J. Physiol. (1975), **252**, *pp.* 379–396
With 5 text-figures
Printed in Great Britain

SECRETION OF ELECTROLYTES BY THE PANCREAS OF THE ANAESTHETIZED RAT

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(*Received 25 March 1975*)

SUMMARY

1. HCO_3^-, Na^+ and K^+ concentrations were measured in bile-free pancreatic juice collected from fasted and fed anaesthetized rats.

2. Resting flow rates averaged 0·62 μl. $g^{-1}.min^{-1}$ (fasted) and 2·8 μl. $g^{-1}.min^{-1}$ (fed) and the mean HCO_3^- concentrations, respectively, were 25·8 and 33·3 mM.

3. In fasted rats, instillation of HCl into the duodenum caused flow rate to increase threefold and HCO_3^- concentrations to double (66 mM). Intravenous infusion of pure natural (GIH) secretin caused a fivefold increase in flow rate; HCO_3^- concentrations, again, doubled (67·5 mM). Infusion of synthetic secretin produced effects essentially the same as those produced by GIH secretin.

4. Infusion of Boots secretin caused a thirteenfold increase in flow rate (8·32 μl. $g^{-1}.min^{-1}$) but HCO_3^- concentrations rose only slightly (43·3 mM). However, following cessation of infusion, when flow rate approximated the maximum obtained with pure secretin, the HCO_3^- concentration was much higher (57·2 mM at 3·19 μl. $g^{-1}.min^{-1}$). In fed animals the responses were similar but maximum flow rates were greater (12 μl. $g^{-1}.min^{-1}$).

5. Infusion of caerulein produced a secretory rate slightly less than with Boots secretin (5·06 μl. $g^{-1}.min^{-1}$) and HCO_3^- concentrations were plasma-like (30·2 mM); infusion of the synthetic octapeptide of cholecystokinin (OP-CCK) gave similar flow rates and HCO_3^- concentrations.

6. Infusion of a mixture of caerulein and GIH secretin mimicked closely the effect of Boots secretin. At maximum flow rates (7·6 μl. $g^{-1}.min^{-1}$) the HCO_3^- concentration was 43·7 mM and at lower flow rates (3·90 μl. $g^{-1}.min^{-1}$) it rose to 54·2 mM.

7. It is concluded that the response of the rat pancreas to secretin is qualitatively similar to that of all other vertebrates so far studied, but, relative to other animals, the response is sluggish. In contrast, the rat pancreas responds well to cholecystokinin (CCK) stimulation, yielding a

juice with plasma-like HCO_3^- concentration. Boots secretin, which is heavily contaminated with CCK, causes a mixed response resembling that of CCK at high secretory rates and that of pure secretin at lower rates.

8. An unexplained feature of rat pancreatic juice was that K^+ concentrations, although plasma-like in unstimulated samples, rose to about 8 mM when flow rate increased as a result of secretin, but not CCK, stimulation. In all other animals so far studied, the K^+ concentration has been found to be independent of flow rate.

INTRODUCTION

In 1902 Bayliss & Starling first demonstrated that mediation of the pancreatic response to acid in the duodenum depended on release of secretin by cells of the intestinal mucosa. The original studies of Bayliss & Starling (1902) were performed in the dog, but, subsequently (Bayliss & Starling, 1903), they were able to demonstrate the presence of the hormone in the intestinal mucosa, not only of many mammals (man, monkey, dog, cat, rabbit, ox, sheep, pig and squirrel) but also of fishes (teleosts and elasmobranchs), frogs and turtles. In the following decades it was realized that, not only did secretin increase flow of pancreatic juice, but that it also increased the HCO_3^- concentration of the juice. Most of these studies were performed on the dog (Ball, 1930; Komarov, Langstroth & McRae, 1939; Hart & Thomas, 1945; Bro-Rasmussen, Killman & Thaysen, 1956) but excellent studies are also available for the cat (Case, Harper & Scratcherd, 1969*a*). In addition, data are available for man (Dreiling & Janowitz, 1959) and for the pig (Hickson, 1970), rabbit (Rothman, 1964), calf (Ternouth & Buttle, 1973) and horse (Comline, Hall, Hickson, Murilio & Walker, 1969). In all these species, HCO_3^- concentrations have been found to be plasma-like at low secretory rates and, following stimulation with secretin, to rise asymptotically to a maximum at high flow rates. In dog, cat, pig and man there is little or no spontaneous flow of juice and, following stimulation, the HCO_3^- concentration approaches an asymptote well in excess of 100 mM; in rabbit, calf and horse, on the other hand, there is a considerable resting flow rate and, although secretin causes HCO_3^- concentrations to rise, the maximum is less than 100 mM.

Technical difficulties associated with collection of bile-free juice impeded investigation in the rat but, eventually, Grossman (1958), using a conscious, chronic-fistula preparation, was able to demonstrate that the rat, like the rabbit, calf and horse, had a high spontaneous secretory rate that could be increased moderately by I.V. infusions of secretin, or by feeding.

There are only a few reports published on the HCO_3^- content of rat

pancreatic juice. Heatley (1968), who used anaesthetized rats, reported a HCO_3^- concentration of 39 mM in resting juice which rose to 58 mM following stimulation of the pancreas with a highly purified secretin preparation. In conscious rats, with chronic in-dwelling cannulae, Shaw & Heath (1972) reported a HCO_3^- concentration of only 18 mM in resting juice, which rose to 40 mM following stimulation with GIH secretin. However, two other studies (Mangos & McSherry, 1971; Mangos, McSherry, Noussia-Arvanitakis & Schilling, 1974) on anaesthetized, fed rats, stimulated with Boots secretin, have yielded startlingly different results. In these studies, HCO_3^- concentrations were found to be very high (70 mM) in samples of juice collected at low flow rates and fell towards plasma-like values as flow rate was increased. Since this finding would seem to imply that the gastrointestinal physiology of the rat was vastly different from that of other animals, we decided to repeat Mangos' experiments and extend them in order to establish what had caused his rats to respond so differently.

Our results show that, when stimulated with pure, natural secretin, or synthetic secretin, the rat pancreas responds poorly but in a qualitatively similar manner to that seen for other animals; the unusual response seen by Mangos is elicited only with Boots secretin (or a mixture of caerulein and GIH secretin) and arises because Boots secretin is heavily contaminated with cholecystokinin (CCK), to which the rat is far more sensitive than it is to secretin.

We have already published a preliminary report of this work (Sewell & Young, 1974).

METHODS

Male albino Wistar rats (180–280 g body weight) were used. 'Fed' animals had access to food and water up to the time of surgery while 'fasted' animals were left overnight without food, but with access to water. All animals were anaesthetized with Inactin^R (Na salt of 5-ethyl-(1-methylpropyl)-2-thiobarbiturate) in a dose of 120 mg.kg^{-1} body weight. Routinely, the animals were tracheostomized and placed on a thermostatically controlled heating table so as to maintain body temperature at 37·5° C.

The surgical technique that we used to permit collection of pancreatic juice from the rat has been described in detail by Grossman (1958). First, the pylorus was exposed through a midline incision and ligated, so as to prevent gastric contents from entering the duodenum. Following this, the posterior surface of the pancreas was exposed and the bile duct located. In the rat there is no main pancreatic duct, rather the pancreas drains by a number of separate ductules into the bile duct. In order to collect the pancreatic juice, free of bile, it was first necessary, either to divert the bile flow by cannulation of the bile duct near the liver, or else, more simply, to tie off the bile duct completely. Since it is much simpler to perform ligation without damage to the pancreas, we adopted this procedure in most experiments. It was considered feasible since the experiments last only a few hours; a comparison of pancreatic responses failed to reveal any significant effect that could be attributed to bile duct ligation.

In all experiments, the juice was collected for about 1 hr before any pancreatic stimulation was undertaken. Stimulation was then begun, usually by i.v. infusion of a polypeptide hormone dissolved in physiological bicarbonate-saline via a catheter placed in the jugular vein. The rate of the infusion was 0·0375 ml.min^{-1} and its duration was 30 min.

Samples of pancreatic juice were collected consecutively during the infusion period and following cessation of infusion, until the flow rate of the juice had returned to control levels (often 200 min); collection time, which we kept as short as possible, was adjusted to ensure that the volume of juice collected (about 20–40 μl.) was adequate for analysis. In one series of experiments, the pancreas was stimulated by infusion of acid into the duodenum via a catheter inserted through the duodenal wall. After collection of samples of unstimulated juice, infusion of isotonic saline into the duodenum was performed for 30 min; the infusion was then changed to an isotonic solution of HCl (0·1 M) and NaCl, and infusion was continued for another 30 min. Samples of juice were collected during and after the infusions.

Juice was collected under paraffin oil in tared polyethylene tubes, and sample volumes were determined, after collection, by weighing (assuming a specific gravity near to unity). Samples were stored frozen pending analysis. Blood samples were collected from the tail vein and, after centrifugation, the plasma was stored frozen.

At the end of each experiment, about 0·1 ml. of a 2 % solution of Lissamin Green was injected retrograde up the duct system so as to outline the pancreatic lobes. The animals were then killed and the green-stained pancreatic tissue was dissected free and weighed. Since unstained tissues were not weighed, pancreatic lobes that drained only by an accessory duct, directly into the duodenum (Herriott, Sinnet & Palmer, 1965) would have been overlooked. The average rate of formation of juice during each collection period was expressed per g wet gland tissue. In our series, the gland weights ranged from 0·70 to 1·40 g (mean 0·96 g for average body weight of 225 g).

The following preparations were used to stimulate pancreatic secretion.

Boots secretin. Prepared by the Boots Company Ltd, Nottingham (batch nos. 129 and 132). This is a crude extract of porcine intestinal mucosa prepared as described by Crick, Harper & Raper (1949); secretin makes up only a minute fraction of the dry powder. In batches nos. 129 and 132 the secretin activities, respectively, were 2·8 and 3·2 Crick–Harper–Raper (CHR) units per mg dry powder. All batches are contaminated with CCK; the exact CCK activity of batch no. 129 is unknown but in batch no. 132 the manufacturers advise us that CCK contamination was exceptionally high, being greater than the 'normal' upper limit of 25 CHR μ. of CCK per 100 CHR μ. of secretin (Dr J. Warwick Butler, The Boots Company Ltd: letters of 17 and 25 October 1974). Solutions of the preparation in bicarbonate-saline were prepared immediately before each experiment.

GIH secretin. Prepared by Professor V. Mutt (Jorpes & Mutt, 1973), Karolinska Institutet, Stockholm (batch nos. 17411 and 17451). Each vial contained about 20 μg of purified porcine secretin (equivalent to 75 clinical units or about 600–650 CHR μ.: *vide* Harper (1972)) and is said not to be contaminated with CCK; the preparation is mixed with cysteine-HCl. Since solutions of GIH secretin in physiological bicarbonate-saline are unstable and lose potency within a few hours, the contents of each ampoule were dissolved in 100 μl. acid-ethanol (75 ml.ethanol, 1·5 ml.conc. HCl, 23·5 ml.water), subdivided into 5 aliquots, and stored at $-20°$ C. Immediately before each experiment, an aliquot was dried by evaporation and dissolved and neutralized in bicarbonate-saline. In order to prevent the positively charged secretin molecules from adhering to the negatively charged glass walls of

the infusion syringe, bovine serum albumin (0·3 g/100 ml.) was added to the solution (Dr M. Coleman, personal communication, 1974).

Synthetic porcine secretin. Prepared by the Schwarz-Mann Company, New York (batch no. ZY-1035), according to Bodanszky (1973). Each vial contains about 100 clinical units mixed with cysteine-HCl and has a calculated activity of 0·267 μmole of peptide per mg dry powder. The material was dispensed exactly as for GIH secretin.

Caerulein. This is a decapeptide amide isolated from the skin of the frog, *Hyla caerulia*, with close structural similarity and almost identical biological properties to the octapeptide of CCK (Bertaccini, 1971). It was prepared by the Calbiochem Company, Los Angeles (batch no. 206411, lot 000081). Each vial, containing 150 μg caerulein mixed with 30 mg mannitol, was dissolved in 5 ml. bicarbonate saline and stored in polystyrene vials at $-20°$ C.

Synthetic octapaptide of cholecystokinin (OP-CCK). Prepared by E. Squibb & Sons, New Jersey (batch no. NN 005NA). The drug was dissolved in bicarbonate saline (0·5 μg/μl.) and stored at $-20°$ C as for caerulein.

Analytical techniques. Na^+ and K^+ were measured with the aid of an Eppendorf^R flame photometer, using samples of volume 3 μl. diluted in 3 ml. water. For approximately plasma-like concentrations, as encountered in our experiments, the sample standard deviation was 2 % for Na^+ and 4 % for K^+. HCO_3^- was determined as total CO_2 with the aid of a Natelson Microgasometer^R (Natelson, 1951) using a sample volume of 10 μl. The sample standard deviation was 2·4 %.

RESULTS

The secretory rate and HCO_3^- concentration for juice collected from glands prior to the administration of any exogenous stimulus are shown in Table 1. In fasted rats the mean flow rate averaged 0·62 μl. $g^{-1}.min^{-1}$ whereas in fed rats it was more than 4 times as great ($P < 0·001$). Similarly, the mean HCO_3^- concentration in samples from fed rats was 7–8 mM higher than from starved animals ($P < 0·01$). The mean plasma HCO_3^- concentration in a series of twenty-eight starved animals was 29·2 mM $\pm$ 0·5 S.E. of mean; a paired t test showed that this value was significantly greater ($P < 0·01$) than the concentration in resting juice from starved animals.

Stimulation with endogenous and exogenous secretin

Fig. 1 depicts results of three sets of experiments. In one group, 0·1 M-HCL was infused intraduodenally to provoke release of the animals' endogenous secretin, and in the other two groups, either GIH secretin or synthetic secretin was infused I.V. at a rate of 0·4 clinical units/min. This dose was arrived at in pilot experiments where supramaximal doses of up to 4·0 μ./min were infused initially and then reduced step-wise; the level was maintained well above the minimum necessary to sustain a maximum secretory response. The mean flow rate and HCO_3^- concentration in samples of resting pancreatic juice and in samples collected during the

maximum secretory response to each stimulant are given in Table 1. In all three cases, the secretory rate increased during stimulation and there was a concomitant increase in juice HCO_3^- concentration to 60–80 mM. The response to intraduodenal HCl was least vigorous. Following cessation of administration of the stimulant, juice flow rates and HCO_3^- concentrations fell again (Fig. 1) but, in general, did not attain pre-stimulus levels.

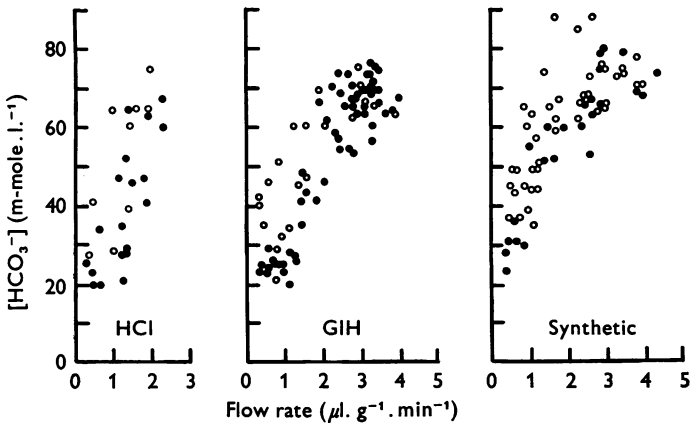

Fig. 1. The relation between secretory rate and HCO_3^- concentration for juice collected from the pancreas of fasted, anaesthetized rats. Secretion was stimulated (*a*) by infusion of HCl intraduodenally, (*b*) by I.V. infusion of GIH secretin, (*c*) by I.V. infusion of synthetic porcine secretin. ● indicates samples collected before and during administration of the stimulus; ○ indicates samples collected after cessation of administration of the stimulus.

Fig. 2 shows the time course of a typical experiment (in this case with GIH secretin). Flow rate and HCO_3^- concentrations rose sharply and remained elevated during secretin infusion. Following cessation of infusion, flow rates and HCO_3^- concentrations had fallen substantially by 40 min, but HCO_3^- concentration still remained above control levels after 165 min.

Stimulation with caerulein or OP-CCK

Fig. 3 depicts results of experiments in which either caerulein or OP-CCK was infused I.V. Choice of a maximum dose presented some problems since excessive doses of either of these compounds were found to cause an irreversible reduction in flow rate. This may have been because the high protein content rendered the juice excessively viscous (Debray, Vaille, de la Tour, Rozé, Souchard, Chariot & Fox, 1973) or because the drugs caused a precipitate fall in blood pressure (Erspamer, 1970). By trial and error, an infusion rate for caerulein of 3·1 p-mole.min^{-1} was

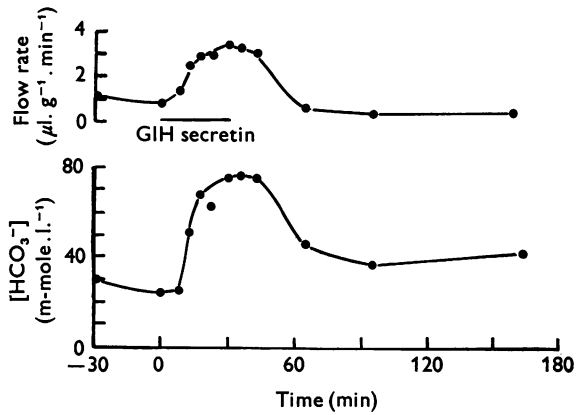

Fig. 2. Time course of a single experiment in which a fasted, anaesthetized rat received I.V. GIH secretin (0·4 clinical μ./min) during the period indicated by the horizontal bar.

TABLE 1. HCO_3^- concentration and flow rate of pancreatic juice collected from anaesthetized rats before and during stimulation with various agents. Mean values ± S.E. of mean are given; *n* indicates the number of observations

	n	Flow rate ($\mu l.\ g^{-1}.min^{-1}$)	$[HCO_3^-]$ (mM)	HCO_3^- output (n-mole. $g^{-1}.min^{-1}$)
Resting secretion				
Fasted rats	51	0·62 ± 0·04	25·8 ± 0·4	16
Fed rats	9	2·80 ± 0·28	33·3 ± 1·5	93
Stimulated secretion (fasted rats)				
Intraduodenal HCl	4	1·96 ± 0·21	66·0 ± 3·2	129
GIH Secretin	13	3·25 ± 0·09	67·5 ± 1·4	219
Synthetic secretin	5	3·27 ± 0·36	70·6 ± 2·9	231
Caerulein	5	5·06 ± 0·52	30·2 ± 1·7	153
OP-CCK	4	4·75 ± 0·18	30·8 ± 2·1	146
Caerulein plus GIH secretin				
Low dose	4	3·51 ± 0·27	63·5 ± 1·5	223
High dose	8	7·60 ± 0·50	43·7 ± 1·4	332
Post inf.*		3·90 ± 0·47	54·2 ± 1·9	211
Boots secretin				
Low dose	6	2·97 ± 0·36	66·8 ± 2·7	198
High dose	10	8·32 ± 0·40	43·3 ± 1·4	360
Post inf.*		3·19 ± 0·45	57·2 ± 2·4	182
Stimulated Secretion (fed rats)				
Boots secretin				
High dose	6	12·02 ± 1·12	48·7 ± 0·8	585
Post inf.*		3·71 ± 0·58	68·3 ± 3·9	253

* Samples collected after cessation of infusion when HCO_3^- concentration was maximal.

found to produce an approximately maximum secretory response; OP-CCK was found to have a similar potency and we infused it at a rate of 3·3 p-mole.min^{-1}. Both drugs caused a marked increase in flow rate (up to 5–6 μl. g^{-1}.min^{-1}) i.e. about half as great again as the maximum response to pure secretin (Fig. 3, Table 1). In contrast to the effect of secretin, the HCO_3^- concentration in the juice averaged about 30 mM and showed no change with increasing secretory rate (Fig. 3, Table 1). The effects of caerulein and OP-CCK on the pancreas were indistinguishable.

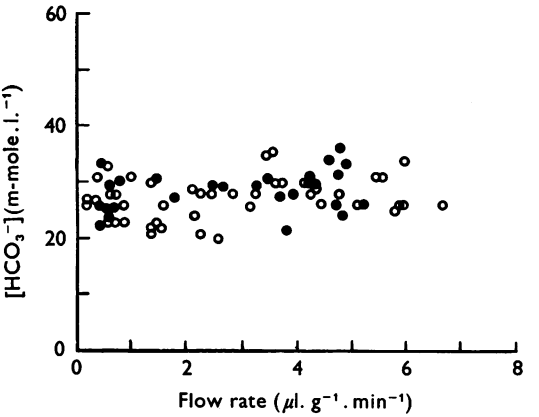

Fig. 3. The relation between secretory rate and HCO_3^- concentration in juice collected from the pancreas of fasted, anaesthetized rats in which secretion was stimulated by I.V. infusion of caerulein (○) or the synthetic octapeptide of cholecystokinin (●).

Stimulation with Boots secretin

Fig. 4 depicts results of experiments with Boots secretin. Infusion at a rate of 0·5 CHR μ./min (approximately 0·06 clinical μ./min: *vide* Harper, 1972), the dose used by Mangos & McSherry, resulted in production of juice with a HCO_3^- concentration of about 43 mM but the flow rate was exceptionally high, averaging 8·32 μl. g^{-1}.min^{-1} (Table 1, Fig. 4). Following cessation of infusion, the flow rate fell, but HCO_3^- concentrations first rose (on average to 57·2 mM: *vide* Table 1) and then, below flow rates of about 3 μl. g^{-1}.min^{-1}, fell towards resting levels (Fig. 4). Infusion of Boots secretin in a much lower dose (0·05 CHR μ./min) gave rise to a pattern almost identical to that obtained with a maximal dose of GIH secretin; the flow rate rose to a maximum of about 3 μl. g^{-1}.min^{-1} and the HCO_3^- concentration increased to 66·8 mM (Table 1). The results obtained using a low dose of Boots secretin were indistinguishable from those obtained in the post-infusion phase of high-dose experiments (Fig. 4).

As was the case in experiments with GIH secretin and synthetic secretin, the post-stimulus flow rates and HCO_3^- concentrations tended to remain slightly above the pre-stimulus values, even 2 hr after stopping of the infusion. Thus, in one series of sixteen experiments, the pre-infusion

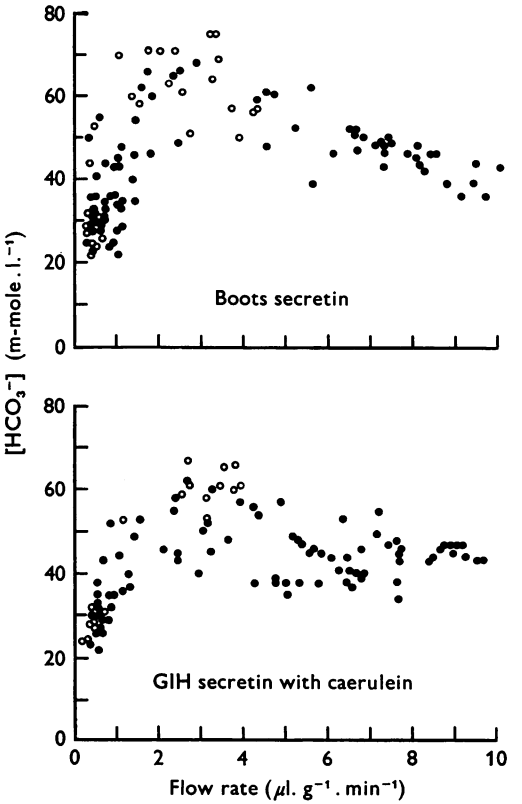

Fig. 4. The relation between secretory rate and HCO_3^- concentration in juice collected from the pancreas of fasted, anaesthetized rats in which secretion was stimulated by I.V. administration of Boots secretin (above) and GIH secretin mixed with caerulein (below). ● indicates samples collected during and after high doses of the mixture; ○ indicates samples collected during and after low doses of the mixture.

flow rate was 0·48 μl. $g^{-1}.min^{-1} \pm 0{\cdot}06$ S.E. of mean and the HCO_3^- concentration was 25·5 mM $\pm$ 0·7 S.E. of mean. However, 70–240 min after cessation of infusion, the mean flow rate was 0·65 μl. $g^{-1}.min^{-1} \pm 0{\cdot}07$ S.E. of mean and the HCO_3^- concentration was 29·6 mM $\pm$ 1·2 S.E. of mean; these values are significantly higher than the pre-stimulus values ($P < 0{\cdot}05$).

Stimulation with a mixture of caerulein and GIH secretin

Also shown in Fig. 4 are the results of experiments in which caerulein was infused together with GIH secretin. Initially, the infusion rates were 0·24 p-mole.min^{-1} for caerulein and 0·03 clinical μ./min for GIH secretin; subsequently, the rates were increased to 3·1 p-mole.min^{-1} and 0·4 clinical μ./min, respectively, and, finally, after 60 min, the infusion was stopped altogether. The pattern of secretory rates and HCO_3^- concentrations obtained were almost identical to those obtained by infusion of Boots secretin (Fig. 4); the mean values at maximum and intermediate flow rates are shown in Table 1.

Effects of feeding

In fed rats the pancreas is known to have a much higher basal secretory rate than in fasted animals (Table 2). Since the maximum secretory rates that we obtained in fasted rats, even using Boots secretin, were less than half those reported by Mangos (Mangos & McSherry, 1971; Mangos *et al.* 1974), who had worked with fed animals, we have repeated our experiments with Boots secretin in fed rats. The results are summarized in Table 1. Under these conditions, Boots secretin produced much greater flow rates (average maximum flow rate was 12·02 μl. g^{-1}.min^{-1}) but the HCO_3^- concentrations were similar to those seen in unfed animals; as was the case with fasted animals, after cessation of infusion of Boots secretin, HCO_3^- concentrations first rose to a maximum as secretory rates declined and, then, at lower rates still, fell towards pre-stimulus concentrations.

Cation concentrations in pancreatic juice

Potassium. In juice collected from unstimulated, fasted animals, the mean K^+ concentration was 4·71 mM $\pm$ 0·14 S.E. of mean (n = 20). In fed animals, the value was significantly ($P < 0·001$) higher, viz. 6·92 mM $\pm$ 0·34 S.E. of mean (n = 14). Stimulation with caerulein caused no significant change in K^+ concentrations, which averaged 4·90 $\pm$ 0·15 S.E. of mean (n = 31) in samples collected at maximum secretory rates. However, stimulation with either GIH secretin or Boots secretin caused a marked rise in K^+ concentration (Fig. 5). Thus, in fasted animals, the K^+ concentration in samples collected at maximum secretory rates averaged 7·61 mM $\pm$ 0·57 S.E. of mean (n = 8) with Boots secretin and 7·98 mM $\pm$ 0·36 (n = 6) with GIH secretin, values that are significantly higher than in control samples ($P < 0·001$). This increase in K^+ concentration during secretin stimulation persisted in all samples collected during the 30 min infusion period and declined, subsequently, in parallel with flow rate.

Sodium. Concentrations of Na^+ in pancreatic juice showed no significant correlation with secretory rate, regardless of whether the stimulant used was caerulein, GIH secretin or Boots secretin, or whether the animals were fed or starved. The average concentration for 198 samples in which Na^+ was determined was 133·02 mM ± 6·46 S.D.

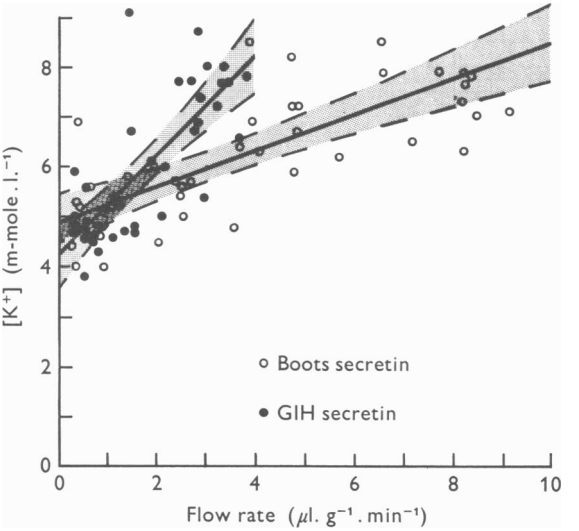

Fig. 5. The relation between K^+ concentration and secretory rate for juice collected from the pancreas of fasted, anaesthetized rats receiving I.V. infusions of GIH secretin (●) or Boots secretin (○). The continuous lines are lines of best fit calculated by the method of least squares and the shaded areas, bounded by interrupted lines, indicate the 95% confidence limits of the lines.

DISCUSSION

The pancreatic secretory rates reported in our experiments are considerably lower than those reported from Mangos' group (Mangos & McSherry, 1971; Mangos *et al.* 1974). In Table 2 are listed all published values for the rat. It can at once be seen that a number of factors contribute substantially to the observed variations in flow rate: whether the animals have been fed or starved, whether they were conscious or anaesthetized, and whether, when stimulated, they have been given pure secretin or an impure preparation. Our own values agree well with previous studies in which similar conditions were employed; the exceptionally high flow rates reported by Mangos can be ascribed with confidence to the effects of feeding and to a high level of contamination with CCK-like compounds

TABLE 2. Comparison of flow rates for rat pancreatic juice as reported in the literature and in the present study. All flow rates have been expressed in μl./min.kg body wt., since gland weights were not always reported

	Fasted	Fed	Reference
Conscious rats			
Resting secretion	30	40	Grossman (1958)
	15	—	Lin & Alphen (1959, 1962)
	18	37	Shaw & Heath (1972)
Stimulated secretion			
Pure secretin	45	—	Shaw & Heath (1972)
Impure secretin	45	—	Grossman (1958)
Anaesthetized rats			
Resting secretion	0·6	—	Hirsch *et al.* (1957)
	1·0–1·5	—	Love (1957)
	0·8	—	Lin & Alphen (1959, 1962)
	3·3	—	Debray *et al.* (1962)
	1·0–2·2	—	Ramirez *et al.* (1966)
	2·6	—	Heatley (1968)
	—	20	Mangos & McSherry (1971)
	2·4–3·0	—	Dockray (1972)
	2·8	15	Present study
Stimulated secretion			
Pure secretins	6–10	—	Ramirez *et al.* (1966)
	10	—	Heatley (1968)
	12	—	Dockray (1972)
	14	—	Present Study
Impure secretins	—	125*	Mangos & McSherry (1971)
	—	85*	Mangos *et al.* (1974)
	38	55	Present study

* These represent the highest values reported, not average maximum responses. The corresponding highest value for Boots (impure) secretin in fed rats in our experiments was 84.

of the secretin preparation that he used (see below). We preferred always to work with starved animals since their response to secretin and CCK is more reproducible.

Effects of secretin

The present studies show clearly that, in the rat, I.V. infusions of porcine secretin, natural or synthetic, cause only small increases in pancreatic secretory rates and moderate rises in juice HCO_3^- concentrations; this confirms the earlier reports of Heatley (1968) and Shaw & Heath (1972). Although it is known that secretin is present in rat intestinal mucosa (Dorchester & Haist, 1952), its precise chemical structure has not been determined. However, the poor response of the rat pancreas to porcine secretin cannot be explained solely on the basis of some structural difference between murine and porcine secretin since the animal does not

respond any more vigorously to its own endogenously released secretin (present studies; *vide* Ramirez, Hubel & Clifton, 1966); indeed, in conscious animals, there is no response at all to intraduodenal HCl administration (Grossman, 1958; Shaw & Heath, 1972). The reason for the rat's poor response to secretin remains only a matter of speculation but it is clear, qualitatively speaking, that the rat's response is similar to the rabbit's (Rothman, 1964), the calf's (Ternouth & Buttle, 1973) and the horse's (Comline *et al.* 1969) and gives no grounds for considering that the gastrointestinal physiology of the rat is strikingly unusual.

The effect of caerulein and OP-CCK

Early reports of the effect of CCK on the secretory rate of the rat pancreas (Lin & Alphen, 1962; Heatley, 1968*a*, *b*) have proven difficult to interpret since pure hormone preparations were not available at that time. Recently, however, Dockray (1972) reported a maximum secretory response of 4 μl. min^{-1} for rats receiving single I.V. injections of either a purified CCK preparation or of caerulein. Our own maximal flow rate responses to a sustained I.V. infusion of OP-CCK or caerulein were about 20% larger, as one might expect. The flow rates are much greater than those induced by secretin but this reflects the poor responsiveness of the rat to secretin at least as much as a good responsiveness to CCK. The dog pancreas also responds vigorously to CCK, caerulein and OP-CCK (Stenning & Grossman, 1969; Debas & Grossman, 1973); however, unlike the rat, it responds even more vigorously to secretin. Dockray (1972) calculates that the CCK responses of the two species, when corrected for pancreatic weights, are of about the same size. In contrast, the cat pancreas does not secrete at all in response to CCK and the effect of the hormone can only be assessed when superimposed on a background secretin response (Brown, Harper & Scratcherd, 1967). Unfortunately, studies in other species, in which preparations with pure CCK activity were used, are not available. Why there should be large species differences in the response to CCK (and secretin) is a matter for speculation.

An interesting finding in the present studies was that HCO_3^- concentrations in rat pancreatic juice were not altered during infusion of OP-CCK or caerulein but, rather, remained plasma-like, regardless of the size of the (CCK-induced) flow response. In the dog (Debas & Grossman, 1973), CCK produces an increase in juice HCO_3^- concentration, although not of the same size as that produced by secretin, while, in the cat, it causes an increase (Brown *et al.* 1967) or a decrease (Case *et al.* 1969*a*) depending on the intensity of the background of secretin stimulation. It has been concluded that CCK acts partly by direct stimulation of HCO_3^- secretion (Debas & Grossman, 1973) but our present studies suggest, at least for the rat, that the two hormones have totally independent actions on two

different receptor systems. Electrophysiological studies support the concept since Petersen & Ueda (1975) have shown that only CCK (but not secretin) can cause electrical changes at the basal plasma membrane of rat pancreatic acinar cells, while Greenwell (1975), in the mouse, has shown that secretin (but not CCK) affects the basal membrane only of duct cells.

Effects of impure secretin preparations and mixtures of caerulein and pure secretin

Boots secretin is a crude intestinal extract in which the active principle comprises less than 0·1% by weight of the dry preparation; up to 25% cross-contamination with CCK is encountered in individual batches (see above, under Methods). In human subjects, where the pancreatic flow response to secretin is vigorous and that to CCK relatively sluggish (Jorpes & Mutt, 1973; Ertan, Brooks, Ostrow, Arvan, Williams & Cerda, 1971), this contamination can make little difference to the maximum pancreatic flow rates obtained. In the rat, however, the position is considerably different. The present studies show that administration of exogenous secretin or provocation of release of endogenous secretin cannot cause the secretory rate to rise much above 3·25 μl. $g^{-1}.min^{-1}$, whereas administration of caerulein or OP-CCK causes flow to reach about 5 μl. $g^{-1}.min^{-1}$ and a mixture of caerulein and GIH secretin had an additive effect, producing flow rates of 7·6 μl. $g^{-1}.min^{-1}$. Since Boots secretin produced a maximum flow rate of 8·3 μl. $g^{-1}.min^{-1}$, the inference is strong that it did so because of its known contamination with CCK.

This inference is even more strongly supported when one examines the total HCO_3^- output of the pancreas (Table 1). The maximum rates of secretion of HCO_3^- in response to GIH secretin and caerulein, given alone, (219 and 153 n-mole.$g^{-1}.min^{-1}$, respectively) were found to be additive when the drugs were given together (332 n-mole. $g^{-1}.min^{-1}$) and this summated response was about the same as that induced by Boots secretin (360 n-mole. $g^{-1}.min^{-1}$).

If one may accept that the secretory response to Boots secretin can be explained as the result of the combined action of secretin and CCK, it becomes quite simple to account for the shape of the curve obtained by plotting HCO_3^- concentration against secretory rate (Fig. 4). During infusion of Boots secretin (or the mixture of caerulein and GIH secretin) the maximum flow rates observed and the HCO_3^- concentrations in the samples are the result of the admixture of two separate fluids, one secretin-induced and the other CCK-induced. Following cessation of the I.V. infusion, flow rate and HCO_3^- concentrations return slowly to resting levels. Although data are not available for the rat, it seems clear, at least for other animals, that CCK has a circulatory half-life considerably shorter

than 2 min whereas secretin has a half-life of 3–18 min (Lagerlöf, Ek & Nyberg, 1962; Case *et al.* 1969*a*; Lehnert, Strahlheber, Forell, Fritz & Werle, 1972). If this holds for the rat, then, as flow rate begins to fall after cessation of infusion, the CCK effect would disappear sooner and HCO_3^- concentrations would rise to typical secretin-stimulated values before falling, once more, to resting levels; this was precisely the pattern that we observed (Fig. 4).

Potassium concentrations

Our finding of plasma-like Na^+ concentrations in rat pancreatic juice, regardless of the secretory rate, or the agent causing secretion, accords with the findings reported for other animals (e.g. Case *et al.* 1969*a*). Our finding with respect to K^+ concentrations, however, is most unusual. In the pancreatic juice of other animals, such as the dog (Bro-Rasmussen *et al.* 1956) and cat (Case *et al.* 1969*a*), the K^+ concentration is plasma-like and independent of flow rate or stimulant; in the rabbit, the juice has a K^+ concentration about 2 mM higher than in the interstitial fluid but flow-rate dependency has not been observed (Swanson & Solomon, 1973). In our studies with the rat, however, we find that although the K^+ concentrations are plasma-like in the resting juice of fasted animals, they rise substantially following administration of secretin (pure or impure), but not following administration of caerulein (or OP-CCK). In resting samples collected from fed animals, the K^+ concentration was also significantly elevated above plasma levels, presumably reflecting the action of endogenously released secretin. A small elevation of K^+ concentrations, analogous to salivary K^+ 'transients' (Burgen, 1956), has been observed in juice from the cat pancreas immediately following the onset of secretin stimulation (Case, Harper & Scratcherd, 1969*b*), but the effect did not last more than 5 min and was extremely small. The increases in K^+ concentration that we have observed were quite substantial (up by 4 mM) and persisted for as long as secretin-stimulated increases in flow rate could be observed. It seems to us quite likely that the increase in K^+ secretion arises secondarily to a change in cation conductance of the plasma membranes of the cells responsible for secreting the HCO_3^--rich fluid (Petersen, 1975); since acinar cells in the rat pancreas respond electrically to administration of CCK but not secretin (Petersen & Ueda, 1975), it seems likely that the release of K^+ must have come from centro-acinar and duct cells rather than acinar cells.

The rat pancreas may well prove to be a most useful tool for the elucidation of pancreatic secretory mechanisms. On the one hand, it responds vigorously to CCK stimulation and on the other hand, the response to secretin, however sluggish, is always distinguishable by the change in

juice K^+ concentrations. Coupled with the fact that only CCK can produce electrical changes in the acinar cells, these findings may well permit a critical evaluation of the admixture hypothesis of pancreatic secretion (Lim, Ling, Liu & Yuan, 1936; Case *et al.* 1969*a*).

We are grateful to E. Squibb & Sons, New Jersey, for the gift of 3 mg. OP-CCK. The project was supported by the National Health & Medical Research Council of Australia to whom one of us (W.A.S.) is also indebted for the award of a B.Sc. (Med.) studentship.

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