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HISTIDYL-PROLINE DIKETOPIPERAZINE CYCLO (HIS-PRO): IDENTIFICATION AND CHARACTERIZATION IN RAT PANCREATIC ISLETS

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SUMMARY: Measurements of cyclo (His-Pro) in the pancreas were carried out in the rat by a specific radioimmunoassay. Cyclo (His-Pro)-like immunoreactivity was identified in pancreatic islets with a mean concentration of 2023 pg/mg protein, 88-fold higher than that of the whole pancreas. Cyclo (His-Pro) immunoreactivity from pancreatic extracts was indistinguishable immunologically and chromatographically from synthetic cyclo (His-Pro). Insulin-induced hypoglycemia caused a significant, 53% decrease in pancreatic cyclo (His-Pro) concentrations, and FLA-63, a dopamine β-oxidase inhibitor, also reduced islet cyclo (His-Pro) concentrations 51%. These data indicate that cyclo (His-Pro) is present in rat pancreatic islets and may play a potential role in modulating pancreatic responses to nutrient and pharmacologic stimuli.

Histidyl-proline diketopiperazine cyclo (His-Pro) is a biologically active peptide derived from thyrotropin-releasing hormone (TRH) (1). Cyclo (His-Pro), like TRH, is present throughout rat and monkey brain (2-4) and the rat gastrointestinal tract (5). Pancreatic islets contain several peptides smaller than insulin and glucagon (e.g., somatostatin, TRH) (6-8). Because both TRH and pyroglutamate aminopeptidase are present in the pancreas (8,9), we wondered whether cyclo (His-Pro) might be present in the endocrine pancreas and whether islet cyclo (His-Pro) could be influenced by nutrient and pharmacologic manipulations.

MATERIALS AND METHODS

Adult male rats of Sprague-Dawley strain were kept at 24°C on an alternating light-dark cycle and fed laboratory chow ad libitum. Animals were decapitated and their pancreata removed. Pancreata were then homogenized in 1 ml of 0.4 M perchloric acid (5) and then neutralized with 4 M KOH for cyclo (His-Pro) and TRH determinations by specific radioimmunoassays (2) and protein (10).

Pancreatic islets were isolated according to the method of Lacy and Kostianovsky (11). Approximately 300 islets were isolated and extracted in 1 ml of chilled 0.4 M perchloric acid as described above (5) for cyclo (His-Pro) and TRH determinations (2).

Pooled samples of pancreatic extracts, containing a tracer amount of ^{3}H cyclo (His-Pro), were applied to a Sephadex G-25 column (1 x 45 cm), equilibrated with 0.01 M phosphate, 0.15 M NaCl (PBS), and to a high pressure liquid chromatography system

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(HPLC) utilizing a μBondapack C-18 column (0.4 x 30 cm), eluted by ammonium acetate buffer (10 mM ammonium acetate in 22% ethanol, pH 4.0) (12). One ml of each fraction was collected, lyophilized, and resuspended in PBS for ^{3}H counting and cyclo (His-Pro) determinations by RIA.

Streptozotocin (STZ, Sigma Chemical Co., St. Louis, MO) was administered at a dosage of 75 mg/kg i.p. vs. vehicle (0.9% NaCl). Animals were sacrificed one week later for blood glucose determinations and pancreatic extractions with 0.4 M perchloric acid (6).

Regular insulin (40 units/kg) was administered to rats i.p. at 7:00 a.m., after overnight fasting. Animals were decapitated 30 min later for blood glucose measurements and cyclo (His-Pro) determinations in pancreatic extracts.

Bis (4-methyl-1-homopiperazinylthiocarbamyl disulfite, FLA-63) (25 mg/kg) was given i.p. b.i.d. x 3 days to 16- to 19-day-old rats. Animals were killed on day 19. Pancreatic homogenization was carried out for dopamine and norepinephrine determinations using 0.1 M HCl, 5 mM EGTA, 5 mM glutathione. Catecholamine was measured by radioenzymatic analysis according to Osterburg et al. (13). TRH and cyclo (His-Pro) were measured in separate pancreata extracted with 0.4 M perchloric acid (5).

EXPERIMENTAL RESULTS

Cyclo (His-Pro) was detected in all pancreatic extracts, in concentrations ranging from 7.5-74 pg/mg protein with a mean of 23.2 ± 2.7 pg/mg protein (Table 1). The corresponding mean TRH concentration was 8.7 ± 1.8 (N=38). Cyclo (His-Pro) concentrations were raised dramatically in islets isolated from exocrine tissues. The mean cyclo (His-Pro) concentration of 2023 pg/mg protein was 88 times higher than that in whole pancreas ($p<0.001$) and islet TRH concentrations of 255 pg/mg protein were 29-fold higher than those of whole pancreas. Islet cyclo (His-Pro) and TRH concentrations were both significantly higher ($p<0.01$) than those of whole brain (line 3, Table 1).

The elution positions of cyclo (His-Pro) in both Sephadex G-25 and HPLC chromatographic systems (Fig. 1) were identical to those of the ^{3}H cyclo (His-Pro)

Table 1. Concentration of Cyclo (His-Pro) and TRH in Rat Pancreatic Islets

	N	Cyclo (His-Pro) (pg/mg protein)	TRH (pg/mg protein)
Whole pancreas	38	23.2 ± 2.7	8.7 ± 1.8
Islets	3	2023 ± 558	255.1 ± 117
Whole brain	10	59.6 ± 3.2	65.6 ± 2.5

Adult male rats were killed between 9:00 and 11:00 a.m. The samples of whole pancreata islet cells from pancreata and brains were extracted in 0.4 M perchloric acid and assayed for cyclo (His-Pro) and TRH by RIA. The concentrations of the two peptides are expressed as mean ± SEM.

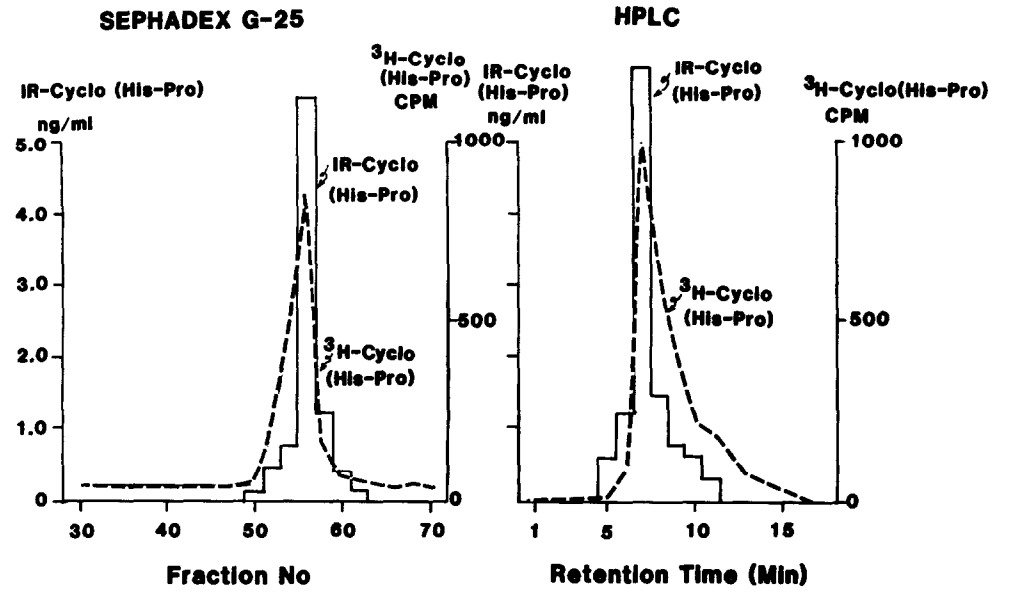

Figure 1. Chromatographic profiles of ^{3}H-cyclo (His-Pro) (broken line) pancreatic cyclo (His-Pro)-like immunoreactivity (base) after Sephadex G-25 (left section) and HPLC (right section). Immunoreactivity co-chromatographed precisely with the tritiated marker peak in both systems.

marker peptide, suggesting structural similarity between endogenous and synthetic cyclo (His-Pro).

STZ administration (75 mg/kg) induced hyperglycemia (blood glucoses from 93 to 257 mg/dl) (Table 2), and a corresponding weight loss of 13%. TRH concentrations declined strikingly and significantly from 9.3 to 3.0 pg/mg protein ($p<0.01$) after STZ. By contrast, cyclo (His-Pro) concentrations remained unchanged (Table 2, line 2).

Table 2. Effect of Streptozotocin (STZ) on Pancreatic Cyclo (His-Pro) and TRH Concentrations

	N	Body Weight (g)	Blood Glucose (mg/dl)	Cyclo (His-Pro) (pg/mg protein)	TRH (pg/mg protein)
Vehicle	8	303.1 ± 7.2	93.0 ± 4.7	26.1 ± 1.8	9.3 ± 0.9
STZ	6	263.3* ± 10.4	257.8* ± 15.7	25.8 ± 1.8	3.0* ± 0.3

Adult male rats were injected i.p. with 75 mg/kg streptozotocin (STZ) or vehicle (control). One week later, animals were killed and pancreatic cyclo (His-Pro) and TRH were determined by RIAs. Values are expressed as mean ± SEM.

* ($p<0.01$) vs. control values

Table 3. Effect of Insulin-Induced Hypoglycemia on Pancreatic Cyclo (His-Pro) and TRH Concentrations

	N	Blood Glucose (mg/dl)	Cyclo (His-Pro) (pg/mg protein)	TRH (pg/mg protein)
Control	9	63.8 ± 0.8	28.7 ± 7.3	14.2 ± 2.7
Insulin	9	26.6* ± 1.7	13.6* ± 1.3	4.2* ± 0.5

Overnight fasted rats were infected with regular insulin, 40 U/kg. These rats were decapitated 30 minutes later. Pancreatic cyclo (His-Pro) and TRH were measured by RIAs. Values are expressed as mean ± SEM.

* ($p<0.01$) vs. control values

Insulin-mediated hypoglycemia (Table 3) was attended by significant reductions in the pancreatic concentrations of both cyclo (His-Pro) and TRH ($P<0.01$) (Table 3).

Inhibition of dopamine conversion to norepinephrine by administration of FLA-63, corroborated by a rise in pancreatic dopamine and a reciprocal decline in pancreatic norepinephrine (Table 4), caused a significant fall in both cyclo (His-Pro) and TRH concentrations ($p<0.01$).

DISCUSSION

Our data show for the first time the cyclo (His-Pro) immunoreactivity is present in rat pancreatic islets and at concentrations 88 times higher than those found in whole

Table 4. Effect of FLA-63 on Pancreatic Dopamine, Norepinephrine, Cyclo (His-Pro) and TRH Concentrations

	Dopamine (pmol/mg protein)	Norepinephrine (pmol/mg protein)	Cyclo (His-Pro) (pg/mg protein)	TRH (pg/mg protein)
Vehicle	1.0 ± (4) 0.4	84.0 ± (4) 7.7	16.6 ± (10) 2.0	21.6 ± (10) 1.6
FLA-63	9.3* ± (4) 1.0	39.1* ± (4) 2.4	8.6* ± (10) 0.8	6.9* ± (10) 0.5

Sixteen day-old rats received either FLA-63 (25 mg/kg) or vehicle alone for 3 days. Animals were killed on day 19, and pancreatic cyclo (His-Pro) and TRH were measured by RIAs. Dopamine and norepinephrine in the pancreatic extracts were measured by a radioenzymatic assay. Values are expressed as mean ± SEM. The number of animals per group is shown in parentheses.

* ($p<0.012$) vs. control values.

pancreas. Moreover, such pancreatic cyclo (His-Pro) immunoreactivity appears to be chromatographically (Fig. 1) and immunologically (Fig. 2) identical to the synthetic dipeptide. TRH-like immunoreactivity was also present in pancreatic islets, in agreement with previous data (8), but in concentrations significantly lower than those of cyclo (His-Pro).

Administration of streptozotocin, a pharmacological agent known to induce diabetes mellitus by selective β-cell injury, resulted, as anticipated, in hyperglycemia and a significant decline in pancreatic TRH concentrations. Under these conditions, however, pancreatic cyclo (His-Pro) concentrations remained completely unaltered, suggesting that islet cyclo (His-Pro) may not be associated with β-cells, as TRH has been associated immunocytochemically (14), but rather with α or δ cells. Because TRH has been reported to stimulate glucagon secretion (15), which can be enhanced by hypoglycemia (16), we wondered whether insulin-mediated hypoglycemia might influence pancreatic peptide concentrations. Insulin administration did cause hypoglycemia and a significant reduction in both pancreatic cyclo (His-Pro), implying that cyclo (His-Pro) may participate also in the modulation of islet hormonal responses to nutrients. Similarly, interference with the local formation of pancreatic norepinephrine from dopamine by blockade of dopamine-β-oxidase with FLA-63 reduced the pancreatic content of both peptides, suggesting that cyclo (His-Pro) may be involved, as well, in the catecholamin-

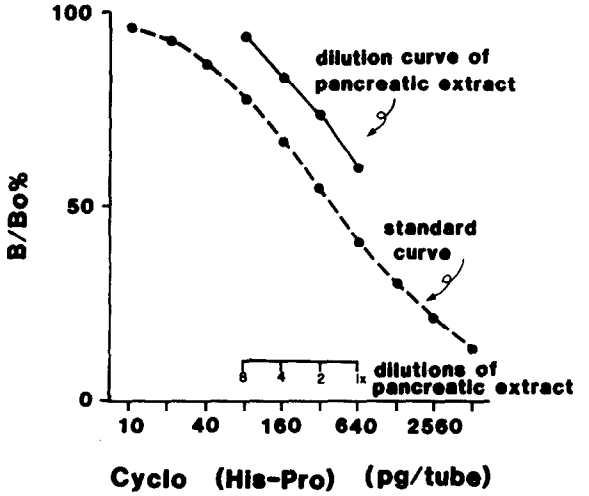

Figure 2. Cyclo (His-Pro) radioimmunoassay standard curve (broken line). The inhibition curve generated with serial dilutions of pancreatic extract (solid line, 1:1 to 1:8) was parallel with the standard curve.

ergic control of islet secretory events. Further studies are currently in progress to examine directly the influence of cyclo (His-Pro) on the secretion of insulin and/or glucagon by rat pancreatic islets.

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