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The Effect of Secretin on Pancreatic Blood Flow in the Awake and Anesthetized Dog (41696)

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Abstract. The canine pancreatic blood flow was studied after iv secretin (resulting in a plasma level commensurate with the postcibal state), and also after larger iv doses and after duodenal acidification. We found that blood flow was unaffected by "physiological" doses of secretin, or by perfusion of duodenum at a pH as low as 2.0, but increased by bolus doses (0.5 CU/kg and above), and by acidification to pH 1.4. Anesthesia does not affect the blood flow response although the bicarbonate response appeared to be blunted under anesthesia. We conclude that increase in pancreatic blood flow is not a physiological effect of secretin.

Although Bayliss and Starling deduced the existence of secretin from their classical observation in 1902, a postprandial rise of plasma immunoreactive secretin was demonstrated only recently with refinement of techniques of measurement (1, 2). In the dog the postprandial plasma level was shown to be in the range of 35–55 pg/ml (2), and this level was further shown to be attainable by iv infusion at 0.125–0.25 CU/kg/hr in 10- to 25-kg dogs. Thus the existing literature on the effect of secretin on pancreatic blood flow consists of observation at doses (and blood levels) that may be regarded as pharmacological compared to what is now known to be "physiological" (3). We undertook to measure the effect of endogenous and exogenous secretin, at doses ranging from one commensurate with a physiological blood level, to one known to generate pharmacological blood levels of secretin, on pancreatic blood flow. In view of the current uncertainty regarding the effect of anesthesia on splanchnic blood flow, we also studied this effect on both the anesthetized and awake dog.

Methods. *Experiments in anesthetized dogs.* Mongrel dogs weighing between 14 and 20 kg were anesthetized with pentobarbital at a dose of 30 mg/kg given intravenously. The dogs breathed spontaneously but the airway was intubated. Through a midline abdominal incision the cystic duct was tied and the common bile was cannulated. The minor pancreatic duct was identified and ligated, and the major pancreatic duct was cannulated extraduodenally with a Teflon cannula equipped with wings for anchorage to the duodenal wall with serosal sutures. The correct position of the pancreatic cannula was ascertained by observing good drainage of basal pancreatic secretion (0.1–0.2 ml/30 min). Ten centimeters of the first portion of the duodenum was then cannulated for luminal perfusion. The inlet cannula was introduced through an incision of the antrum and was held in place by a circumferential ligature at the pylorus without disturbance of the blood supply. The outlet cannula was larger than the inlet cannula, and was similarly secured with an occluding suture 10 cm distal to the pyloric ligature. Duodenal luminal perfusion was maintained at 8 ml/min in a recirculating circuit containing Ringer's solution kept at 37°C with a thermostat. The pH of this perfusion system was regulated by means of a pH stat (Radiometer).

Through an incision in the neck the right carotid artery was exposed and a pigtail catheter (8 Fr.) was introduced and advanced into the left ventricle. The correct position was verified by pressure tracing. Blood flow was determined with the dual reference sample technique, using a left femoral artery catheter and a distal abdominal aortic catheter via the right femoral artery. Radioactive microspheres of 15 ± 5 μm in diameter were used, labeled with radioactive isotopes of ^{141}Ce, ^{85}Sr, ^{51}Cr, or ^{46}Sc. The dose was calculated to deliver 400 spheres/g of tissue.

The experiments were conducted by perfusing the duodenum at pH 7 for 60 min as baseline. Blood flow was measured at 30 min into the experiment. The perfusate was then

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kept at either pH 4.0, 3.0, 2.0, or 1.4 in groups of five dogs each. Pancreatic blood flow was measured again 10 min after achieving the new pH. Exogenous secretin (GIH, Karlinska Institute, Copenhagen) was given either as a bolus at 0.5–5 CU/kg, or infused continuously at a dose of 0.03–0.12 CU/kg/hr, dissolving the GIH secretin in 2% canine albumin to reduce loss due to surface adsorption. Blood flow in these experiments were measured 5 min after the secretin stimulus.

Additional experiments were done in two dogs in which plasma secretin level was determined when the duodenum was perfused at pH 7.0, 4.0, and 2.0, with simultaneous determination of pancreatic blood flow. In two other dogs given iv infusion of secretin in 2% albumin at 0.12 and 0.25 CU/kg/hr, plasma secretin levels and pancreatic blood flow was also measured. The method of secretin assay was that described by Tai and Chey (4), a modified radioimmunoassay. Specificity of the antisecretin serum had been established previously showing no cross-reactivity with glucagon and insulin, and under 1% with cholecystokinin (CCK). The purity of synthetic porcine secretin used for the assay had also been verified by electrophoresis and TCA precipitability. Heparinized venous samples were immediately centrifuged at 4°C for the plasma which was treated with Trasylol and stored in deep refrigeration. Prior to the assay, the samples were concentrated by percolating 3 times through XAD-2 resin columns (using only columns of the same lot number), and the adsorbed secretin was recovered by eluting with acid methanol. The percentage recovery of secretin adsorbed in the batch of XAD-resin column used was 66 ± 0.8%. The 95% confidence limit for the assay was 3 pg/ml.

At the end of the experiment, the dog was sacrificed with iv KCl and the pancreas was dissected free of mesentary and fat. The wet weight was obtained and the whole pancreas was counted for radioactivity of the isotopes, as were the reference blood samples. The blood flow was calculated according to the equation: $BF - (C_t \times RBF)/C_r$, where *BF*-blood flow in ml/100 g/min, C_t = tissue counts/min/100 g, *RBF* = reference blood flow (rate of withdrawal in femoral arteries) and C_r = total counts in reference blood.

Experiments on awake dogs. Chronic mongrel dogs were prepared with a Thomas cannula-equipped duodenal pouch, into which opened the pancreatic ducts. Another Thomas cannula was implanted into the dependent portion of the stomach. Three weeks were allowed for recovery. Prior to experiments the carotid artery was cannulated for access to the left ventricle as described above, and the femoral artery was similarly catheterized using local anesthesia. Blood flow was measured on conscious dogs in a Pavlov stand. Pancreatic secretion was collected from the pouch by means of ductal cannulation with the external catheter tip immersed under a layer of mineral oil. Duodenal acidification was achieved by irrigation of the duodenal pouch with 0.04 N HCl, delivering an acid load of 2 m*M*/15 min. Blood flow, measured by the above described technique, was determined for baseline, after 15 min of acid irrigation, add 5 min after iv bolus doses or continuous infusion of secretin, using a different isotope-labeled microspheres for each determination. The dogs were sacrificed at the end of the experiment and the pancreas was counted for radioactivity. Not more than two secretin dosages (in random order) were used in any one dog.

Plasma secretin and pancreatic blood flow was measured in two awake dogs when the duodenum was acidified (2 m*M*/15 min of 0.04 *N* HCl) and after continuous iv infusion of secretin in dog albumin at 0.12 and 0.5 CU/kg/hr as described above.

Statistics. Results were expressed as mean ± SE. Analysis of variance was performed. Student's *t* tests were used for paired and unpaired variates.

Results. Perfusion of the duodenal lumen at pH 7 did not alter pancreatic basal secretion in either the awake or anesthetized dogs. Blood flow measurement obtained during this period was taken as the basal stage. The basal pancreatic blood flow did not differ significantly in the awake or anesthetized dog (Fig. 1). A greater variation was observed in the awake state as evidenced by a greater standard error. A plasma secretin concentration of 4 to 6 pg/ml when the duodenum was perfused at pH 7 was probably not significantly different from zero as it approached the limit of sensitivity of the radioimmunoassay.

Effect of acidification of the duodenum. In anesthetized dogs, perfusion of the duodenal

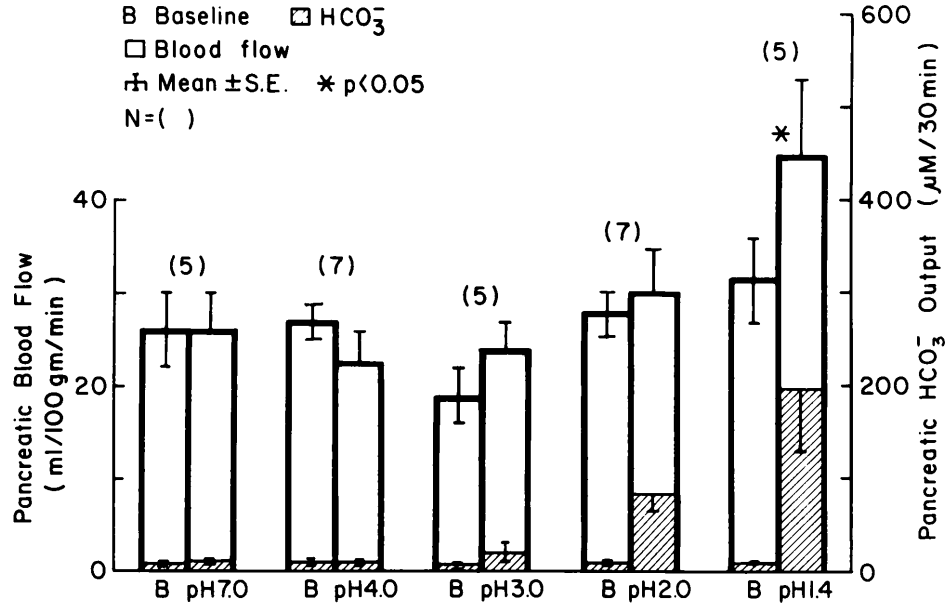

FIG. 1. Effect of duodenal acidification on pancreatic HCO^- output and blood flow in anesthetized dogs.
* Increased over baseline B ($P < 0.05$).

lumen at pH 4.0, 3.0, or 2.0 did not significantly increase pancreatic blood flow (Fig. 1). Pancreatic exocrine secretion (measured as bicarbonate output) increased mildly beginning at pH 2. Perfusion at pH 1.4 resulted in a prompt increase in bicarbonate output, but only marginal increase in pancreatic blood flow (Fig. 1). In the awake dogs, duodenal perfusion at an acid load of 2 m*M*/15 min yielded a markedly increased bicarbonate output, although blood flow increased only moderately. Perfusing the duodenum at pH 4.0 in our experiments barely raised the plasma secretin from baseline to 14–26 pg/ml, a value lower than postprandial but compatible with "physiological" range. At pH 2 the plasma secretin attained (92–106 pg/ml) was several times higher than that observed after a meat meal, without demonstrable increase in pancreatic blood flow.

Effect of exogenous secretin. In the anesthetized dog, exogenous secretin had no effect on pancreatic blood flow between the dose of 0.03–0.25 CU/kg/hr (Fig. 2). As shown in Table I, plasma secretin compatible with "physiological" was attained at these dosages. At 0.5–1 CU/kg given as bolus doses, a moderate increase in blood flow was observed. When given as a bolus at 2 CU/kg, a fourfold increase was seen. Plasma secretin was not measured with bolus doses.

Significant increase in bicarbonate output was observed at 0.12 and 0.25 CU/kg/hr of iv secretin infusion. We did not see any bicarbonate response at the two lower doses in the anesthetized dog.

In the awake dog, increased blood flow was seen only at 2 and 5 CU/kg of bolus iv secretin (Fig. 3). Continuous iv secretin infusion up to a dose of 0.5 CU/kg/hr had no effect on blood flow. Plasma secretin was between 19 and 92 pg/ml when iv secretin was infusing at 0.12 and 0.25 CU/kg/hr (Tables I and II) in anesthetized or awake dogs, and these levels are compatible with those observed after a meat meal (2). The bicarbonate output, however, increased beginning at a dose of 0.06 CU/kg hr. The customary bicarbonate response was observed in the rest of the dosages.

Effect of anesthesia on blood flow. Comparing the data in awake and anesthetized dogs, there is no difference in basal pancreatic blood flow (Figs. 1 and 3). Acidification of the duodenum to pH 1.4 produced a larger increase in blood flow in the awake dog but this was not statistically significant. We also observed no difference in blood flow when secretin was infused from 0.03 to 0.25 CU/kg/

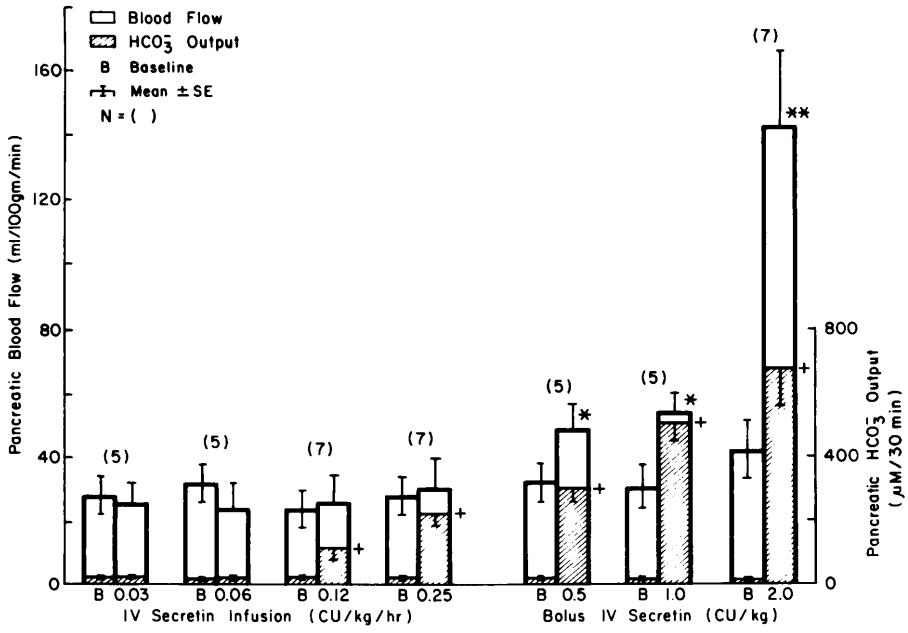

FIG. 2. Effect of iv secretin on pancreatic HCO_3^- output and blood flow in anesthetized dogs. **Increased over baseline B ($P < 0.01$); *increased over baseline B ($P < 0.05$); +increased over baseline B ($P < 0.025$).

hr, whether the dog was anesthetized or not (Figs. 2 and 3). At clearly pharmacological doses (2 CU/kg bolus and above) we actually found a larger increase in blood flow under anesthesia, although this also was not statistically significant.

There seems to be a significant blunting of the bicarbonate response by anesthesia as seen by comparing equal doses of secretin in the awake and anesthetized dogs (Figs. 2 and 3). Also, significantly larger bicarbonate response was seen in awake dogs in response to acidifying the duodenum (Figs. 1 and 3).

TABLE I. PLASMA SECRETIN LEVEL (pg/ml) IN FOUR ANESTHETIZED DOGS

Duodenal perfusion	Dog 1	Dog 2
pH 7.0	4	6
pH 4.0	14	26
pH 2.0	92	106
iv secretin (CU/kg/hr)	Dog 3	Dog 4
0	0	5
0.12	19	28
0.25	68	82

Discussion. Pharmacological doses of secretin have been shown to increase pancreatic blood flow (3, 5–7). It is not known, however, what role secretin plays, if any, in the physiological regulation of pancreatic blood flow. The response to a meal is generally taken as the yardstick of a physiological event, but that is difficult to incorporate into the experimental design as neural influences cannot be easily separated. The major problem had been in determination of the physiological level of secretin as it was established only in recent years that plasma concentration of immunoreactive secretin increases significantly after meals in dogs. Kim *et al.* have shown that a plasma level of secretin comparable to that seen after meals can be achieved by intravenous infusion of secretin at a dose of 0.25 CU/kg/hr (2). Exogenous secretin administered in this fashion at this dose range is arbitrarily termed "physiological" in our study. On this premise our experiments were first done without simultaneous plasma secretin measurements. In the absence of data of plasma level, it is difficult to ascribe the lack of effect to a true lack of response, as such may occur spuriously from nondelivery of secretin, or from loss of activity during delivery, although the possi-

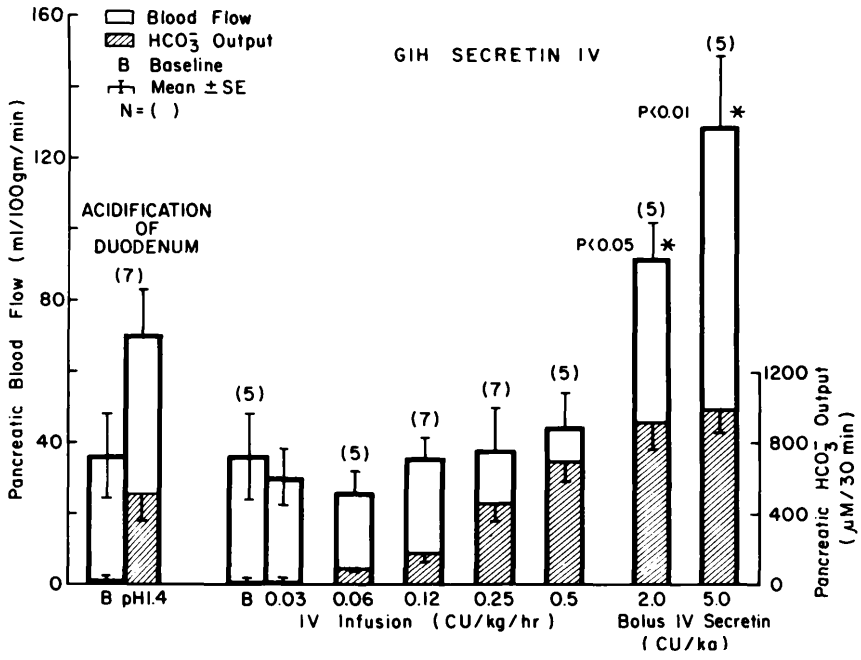

FIG. 3. Effect of duodenal acidification and iv secretin in pancreatic HCO_3^- output and blood flow in awake dogs. *Increased over baseline B ($P < 0.025$)

bility was diminished somewhat due to the customary bicarbonate response being observed. We therefore conducted more experiments in which the plasma secretin was measured when exogenous and endogenous secretin was given in both anesthetized and awake dogs. We confirmed that a "physiological" level of secretin was indeed attained during the experimental conditions, and we therefore conclude that increase in pancreatic blood flow is not a physiological role of secretin. A marked increase in pancreatic blood flow was not seen until a bolus dose of 2 CU/kg, as had been observed previously (3, 6). Comparing the exocrine and blood flow responses to secretin, it seems likely that if there is a maximal dose for blood flow it would be much higher than that for bicarbonate output.

TABLE II. PLASMA SECRETIN LEVEL (pg/ml) IN TWO AWAKE DOGS

	Dog 5	Dog 6
Duodenal acidification, Mean ± SE, $N = 4$	272 ± 64	182 ± 36
iv secretin (CU/kg/hr)		
0.12	33	24
0.25	92	84

It has been shown that the amount of secretin released from the canine duodenum is dependent on the acid load and length of intestine acidified (7). In our experiments in both the awake and anesthetized dogs, acidification was limited to 10 cm of the intestine, in contrast to other perfusion experiments where the length of intestine acidified was a function of the amount of acid instilled (7), so that the amount of releasable secretin was also limited. Since the duodenum perfused in both the awake and anesthetized dogs had similar lengths, but a larger exocrine response was seen in the awake dogs ($P < 0.05$), it is likely that pentobarbital anesthesia did depress either secretin release or the exocrine pancreatic response to secretin, as has been shown previously (9).

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