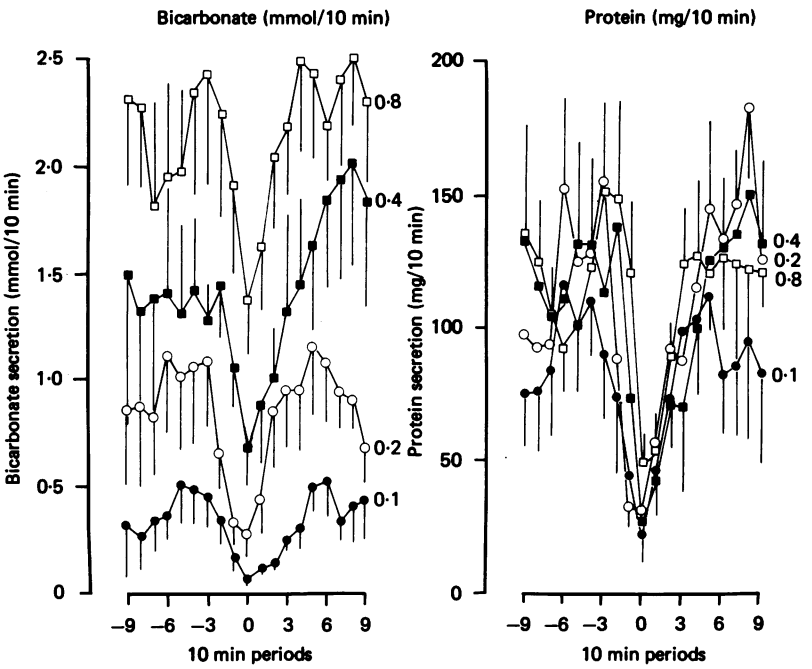

Fig. 1. The bicarbonate and protein response of the pancreas to secretin at 0·1, 0·2, 0·4 and 0·8 c.u./kg per hour in six dogs. The nadirs are designated time zero. Means ± S.E. of the mean are shown ($n = 6$).

the duodenal peaks. The peak/nadir ratios for bicarbonate were 18·6, 5·3, 3·6 and 2·1 to 0·1, 0·2, 0·4 and 0·8 c.u./kg per hour of secretin, respectively. Protein responses at the peak or the nadir were not significantly different among the different doses of secretin. At the low dose there was a significant ($P < 0·01$) correlation between volume or bicarbonate augmentation and protein secretion but this had disappeared at 1·6 c.u./kg per hour (Fig. 2). As the dose of secretin was increased, the intervals between duodenal peaks were prolonged: 112 ± 8, 123 ± 8, 128 ± 10 and 137 ± 10 min for 0·1, 0·2, 0·4 and 0·8 c.u. secretin kg/h, respectively. This was due to an increase in the period between duodenal motor quiescence and the peak. With increasing doses of secretin, pancreatic secretion during the quiescent phase was progressively raised towards the level of the peak, so that when the dose of 1·6 c.u./kg per hour was reached the nadirs were no longer seen, the periodicity was abolished and the relationship between volume and protein was no longer significant (Fig. 2).

Effect of vagal blockade and atropine. Bilateral cervical vagal blockade with local anaesthetics reduced the duodenal motility preceding the peak but the peak activity remained unchanged. The volume, bicarbonate and protein responses to secretin

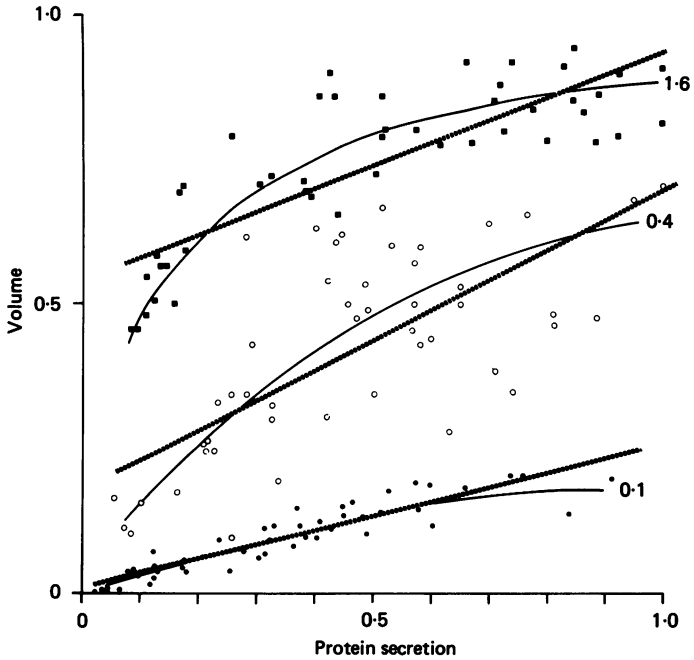

Fig. 2. The relationship between volume and protein secretion during infusion of secretin at 0·1, 0·4, 1·6 c.u./kg per hour in six dogs. The results are expressed as the fraction of the maximal secretion for volume and of the peak secretion for protein in each animal. Dotted lines were obtained by linear regression and continuous lines by visual inspection. P for correlation coefficients (r) are less than 0·01 for regression lines 0·1 and 0·4 and more than 0·1 for 1·6.

TABLE 1. Hill coefficient (n_H) and apparent Michaelis constant (K_m) in five dogs

	Volume n_H	Apparent K_m (c.u./kg per hour)	Bicarbonate n_H	Apparent K_m (c.u./kg per hour)
Peak	1·07	0·35	1·02	0·38
Nadir	1·51	1·39	1·73	1·32
Atropine	1·64	1·05	2·00	0·92
Hexamethonium	1·63	2·95	2·19	3·09
Calculated V_{max}	28·5 ml/10 min		4·17 mmol/10 min	

(0·2 c.u./kg per hour) were reduced, but the augmentation at the peak activity remained following this treatment (Fig. 3). Atropine completely abolished the periodic motility of the gut and the periodic augmentation of secretin-stimulated secretion (Fig. 4). Thus, as a whole, vagal blockade reduced the volume, bicarbonate and protein secretion in one cycle by 42, 49 and 51 % and atropine by 71, 83 and 93 % respectively.

Kinetic analysis. Since no single value can represent the response to a specific dose of secretin due to the periodic change, those at the peak and at the nadir were chosen for analysis. The peak volume and bicarbonate responses to secretin showed the Michaelis–Menten relationship, i.e. rectangular hyperbolas in linear plots (Fig. 5 and

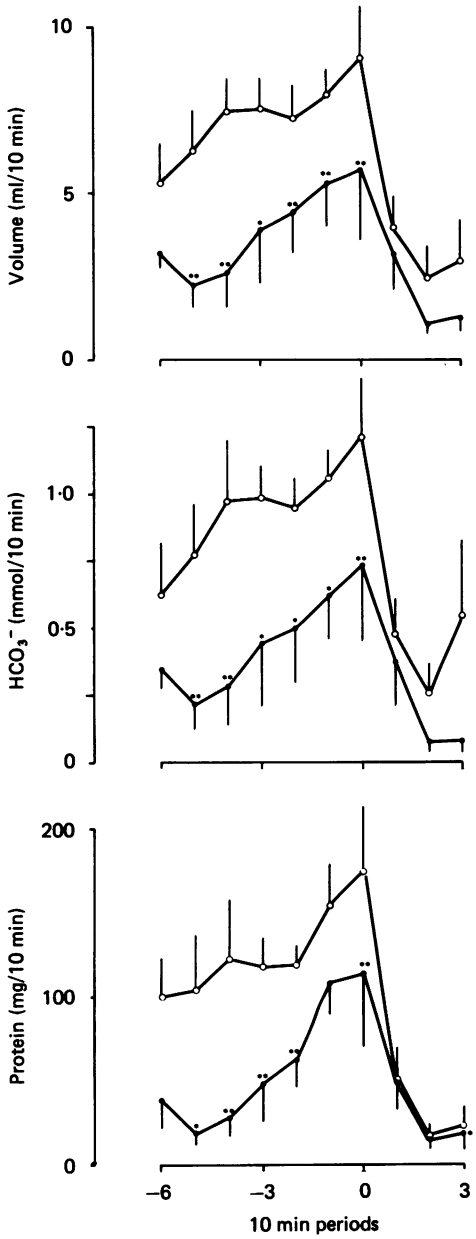

Fig. 3. The effect of bilateral cervical vagal blockade by lidocaine on pancreatic secretion to secretin (0·2 c.u./kg per hour) in five dogs. Open and filled circles indicate control (secretin alone) and vagal blockade respectively. The pancreatic peaks are designated as time zero. Means ± S.E. of the mean are shown ($n = 5$). Asterisks indicate significant difference ($*P < 0·05$, $**P < 0·01$) from control.

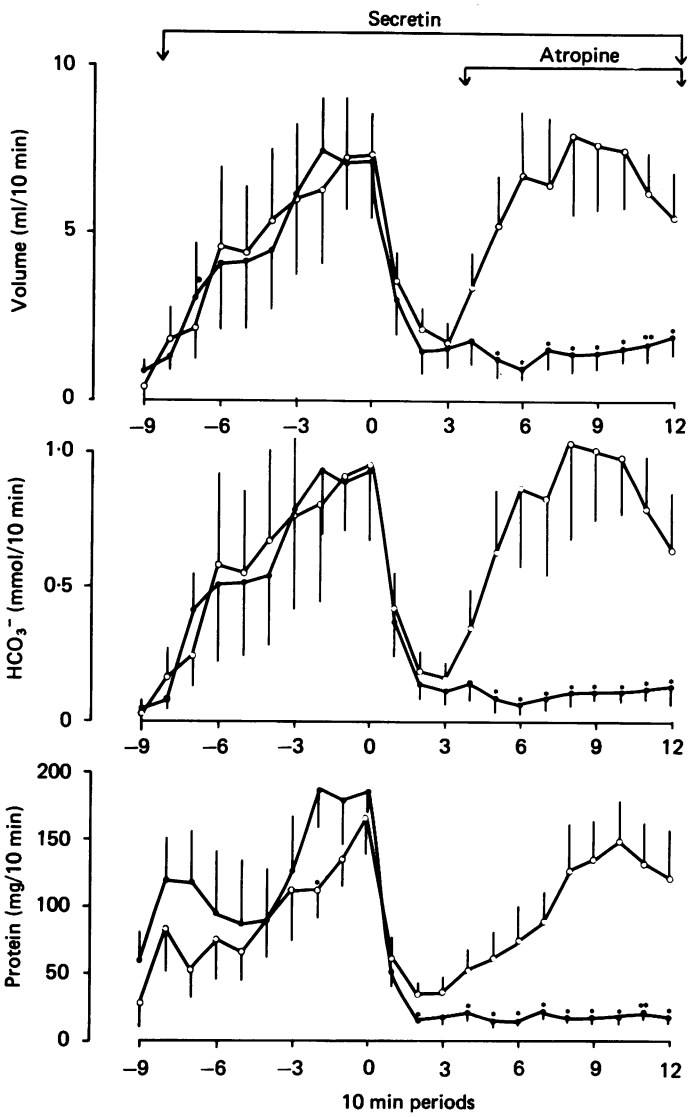

Fig. 4. The effect of atropine (25 μg/kg I.V. followed by 25 μg/kg per hour) on pancreatic secretion during secretin (0·2 c.u./kg per hour) in five dogs. The first pancreatic peaks are designated as time zero. Open and filled circles indicate control (secretin alone) and atropine respectively. Means ± S.E. of the means are shown ($n = 5$). Asterisks indicate significant difference (*$P < 0·05$; **$P < 0·01$) from control.

Table 1) and straight lines in Lineweaver–Burk plots (Fig. 6 and Table 1). The nadir secretin dose responses were sigmoid curves with Hill coefficients larger than one, indicating that they do not obey Michaelis–Menten kinetics. The action of atropine was to shift the peak curves to the nadir ones. Hexamethonium shifted them even further to the right (Fig. 5 and Table 1).

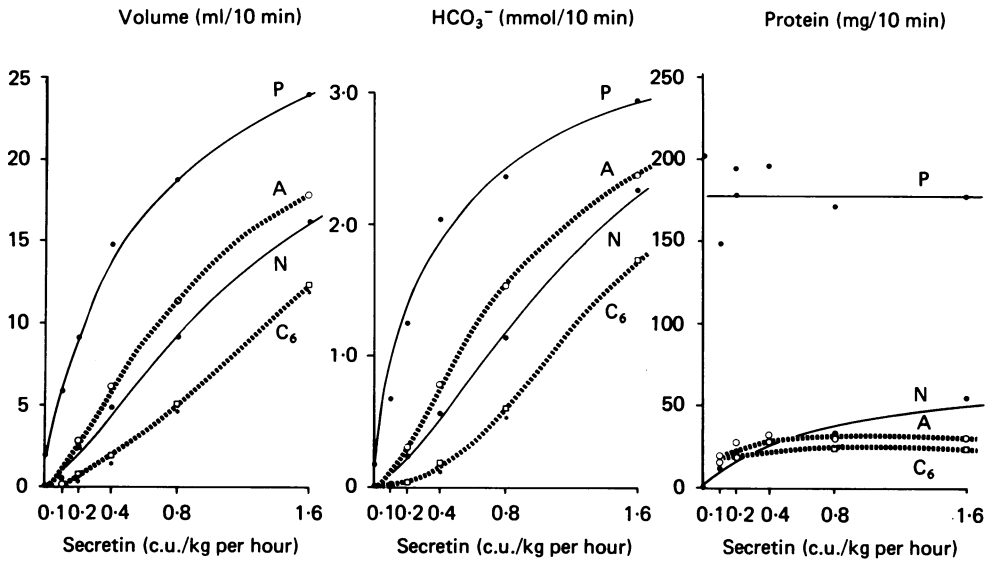

Fig. 5. Dose–response curves of the pancreas to secretin. Mean responses of five dogs are shown. P: peak response: N: nadir response, A: response with atropine (25 μg/kg per hour), C_6: response with hexamethonium (2 mg/kg per hour).

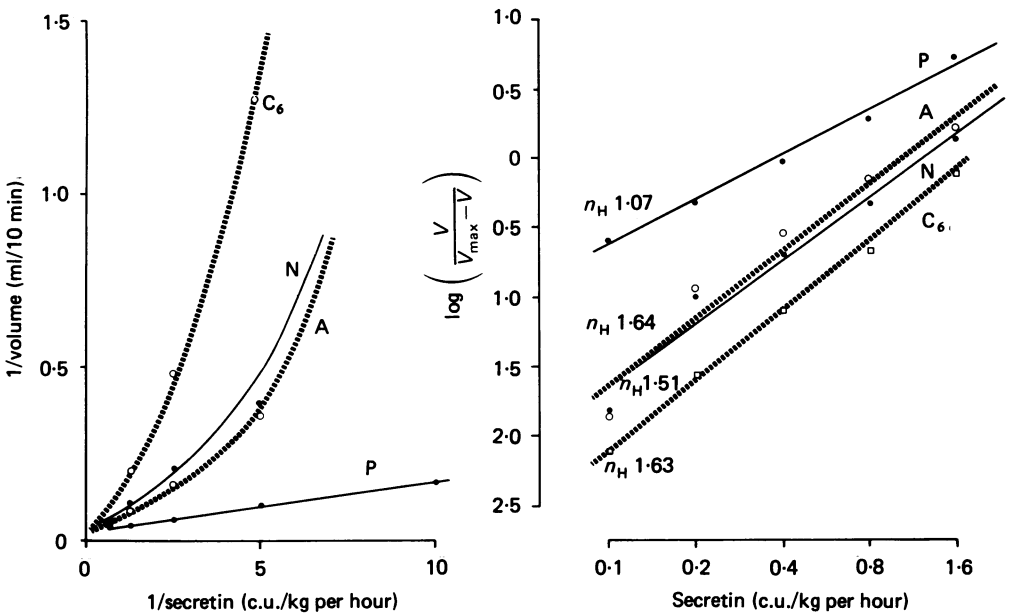

Fig. 6. Lineweaver–Burk and Hill plots of the volume response to secretin. Means of five dogs are given. Abbreviations are same as in Fig. 5.

DISCUSSION

It is now clear that pancreatic secretory fluctuations during infusion of a fixed dose of secretin are not due to technical faults, but to the spontaneous intrinsic activity of the gland. Protein secretion maintained its characteristic interdigestive periodicity during secretin. The augmentation of secretin-stimulated water and bicarbonate secretion was significantly related to this protein periodicity. As the dose of the hormone was increased the intervals between peaks were prolonged, the secretory peaks became less sharp, the nadirs were raised and finally the periodic activity was no longer seen; since periodic protein secretion continued, the straight line relationship between water and protein secretion was no longer evident (Fig. 2). The inhibitory effect of this hormone on the periodic interdigestive motility of the gut has been reported when large doses are used (Mukhopadhyay, Johnson, Copeland & Weisbrodt, 1975). These actions seemed to be pharmacological, but they indicate that secretin can act on the spontaneous intrinsic activity.

Many of the past controversies on the effect of vagotomy and atropine on pancreatic secretion (Magee, 1982) seem to result from the failure to consider this phenomenon when analysing the results. Atropine or ganglion blockade abolished interdigestive periodic pancreatic secretion (Magee & Naruse, 1983) and, as expected, abolished the periodic augmentation of secretin-stimulated water and bicarbonate secretion. Thus, there would be no inhibition of the secretion by these drugs during the quiescent period, but a profound one during the peak. Simple amalgamation of all these results may lead to the conclusion that these drugs have no effect. The effect of vagotomy on pancreatic secretion is a little more complicated because vagal blockade depressed the secretion, but did not abolish the periodic pancreatic peak (Magee & Naruse, 1983). Therefore secretin-stimulated secretion was reduced as a whole, but the augmentation at the peak period remained.

What is the nature of this periodic augmentation of secretin-stimulated pancreatic secretion? Pharmacological evidence indicated that it was neural. Control by a periodic release of cholecystokinin (CCK) is suggested in a preliminary report by Chen, Chey, Lee & Chang (1983). It seems that this CCK mechanism is also under a cholinergic control because it is suppressed by atropine. Whether such a small increase of CCK (about 20 pg/ml) can explain a large increase of protein secretion awaits further studies. A close correlation between protein secretion and the degree of water and bicarbonate augmentation indicates that this neural mechanism, when acting on acinar cells either directly or indirectly via CCK, results in protein secretion and, when acting on duct cells, results in augmentation of water and bicarbonate secretion.

The kinetic analysis showed that the peak response of volume and bicarbonate to secretin obeys the classical Michaelis–Menten relationship. The nadir secretin dose response, however, was a sigmoid curve, suggesting co-operative binding of secretin to its receptor on the pancreas (Dixon & Webb, 1979). The action of atropine was to shift the peak curve to the nadir one. A further shift by hexamethonium indicates the importance of nicotinic mechanisms (Hong & Magee, 1970; Devaux, Diaz, Kubota, Magee & Sarles, 1983 (Fig. 5 and Table 1)). Maximal pancreatic secretion in response to secretin is not changed by atropine (Magee, Fragola & White, 1965; Singer, Solomon, Rammert, Caspary, Niebel, Goebell & Grossman, 1981) or by

cholinomimetics (Vaysse, Bastie, Pascal, Roux, Martinel, Lacroix & Ribet, 1975). It seems, therefore, that nerves control the sensitivity of the pancreas as a positive modulator of the secretin effect by changing the aparent K_m and n_H without changing the V_{max}. Furthermore, the relationship between bicarbonate and protein secretion (Fig. 2) indicates that delicate control by this mechanism is possible over a wide range of secretory rates. Thus the kinetic behaviour of the pancreas to secretin and nervous stimulation is quite similar to that of so-called allosteric enzymes, which play specific roles in the regulation of metabolism (Monod, Changeux & Jacob, 1963).

Plasma levels of secretin following meals are very low. According to Kim, Lee & Chey (1979) the peak level of 55 pg/ml following meals can be attained by 0·25 c.u. secretin/kg per hour or 0·55 mmol intraduodenal HCl/10 min and plateau levels by 0·125 c.u. secretin/kg per hour. The pancreatic bicarbonate response at the nadir to 0·2 c.u./kg per hour of secretin alone, is only 0·24 mmol/10 min (Fig. 1). The activation of the nervous mechanism at the peak shifts the apparent K_m for secretin from the pharmacological (1·32 c.u./kg per hour) towards the physiological range (0·38 c.u./kg per hour) (Table 1). The pancreas can now secrete 1·26 mmol bicarbonate/10 min, which is sufficient to neutralize the acid which liberates secretin. It seems, therefore, that this neural modulation is especially important for the physiological action of secretin.

This work was supported by grant no. PCM8003446 from the National Science Foundation of the United States.

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