Regional Distribution and Concentration of Pancreatic Polypeptide in the Human and Canine Pancreas

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SUMMARY

The regional concentrations of pancreatic polypeptide (PP), insulin, and glucagon and the cellular distribution of PP were studied in 13 human and nine canine pancreases by radioimmunoassay, immunoperoxidase localization, and cell quantitation. PP concentration was highest in both the uncinate process and the head of the human pancreas and in the right lobe of the canine pancreas. In contrast, glucagon and insulin levels were higher in the body and tail of both the human and canine pancreases.

Human F-cells, which contain PP, were located primarily at the periphery of the islets, although a few F-cells were scattered throughout the ducts and acini. Canine F-cells were located in ducts, acini, and islets; the relative proportion of canine F-cells in the endocrine and exocrine tissues differed according to location.

Cellular quantitation of F-cells in both species correlated significantly with the tissue concentration of PP in all regions studied, validating the use of morphometric techniques to quantitate the regional distribution of PP. DIABETES 28:11–15, January 1979.

In a previous study, canine pancreatic polypeptide (cPP) was shown to be regionally more concentrated in the right lobe (uncinate process), whereas insulin and glucagon predominated in the body and tail.[1] A correlative assessment of the regional distribution of the pancreatic F-cell, which contains PP,[2] was not done at that time. There have been no reports concerning the regional concentration and cellular distribution of human pancreatic polypeptide (hPP) in the human pancreas. The purpose of this study was threefold: (1) to determine whether a regional concentration of hPP, insulin, and glucagon exists in the human pancreas; (2) to compare the regional distribution of F-cells in the human pancreas with hPP tissue concentration; and (3) to compare the regional distribution of F-cells in the canine pancreas with cPP tissue concentration.

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MATERIALS AND METHODS

Tissue collection. Human pancreases were obtained at autopsy, within 6 h after death, from 13 patients without pancreatic disease. These included 12 adults (age range, 35–72 yr) and one child (age 12). A review of the patients' charts and (H-E) hematoxylin-eosin–stained sections revealed no pathologic involvement of the pancreases. Nine of the patients died after prolonged illness, and the remaining four patients died after an acute illness. Canine pancreases were removed from nine conditioned, mongrel, adult dogs (10–20 kg, fed ad libitum) that were killed with an overdose of sodium pentobarbital (50 mg/kg i.v.).

Pieces of tissue from the human pancreases were removed from the following regions: (1) end of uncinate process, defined as that portion of the head of the pancreas projecting to the left behind the superior mesenteric artery; (2) head, the area immediately surrounding the common bile duct; (3) body, midway in the gland; and (4) end of tail. Pieces of the canine pancreases were removed from the distal end of the right lobe (duodenal lobe), the body, and the tail of the left lobe. (The uncinate process of the human pancreas has no anatomic counterpart in the canine pancreas.) Pieces from each region were placed in Bouin's solution, fixed for 24 h, and then processed for light microscopy. Separate fresh pieces were quick-frozen in liquid nitrogen and stored at −70°C until extraction for total hormone content.

Radioimmunoassay procedures. Two or three pieces (100–200 mg) of the frozen tissues were selected randomly from each region and homogenized in 1.0 ml of acidified alcohol[3] using a motor-driven, glass, homogenizing system. Aliquots of the tissue extracts were assayed for PP by a heterologous two-antibody technique developed in our laboratory.[1] Insulin was assayed by the alcohol precipitation technique of Wright et al.[4] and glucagon by the two-antibody technique of Leichter et al.[5]

Immunocytochemical techniques. Bouin-fixed, decerated, paraffin sections of human and canine pancreases were stained by the direct immunofluorescent-staining method and by the unlabeled antibody–enzyme method,[6] using a 1:500 dilution of rabbit serum containing antibodies to bo-

TABLE 1
Concentrations of human pancreatic polypeptide (HPP), insulin, and glucagon

No.	Age	Sex	HPP (μg/g)				Insulin (μg/g)				Glucagon (μg/g)			
			UP*	Head	Body	Tail	UP*	Head	Body	Tail	UP*	Head	Body	Tail
1	67	F	22.9	903.6	34.7	12.3	144.0	74.4	72.8	172.4	10.36	2.06	9.94	39.10
2	72	F	147.6	39.2	3.6	37.0	151.2	126.8	57.6	216.8	5.49	3.82	13.67	21.24
3	66	M	29.2	58.6	3.3	4.6	56.8	92.4	88.4	82.8	17.82	9.15	15.05	10.77
4	62	F	39.4	56.5	0.7	0.4	75.6	46.4	99.6	156.0	3.64	0.70	7.46	7.51
5	58	M	251.8	223.0	13.1	6.6	25.6	30.4	42.8	45.2	0.32	0.15	1.14	1.83
6	75	F	0	11.6	6.5	34.0	94.4	109.6	118.8	163.2	5.18	5.17	12.86	14.04
7	60	F	16.9	0	0.8	9.6	149.1	62.8	135.7	428.4	10.72	5.61	7.25	29.97
8	58	F	28.7	0	2.2	3.6	318.2	121.4	223.6	384.3	15.90	3.40	5.84	25.45
9	46	F	7.5	13.5	5.2	6.7	214.6	98.5	101.6	86.2	32.09	21.67	12.00	5.38
10	12	F	103.3	3.9	1.1	0.9	59.4	44.4	64.0	65.4	4.71	7.92	19.66	19.82
11	35	M	0	0	1.1	1.4	134.7	153.4	168.6	284.9	9.02	6.04	8.86	16.80
12	62	M	308.8	245.4	9.8	5.6	48.0	24.1	105.9	136.3	1.42	0.47	7.38	9.62
13	66	F	76.6	4.3	1.9	3.0	102.5	62.0	87.3	113.1	16.91	8.10	17.23	29.45
Mean			79.4	119.9	6.5	9.7	121.1	80.5	105.1	179.6	10.28	5.71	10.64	17.77
SEM			27.64	69.23	2.57	3.32	22.00	11.12	13.57	33.27	2.42	1.57	1.41	3.06

* UP, uncinate process.

vine PP (bPP) (Lilly lot 615-R110-146-6, a gift from Dr. Ronald Chance, Eli Lilly Research Laboratories, Indianapolis) overnight at 4°C. Controls included (1) the use of aliquots of bPP antiserum diluted 1:500 with 0.01 phosphate buffer–0.15 M sodium chloride, pH 7.2, and preincubated overnight at 4°C with an excess of either bPP (10×), glucagon, gastrin, insulin, or somatostatin; (2) the use of normal rabbit serum instead of the primary antiserum; (3) the elimination of the peroxidase-antiperoxidase complex (PAP) in the third incubation medium of this method; and (4) the use of dilutions of 1:500, 1:1000, 1:2000, and 1:5000 of the primary bPP antiserum on adjacent sections.

The light microscopic–staining properties of the cPP cells were studied by obtaining a photomicrograph of the cells stained with fluorescein-labeled antibodies and then treating the section with one of the following stains: aldehyde-fuchsin-trichrome, phosphotungstic acid-hematoxylin, Hellerstrom-Hellman silver method, or Grimelius' silver method. Areas previously photographed were relocated and the histochemical-staining property of the cells determined.

TABLE 2
Counts of human pancreatic polypeptide cells per 200 fields at ×400 for each region

No.	Uncinate process	Head	Body	Tail
1	478	622	7	0
2	297	0	7	69
3	18	10	6	1
4	254	69	0	5
5	197	570	6	2
6	188	11	14	33
7	0	416	0	3
8	63	11	12	15
9	5	7	8	3
10	179	0	3	17
11	5	1	0	11
12	166	224	5	10
13	0	0	0	4
Mean	142.3	149.3	5.2	13.3
SEM	40.51	64.57	1.27	5.27

In sections stained with the PAP procedure, cell counts were made of 200 contiguous fields that contained both islet and acinar tissue, as limited by an eye piece reticle (7 × 7 mm), and magnified 400 times. Duplication of counts was prevented by not counting those cells that fell on the bottom and advancing boundary lines of the reticle.

Comparison of F-cell counts with PP concentration. To compare the number of F-cells with the concentration of PP in the regions evaluated, the absolute values for each region of each pancreas (Tables 1–4) were totaled and equated to 100%. The individual values (counts and concentration) were then converted to a percentage of the total. These data were defined as relative proportion, and the means are shown in Figures 1 and 3.

The radioimmunoassay determinations and cell counts were done as a double-blind study. Results were compared after all data were collected. The data were tested for statistical significance by the paired Student's *t* test and by analysis of variance.

RESULTS

Human pancreas. The concentrations of hPP, insulin, and glucagon in four areas of 13 human pancreases are shown in Table 1. The pattern of distribution of hPP in the four regions was different from that of insulin and glucagon. HPP was significantly more concentrated in both the uncinate process and head than in the body and tail ($P < 0.025$). In contrast, the concentrations of both insulin and glucagon were lowest in the head ($P < 0.005$) and highest in the tail ($P < 0.001$).

The hPP-cell counts for the four regions of the 13 pancreases are presented in Table 2. HPP cells were significantly more concentrated in both the uncinate process and head than in the body and tail ($P < 0.001$). A comparison of the relative proportion of F-cells per region and the hPP concentration (Figure 1) indicated a good correlation between the radioimmunoassay and morphometric results.

The F-cells were located primarily near the periphery of the islets. Occasionally, F-cells were present within acini and ducts. In the uncinate process or head region in several

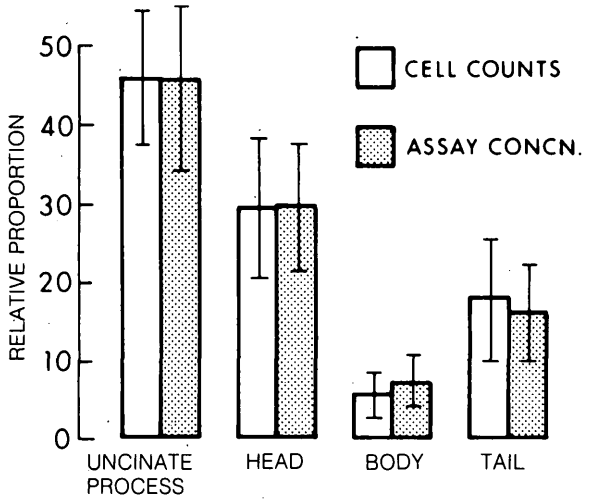

FIGURE 1. A comparison of F-cell counts with hPP concentration in the four areas of the human pancreas. The absolute values for each region of each human pancreas (tables 1 and 2) were totaled, and each total was equated to 100%. The individual values were than converted to the percentage of the total. Means and SEM for the 13 pancreases were then calculated for each region. The data, defined as the relative proportion of F-cells and hPP concentration per region, indicated a good correlation between radioimmunoassay and morphometric results.

pancreases the F-cell was the predominant endocrine cell in many islets (Figure 2).

Dog pancreas. The concentrations of cPP, insulin, and glucagon in three areas of nine canine pancreases are shown in Table 3. Again, the pattern of distribution of cPP in the three regions was different from that of insulin and glucagon. CPP was six times more concentrated in the right lobe than in the body ($P < 0.001$) and eight times more concentrated in the right lobe than in the tail ($P < 0.001$). Insulin and glucagon were least concentrated in the right lobe ($P < 0.001$). Glucagon was 12 times more concentrated both in the body and in the tail than in the right lobe; however, insulin was only about twice as concentrated in the body and tail.

The counts of F-cells in the three regions are presented in Table 4. F-cells were significantly more concentrated in the right lobe than in the body and tail ($P < 0.001$). The distribution of the cells in terms of the relative proportion per region is compared with the relative proportion of cPP concentration per region in Figure 3. The close correspondence of these results indicates an excellent correlation between radioimmunoassay and morphometry.

A comparison of Figures 1 and 3 indicates that the relative concentrations of PP, insulin, and glucagon and the cellular distribution of PP in the canine pancreas and human pancreas were similar.

Canine F-cells were located in islets, acini, and ducts. The relative proportion of cPP cells in the endocrine and exocrine tissues differed according to location. In the right lobe the majority of cPP cells was in acinar tissue, whereas, in the body and tail, F-cells appeared to be about equally distributed between acinar and islet tissue.

The same population of cells was stained by both the direct immunofluorescent and the PAP technique. When immuno-stained sections were restained with conventional histochemical stains, F-cells were not reactive or were only weakly reactive to Grimelius' silver stain. The cells did not stain with aldehyde-fuchsin-trichrome, phosphotungstic-acid-hematoxylin, or Hellerstrom-Hellman silver stains.

The specificity of the immunocytochemical-staining reaction was confirmed by the following observations: a discrete cell population had cytoplasmic staining in sections incubated in a 1:500 dilution of bPP antiserum: no cells were stained in adjacent serial sections incubated in a 1:500 dilution of bPP antiserum pretreated with excess bPP, or in serial sections incubated in normal rabbit serum in place of the antiserum, or in adjacent serial sections not covered

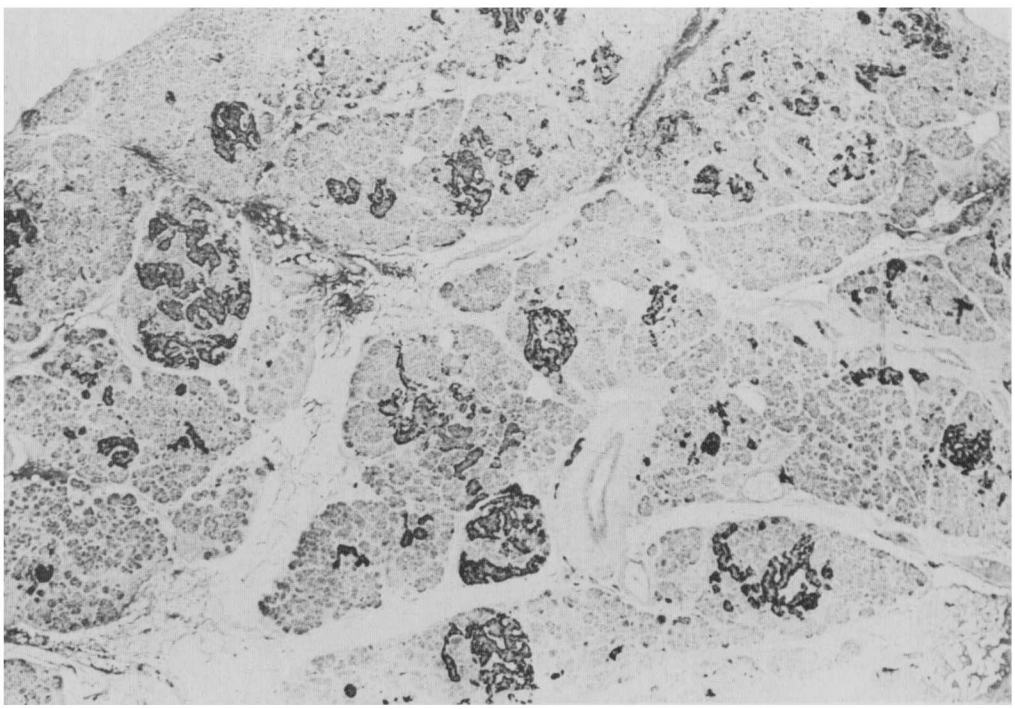

FIGURE 2. An area of the head region of the human pancreas, in which the hPP cells, stained black, make up a high percentage of the endocrine cell population. ×300.

TABLE 3
Concentrations of canine pancreatic polypeptide (cPP), insulin, and glucagon

No.	cPP (μg/g)			Insulin (μg/g)			Glucagon (μg/g)		
	RL*	Body	Tail	RL*	Body	Tail	RL*	Body	Tail
1	96.57	16.81	16.17	49.0	115.0	110.8	1.66	5.04	4.98
2	80.20	19.22	8.70	69.8	114.6	122.2	0.21	4.95	7.05
3	157.10	15.65	19.59	72.4	148.8	116.2	0.32	4.40	4.75
4	74.80	13.00	10.87	43.8	80.8	97.0	0.12	2.15	2.96
5	108.68	8.55	11.05	75.2	85.8	111.2	0.06	5.47	9.74
6	122.29	22.88	17.14	70.8	120.6	141.8	0.28	6.02	7.78
7	179.19	36.64	20.38	93.2	190.0	112.6	0.43	11.86	10.45
8	122.81	16.45	13.41	87.0	162.4	162.8	0.37	8.28	8.15
9	124.68	12.96	9.76	67.6	104.0	113.8	0.38	7.15	5.57
Mean	118.48	18.02	14.12	69.9	124.7	120.9	0.43	6.15	6.83
SEM	11.30	2.69	1.45	5.3	12.0	6.6	0.16	0.92	0.82

* RL, distal portion of right lobe.

with the PAP complex. A gradual decrease in staining reaction was observed in serial sections treated with 1:500, 1:1000, and 1:2000 dilutions of the bPP antiserum, and no staining was observed in sections treated with a 1:5000 dilution of bPP antiserum. Stained cells were present in serial sections incubated in a dilution of 1:500 bPP antiserum pretreated overnight with insulin, glucagon, gastrin, or somatostatin.

DISCUSSION

The results of this study show that a distinct regional concentration of PP, insulin, and glucagon is present in the human pancreas. Human PP concentration was highest in the uncinate process and head, whereas insulin and glucagon were lowest in the head and highest in the tail. The results have also demonstrated that the mean PP cell distribution corresponded with the mean hPP tissue concentration in the four regions. Rastogi et al.[7] reported that the mean insulin content of the tail of 32 nondiabetic pancreases was significantly higher than that in the head but not significantly different from that in the body. Data in the present study are consistent with their data in terms of quantitation and distribution of insulin.

Some inconsistencies were noted in correlation of cell counts and PP tissue concentration in cases 6, 7, and 13 (Tables 1 and 2). This may be explained in part by a distinct localization of PP within the tissues sampled. This unequal distribution was more pronounced in human than in canine pancreases. The presence of large islets with numerous PP cells in the uncinate process and head region of several human pancreases was an unexpected observation. Similar islets, described as hPP hyperplasia, have been seen in pancreases containing pancreatic endocrine tumors[8,9] and in the juvenile diabetic pancreas.[10] Gepts et al.[10] also described less extensive hPP hyperplasia of pancreatic islets in other forms of pancreatic injury (e.g., pancreatitis, tumors of exocrine and endocrine tissue, angiosclerosis). The results of this study have shown such islets to be present in the nondiseased pancreas; thus, their presence may not represent an abnormal condition.

TABLE 4
Counts of canine pancreatic polypeptide cells per 200 fields at ×400 for each region

No.	Right lobe	Body	Tail
1	127	67	38
2	114	31	20
3	63	61	21
4	175	57	44
5	127	21	34
6	143	53	11
7	300	53	25
8	101	44	9
9	68	3	3
Mean	135.3	43.3	22.8
SEM	23.7	7.0	4.6

The regional distribution of PP, insulin, and glucagon in the canine pancreas, previously reported,[1] was shown to correspond well with the distribution of F-cells in the same canine pancreases. These data substantiate and expand the studies that have dealt only with a biregional distribution of PP, insulin, or glucagon in pancreases of experimental animals.[11–17] Several studies have reported that F-cells are more numerous in the right lobe than in the tail half of the pancreas of adult dog, cat, rabbit, hamster, mouse, and rat.[11–14] Gingerich et al.[13] reported that the concentration of PP and of F-cells in the pancreas of *ob/ob* mice was greater in both the duodenal lobe and the tail half when compared with identical areas of control +/+ pancreases. The present study compares F-cells and PP concentrations directly in the same pancreases of dog and man.

The physiologic significance of the regional distribution of PP and other pancreatic hormones is not known. There are data indicating that the differential localization of glucagon to the tail of the pancreas originates during embryonic development. The mammalian pancreas develops from two diverticula of the duodenum—a ventral and a dorsal lobe. In a unique study of the fetal rat pancreas in vitro, Spooner et al.[18] demonstrated that the dorsal lobe contains five to six times more glucagon than the ventral lobe. In man, the ventral lobe forms the uncinate process and part of the head, and the dorsal lobe forms the rest of the head and the body and tail of the pancreas. Perhaps studies of PP concentration in the developing murine or canine pancreas would provide embryologic evidence for its regional distribution.

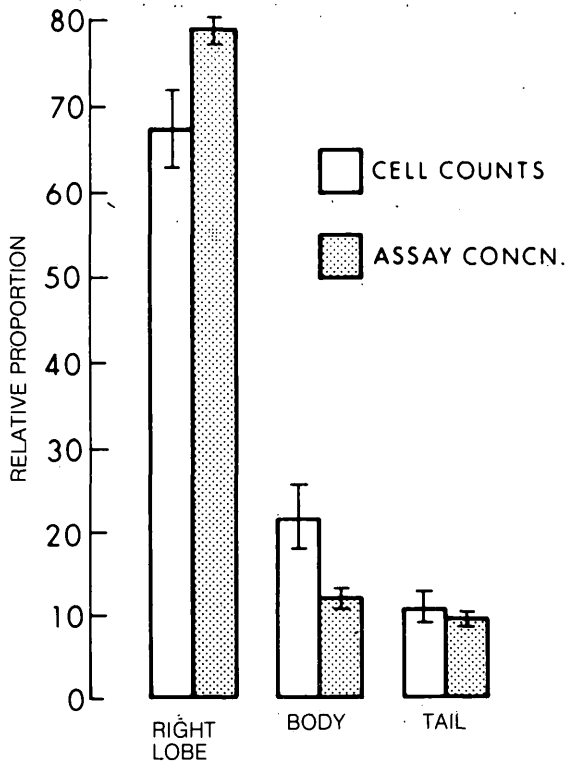

FIGURE 3. A comparison of F-cell counts with cPP concentration in three areas of the canine pancreas. The absolute values for each region of each canine pancreas (Tables 3 and 4) were totaled and each total equated to 100%. The individual values were then converted to a percentage of the total. Means and SEM for the nine pancreases were calculated for each region. The data, defined as relative proportion of F cells and cPP concentration per region, indicated a good correlation between the radioimmunoassay and morphometric results.

The regional distribution of PP, glucagon, and insulin in the pancreas demonstrates the importance of knowing the area of the pancreas sampled for quantitative studies of these endocrine polypeptides.

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