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Transductal Fluxes of Anions in the Rat Pancreas (38097)

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Recent studies of the rat pancreas (1) have shown that during stimulation of the gland with exogenous secretin, the primary secretory fluid which was produced in the acini-intercalated ducts was isotonic to plasma. The concentrations of Na and K of this fluid were identical to those of plasma while the concentrations of Cl and HCO_3 were higher. Simultaneous measurements of the concentrations of these ions in the primary secretory fluid and in the final pancreatic juice revealed that exchanges of Cl and HCO_3 took place across the ducts of the gland while there were no net transductal fluxes of Na and K.

The present experiments demonstrate that the transductal fluxes of anions in the rat pancreas during stimulation with exogenous secretin are regulated by this hormone. In the absence of exogenous secretin in the unstimulated gland or during stimulation of secretion with pilocarpine or pancreozymin (CCK-PZ) there are no transductal fluxes of anions both in the whole gland or in segments of the interlobular ducts perfused by the split-oil droplet method of stationary microperfusion. Under these conditions, the ionic composition of the final pancreatic juice is identical to that of the primary secretory fluid. During stimulation of secretion with porcine secretin, infused intravenously, activation of a transductal mechanism of exchange of anions against concentration gradients appears to regulate the final anionic composition of the rat pancreatic juice.

Methods and Materials. Male, albino rats of the Sprague–Dawley strain, weighing approximately 200 g were used in these experiments. The methods of surgical preparation of the animals, stimulation of pancreatic secretion, collection of samples, and calculation of the flow rates have been described in previous publications (1, 2). The method of split-oil droplet stationary microperfusion developed by Shipp *et al.* (3) as modified by Gertz *et al.* (4) was used for the study of chloride fluxes in segments of the pancreatic ducts *in situ*. The ducts perfused were interlobular ducts of 60–90 μm external diameter. Each duct was ligated at the lobule hilum with a 9-0 special silk ligature in order to prevent leakage of fluid into the ducts during secretory stimulation. Each double-barreled siliconized glass capillary used for microperfusion was ground to a beveled tip of 12–18 μm external diameter. The method of preparation of the pancreas for microperfusion was identical to that previously reported for the rat parotid and pancreas (1, 2). The microperfusion fluid was pancreatic juice obtained during maximal stimulation of the pancreas of two sets of four rats with either secretin or pilocarpine. The ionic composition of the pooled pancreatic juice was adjusted to resemble primary secretory fluid by dialysis against proper solutions of electrolytes. The mean composition was Na:146.5, K:7.5, Cl:113.2 and HCO_3:34.5 mequiv/liter. After dialysis, aliquots ($\simeq$100 μliters) of the pancreatic juice were placed in Spinco microcentrifuge tubes and were kept at -60°C until used. Micropuncture of the acini of the rat pancreas was performed as previously described from this laboratory

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(1, 2). The microcapillaries used for micropuncture had a beveled tip of approximately 5–8 μm in diameter and were filled with mineral oil colored with Sudan Black. Upon insertion of the tip in the acinus, a small droplet of colored oil was ejected into the acinar lumen. In the stimulated pancreas, the droplet was seen to move distally thus identifying the patent lumen. The depth of insertion until the acinar lumen was reached varied from acinus to acinus but in general it was 30–40 μm from the contraluminal cell surface.

The concentrations of chloride in the samples of acinar fluid were determined directly using the ultramicrotitration method of Ramsay *et al.* (5) while the same concentrations in the final pancreatic juice were determined by the electrometric titration method using the Cotlove Chloridometer. The concentrations of bicarbonate in the primary secretory fluid were measured indirectly by the method of retrograde perfusion of the duct system of the gland with a solution of poly-L-lysine (5×10^{-7} *M*) in isotonic saline which eliminates the transductal fluxes of anions and results in the excretion of pancreatic juice that is identical in ionic composition to the primary secretory fluid of the gland, as previously reported from this laboratory (1, 2).

The concentration of inulin-^{14}C in the microsamples was determined by pipetting a 10–30 nliter sample in 5 ml of Bray's liquid scintillation solution and counting to 10,000 cpm in a Tricarb Packard Liquid Scintillation Spectrometer, model 3375, with automatic correction for background radiation and quenching.

In the initial experiments, secretin (Boots Pure Drugs, Inc., Nottingham, England) was used by intravenous infusion (0.5 μliters/min) to stimulate pancreatic secretion in the rat. Since this preparation is known to contain other peptides, such as cholecystokinin-pancreozymin (CCK-PZ), at the later stages of this work we obtained the purified secretin from Professor Jorpes (G.I.H. Research Unit, Chemistry Department, Karolinska Institute, Stockholm, Sweden, lot no. 27132). The studies of secretin stimulation were repeated with the purified hormone and we could not detect any differences in the results. Thus, in this work "secretin" refers to both products. Purified CCK-PZ (from the Karolinska Institute) in the initial experiments was kindly supplied by Dr. A. Hoffman (Mayo Clinic, Rochester, Minn.) while later in the study it was obtained directly from Prof. Jorpes, Karolinska Institute, Sweden. Secretin was used in an intravenous infusion at the rate of 0.5 units/min while CCK-PZ was infused at the rate of 0.3 units/min. The stimulation of pancreatic secretion with pilocarpine was performed by injecting the drug intravenously (0.2 mg/100 g body wt).

Calculations of *net* transductal fluxes of anions in the rat pancreas: (a) The amount of total Cl or HCO_3 secreted in the acini per minute (Sc) from the equation:

$$Sc = F \cdot A \qquad (\text{nequiv/min} \cdot \text{g wgt}),$$

where F is the flow rate and A is the concentration of the anion (Cl or HCO_3) in the primary secretory fluid of the gland. (b) The amount of total Cl or HCO_3 excreted by the pancreas per minute (Ex) from the equation:

$$Ex = F \cdot B \qquad (\text{nequiv/min} \cdot \text{g wgt}),$$

where B is the concentration of the anion in the final pancreatic juice. (c) The net amount of Cl or HCO_3 reabsorbed in the ducts (Rnet) from the equation:

$$Rnet = Sc - Ex \ (\text{nequiv/min} \cdot \text{g wgt}).$$

Results. During stimulation of the rat pancreas with secretin (Fig. 1), the Cl concentrations in the pancreatic juice were elevated at the high flow rates approaching those found in the acinar fluid of the gland. With decreasing flow rates toward prestimulation levels a gradual decrease in the Cl concentrations to approximately 70.0 mequiv/liter was observed. The change in the HCO_3 concentrations were in the opposite direction to those of Cl. At high flow rates, the HCO_3 concentrations were low and approached those found in the acinar fluid. With gradually decreasing flow rates the HCO_3 concentrations rose up to 74.0 mequiv/liter.

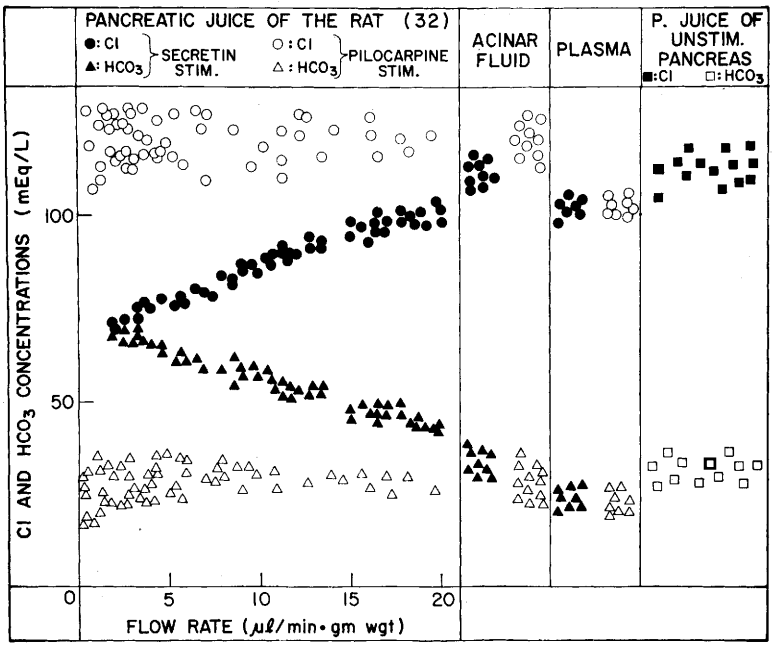

FIG. 1. The concentrations of Cl and HCO_3 in ductal fluid of the rat pancreas and rat plasma during stimulation of secretion with either porcine secretin (dark symbols) or pilocarpine (open symbols). Left column: relationship of Cl and HCO_3 concentrations to flow rate in final pancreatic juice. Second column: concentrations of same ions in acinar fluid of stimulated pancreas. Third column: concentrations of Cl and HCO_3 in plasma. Fourth column: concentration of Cl (dark squares) and HCO_3 (open squares) in the final pancreatic juice of the unstimulated pancreas at flow rates from 0 to 2.0 μliters/min·g wt.

During stimulation of rat pancreatic secretion with pilocarpine, the Cl concentrations in the pancreatic juice did not change with changes in the flow rates and remained at the mean level of approximately 120.0 mequiv/liter which is identical to the concentrations of Cl found in the acinar fluid during this form of secretory stimulation. Similarly, HCO_3 concentrations remained at the levels of approximately 29.0 mequiv/liter which were identical to those determined in the acinar fluid. In the acinar fluid of the secretin-stimulated rat pancreas the mean Cl concentration was 112.8 ± 3.4*[1] mequiv/liter while the indirectly determined mean HCO_3 concentration was 35.9 ± 4.1* mequiv/liter. In the acinar fluid of the pilocarpine-stimulated rat pancreas the concentrations of Cl and HCO_3 were 120.8 ± 4.2* and 29.2 ± 4.7* mequiv/liter, respectively. In the pancreatic juice of the unstimulated rat pancreas exhibiting spontaneous excretion at flow rates 0–2.0 μliters/min·g wt the mean concentrations of Cl and HCO_3 were 115.7 ± 2.9* and 32.1 ± 3.0* mequiv/liter, respectively. The concentrations of the same ions at flow rates of 0–2.0 μliters/min·g wt following stimulation of secretion with secretin and upon return of flow rates to prestimulation levels, were identical to the above values for the pancreatic juice of the unstimulated pancreas (not shown on Fig. 1).

The anionic composition of the primary secretory fluid and of the final pancreatic juice in the rat pancreas during stimulation of secretion with CCK-PZ is shown in Fig. 2. The shaded areas represent the values obtained from Fig. 1, for the secretin-

[1] Values marked with an asterisk (*) represent "mean values ± 1 SD from the mean."

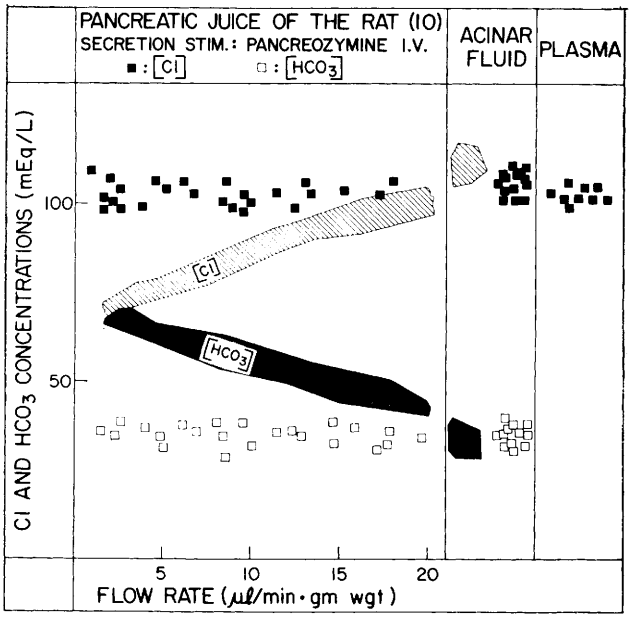

FIG. 2. The concentrations of Cl and HCO_3 in ductal fluid of the rat pancreas and in rat plasma during stimulation of secretion with either pancreozymin (open and dark squares) or secretin (shaded areas). The "secretin data" have been obtained from Fig. 1. Left column: relationship of Cl and HCO_3 concentrations to flow rate in the final pancreatic juice. Concentrations of same ions in acinar fluid (middle column) and in plasma (right column).

stimulated pancreas and have been included for comparative reasons. It is clear that during stimulation of the rat pancreas with CCK-PZ there were no net transductal fluxes of anions and the anionic composition of the final pancreatic juice was identical to that of the primary secretory fluid of the gland. Figure 3 shows the changes in the concentrations of Cl and inulin-^{14}C of pancreatic fluid injected into interlobular ducts by the split-oil droplet method and aspirated at 3, 9, and 21 min. The mean chloride concentration of the pancreatic juice used for stationary microperfusion was 113.2 ± 4.0 mequiv/liter. In the secretin stimulated pancreas the Cl concentration of the aspirated fluid decreased to 101.6, 89.5, and 81.2 mequiv/liter at 3, 9, and 21 min, respectively. In the pilocarpine-stimulated pancreas there was essentially no change of the concentration of Cl in the fluid injected into the interlobular ducts and aspirated for analysis. In the secretin-stimulated pancreas, the inulin-^{14}C of the fluid aspirated-to-fluid injected concentration ratios showed no changes during the 21-min period of the stationary microperfusion. Similarly, but not shown in the figure, there were no changes in the inulin-^{14}C concentration ratios in the pilocarpine-stimulated pancreas.

As shown on Table I, no decreases in the Cl concentrations or changes in inulin-^{14}C concentration ratios of the intraluminal fluid took place during stationary microperfusion of interlobular ducts in the unstimulated rat pancreas or during stimulation with CCK-PZ or pilocarpine. Similarly no changes occurred when secretin was added to the injected fluid in concentrations of 0.01 and 0.1 units/ml. On the other hand, when secretin was infused intravenously the Cl concentration of the injected fluid decreased from 118.6 to 83.1 mequiv/liter in 21 min.

Figure 4 shows that during stimulation of the pancreas with secretin, the concentrations of Cl in the acinar fluid did not change with changes in the flow rate while in the simultaneously collected samples of final

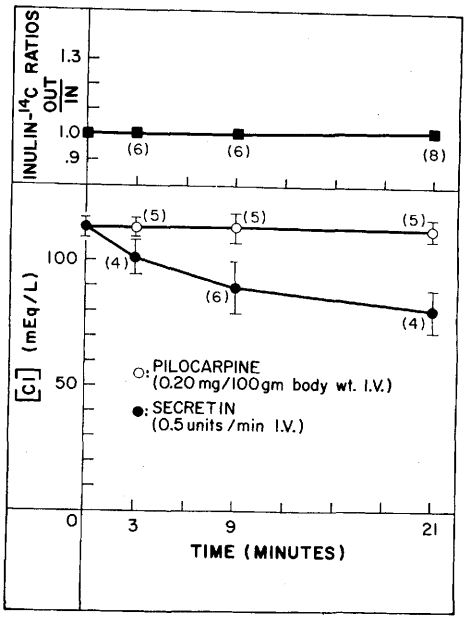

FIG. 3. Split-oil droplet stationary microperfusion of interlobular ducts in the rat pancreas. The upper part shows the inulin-^{14}C concentration ratios of the fluid recovered from (out) over the fluid injected into the ducts (in) during stimulation of the pancreas with secretin. The lower part of the graph shows the changes in the Cl concentrations of the injected fluid after 3, 9, and 21 min in the secretin-stimulated rat pancreas (open circles). Vertical lines indicate ± 1 SD from the mean values.

pancreatic juice, the concentrations of Cl were significantly lower than in the acinar fluid at each flow rate.

The calculated net transductal fluxes of anions in the secretin-stimulated rat pancreas are shown on Table II. The efflux of Cl was 261 ± 37* nequiv/min/g of wet gland tissue from 43 determinations in 11 rats. The influx of HCO_3 was 238 ± 57* nequiv/min·g wt from 36 determinations in 14 rats. These values were not statistically different as indicated by a $p > 0.5$.

Discussion. The present experiments demonstrate that transductal fluxes of anions against concentration gradients occur in the duct system of the rat pancreas during stimulation of secretion with exogenous secretin injected intravenously while these fluxes cannot be demonstrated in the unstimulated and in the CCK-PZ or pilocarpine stimulated pancreas. In a previous study of rat pancreatic function from this laboratory (1), it was demonstrated that the rat pancreas produced a primary secretory fluid with high Cl and low HCO_3 concentrations before and during stimulation with porcine secretin. The concentrations of these anions in the primary secretory fluid were significantly higher than in plasma indicating transacinar fluxes of anions against concentration gradients as a component of the secretory process. It was also shown that as the primary secretory fluid flowed along the duct system of the rat pancreas its anionic composition was modified through transductal fluxes of anions. The nature, mode of stimulation and directions of these fluxes were studied in the present experiments.

In order to study the fluxes of ions across the pancreatic ducts, the water fluxes during secretion had to be calculated. In the previous study of the rat pancreas (1), simultaneous measurements of amylase concentrations in the pancreatic juice and in the acinar secretory fluid demonstrated that no fluxes of water in or out of the ducts occurred during secretion. In the present study, microperfusion of the ducts with inulin-^{14}C containing fluid directly demonstrated the absence of water fluxes in or out of the interlobular ducts during stimulation of the pancreas with secretin. In the absence of water fluxes, the changes of the anionic composition of the luminal fluid were representative of the net fluxes of anions in these ducts.

The rat pancreas, is an organ suitable for the study of the transductal fluxes of Cl (1) as the movements of this anion occur from the ducts to the interstitium and plasma. Since the lowest Cl concentrations of the intraluminal fluid were significantly lower than the mean Cl concentration of 103.2 mequiv/liter in rat plasma, it was concluded that the transductal fluxes of this ion are the results of an efflux mechanism operating against concentration gradients across the duct wall. Furthermore, it was demonstrated that this mechanism was operative only during infusion of exogenous secretin. This transductal efflux of Cl was demonstrated by the microperfusion experiments, by the

TABLE I. Stationary Microperfusion of Interlobular Ducts of Rat Pancreas. Changes in Cl and Inulin-^{14}C (Out/In) Ratios During Different Modes of Stimulation. Numbers in Parentheses Indicate Number of Experiments.

	Cl mequiv/liter			Inulin-^{14}C ((Out/In) Ratio)		
	In	Out		In	Out	
Expt. design / Time (min)	0	9	21	0	9	21
Unstimulated pancreas (5)	113.2	113.9	113.6	—	1.00	1.01
CCK-PZ stimulation (5)	113.2	112.6	113.2	—	1.01	1.00
Pilocarpine stimulation (5)	113.2	113.0	113.5	—	0.98	1.01
Secretin (0.01 μl/ml) in luminal fluid (5)	118.6	118.0	119.2	—	1.00	1.00
Secretin (0.1 μl/ml) in luminal fluid (8)	118.6	119.0	118.5	—	—	—
Secretin iv (0.5 μl/min) (8)	118.6	92.5	83.1	—	—	—

simultaneous measurements of this anion in the acinar fluid and pancreatic juice and by the relationship of the pancreatic juice Cl concentrations to the flow rate. In the case of HCO_3 the intraluminal fluid HCO_3 concentrations started in the acini at levels significantly higher than in rat plasma and continued to increase to levels as high as 74.0 mequiv/liter in response to stimulation with secretin. The movements of HCO_3 were

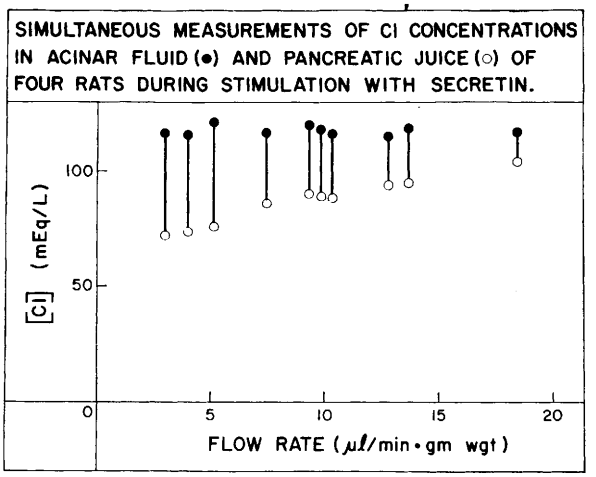

Fig. 4. The relationship of pancreatic flow rate to simultaneously measured Cl concentration in the acinar fluid and pancreatic juice of the secretin-stimulated rat pancreas. Each pair of points connected with a vertical line represents simultaneous measurements in one rat at one flow rate.

TABLE II. The Calculated Transductal Fluxes of Cl and HCO_3 in the Secretin-Stimulated Rat Pancreas (11 Experiments) at Mean Flow Rates of 7.6 ± 2.9 μliters/min·g wt.

Fluxes (nequiv/min·g wt)
Cl – Efflux: 261 + 37
HCO_3 – Influx: 238 + 57
$p > 0.5$

from plasma-to-interstitium-to ductal fluid (influx) and occurred against large concentration gradients exactly as in the case of Cl. The fact that these fluxes of anions occur in the rat pancreatic ducts only in response to administration of secretin and not in the unstimulated or in the CCK-PZ or pilocarpine-stimulated pancreas suggests that the mechanism of modification of pancreatic fluid through transductal anionic exchanges is activated by secretin, the secretagogue hormone for this gland. Furthermore, these experiments show that this mechanism is basically a 1:1 net exchange of Cl for HCO_3 with both ions moving against strong concentration gradients. This control of the transductal fluxes of ions by an agent which also controls the secretory mechanism in the gland is not unique for the rat pancreas as Martin and Young (6) found that in the main duct of the rat submaxillary gland the secretagogue neurotransmitter analogs carbamyl choline and isoproterenol stimulated transductal secretion of K and HCO_3.

In the past, it was felt that in exocrine glands the proceses of ductal fluid modification through transductal fluxes of ions were operative at all times upon availability of primary secretory fluid delivered to the ducts by the secretory segments. Flow rate of primary fluid delivery to the duct, transductal transport of fixed capacities and "steady-state concentrations" of ions were thought to be the only determinants of the composition of the final excretory products. The present experiments as well as the work of Martin and Young demonstrate the existence of states of "secretion-transductal transport coupling" in exocrine glands where the secretagogues controlling secretory processes are also involved in the regulation of the transductal transport of ions.

The mode of action of secretin on the transductal fluxes of anions is not clear. The lack of stimulation of Cl efflux when secretin was added to the fluid injected during stationary microperfusion of the interlobular ducts does not necessarily negate the possibility of a direct action of secretin on the ductal cells. It is possible that secretin must reach the ductal cells from the contraluminal side in order to stimulate the transductal exchange of anions. However, it is also possible that secretin does not act directly on the ductal cells but indirectly causes the intrapancreatic or extrapancreatic production or release of another substance which in turn stimulates the anionic exchanges in the pancreatic ducts.

It could be argued that the changes in the anionic composition of the pancreatic ductal fluid during stimulation with secretin are due to stimulation of transacinar secretion of a primary secretory fluid with lower Cl and high HCO_3 concentrations which would modify the final product of the gland. However, by microsampling acinar fluid during secretion at different flow rates, constancy of the concentrations of Cl in the acinar fluid could be demonstrated. Since the anionic composition of the primary secretory fluid remains constant during stimulation with secretin, one could invoke ductal secretion of a fluid with high HCO_3 and low Cl concentrations in response to secretin. Such a fluid could be added to the intraluminal fluid and decrease its Cl and increase its HCO_3 concentrations. Such a mechanism has been speculated by Schulz *et al.* for the rabbit pancreas (7). That ductal addition of a fluid rich in HCO_3 and poor in Cl is not an explanation for the changes in the anionic composition of the ductal fluid in the secretin-stimulated rat pancreas is clearly shown by the present experiments of stationary microperfusion of the interlobular ducts: if addition of a secretory fluid was responsible for the changes in Cl concentrations, changes in the inulin-^{14}C (out/in) concentration ratios should have been seen; the fact that these ratios remained approximately 1.0 while the concentrations of Cl in the injected fluid decreased from 113.2 to 81.2 mequiv/liter in

the 21 min of observation speaks against transductal secretion of water and addition of any fluid to the ductal contents. Thus, a secretin-stimulated transductal exchange of Cl and HCO_3 appears to be the mechanism for the anionic modification of the ductal fluid in the rat pancreas.

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