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Diabetogenic Polypeptide From Human Pituitaries Similar to That Excreted by Proteinuric Diabetic Patients

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A polypeptide exhibiting diabetogenic and antiinsulin properties has been isolated from human pituitary glands by a procedure slightly modified from that with which a similar polypeptide was previously isolated from the adenohypophysis of cattle, sheep and pigs. The isoelectric point of the compound is approximately pH 4.1 which is the same as that of the compound isolated from the adenohypophysis of the other three species and from urine of patients with lipoatrophic diabetes and proteinuric diabetics without lipoatrophy. Administration of 1 mg polypeptide per kilogram of body weight to dogs resulted in significant intolerance to glucose and resistance to exogenous insulin 10 and 34 hours after injection. Comparison of glucose tolerance tests in the same animals discloses that the diabetogenic potency of the substance from human pituitaries is much greater than that of human growth hormone or ovine prolactin. The molecular weight of the substance as determined by the dodecyl sulfate-polyacrylamide gel electrophoresis method is 20,600. A modified procedure for the isolation and purification of the polypeptide has been outlined.

WE HAVE REPORTED in previous communications the isolation of a diabetogenic polypeptide from the urine of patients with lipoatrophic diabetes,[1] the urine of proteinuric diabetic patients without lipoatrophy,[2] and the adenohypophysis of beef,[3] sheep and pigs.[4] The peptide from all these sources has been shown to induce hyperglycemia and resistance to exogenous

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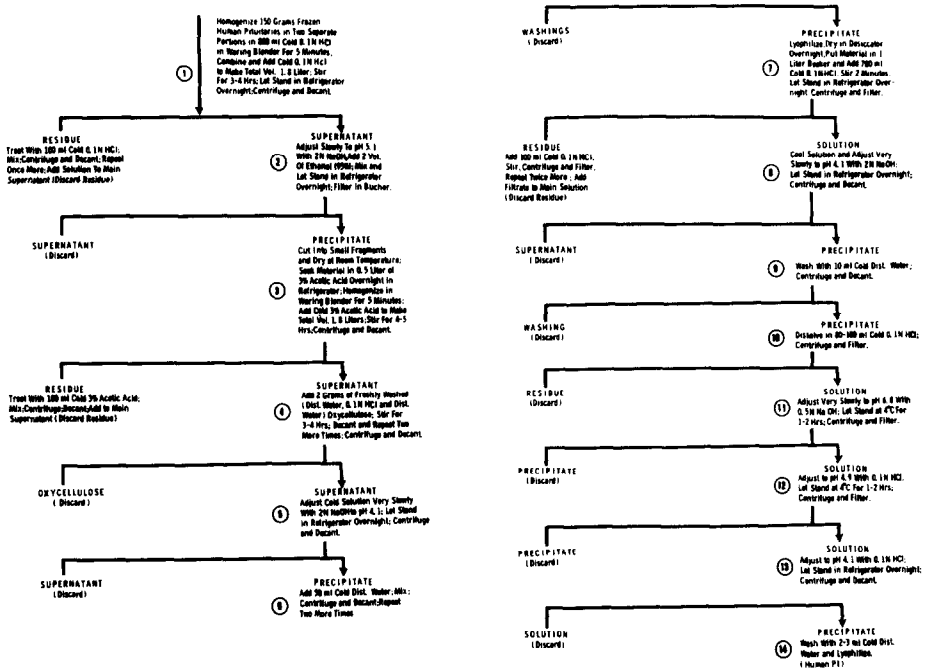

Fig. 1.—Procedure for isolation of diabetogenic polypeptide (human PI) from human pituitaries.

insulin when administered to dogs or humans. Because the compound obtained from each of the above mentioned sources exhibits similar physicochemical and biological properties, it was concluded that the origin of the polypeptide found in the urine of diabetic patients is probably the pituitary gland. Since this conclusion was based on the isolation of a similar peptide from pituitary glands of cattle, sheep and pigs,[3,4] it became important to carry out parallel studies employing pituitary glands of man.

The purposes of the present paper are to outline a slightly modified procedure for the isolation and purification of the diabetogenic polypeptide from human pituitary glands, to demonstrate its antiinsulin and diabetogenic effects in dogs, and to compare its diabetogenic potency with that of human growth hormone and ovine prolactin.

Materials and Methods

After a 2-yr period a sufficient number of human pituitary glands were collected from local hospitals to carry out these studies. After removal the glands were frozen. The active peptide was isolated according to the procedure outlined in Fig. 1. Our previously published method for the extraction of bovine diabetogenic peptide is also suitable.[3] Its isoelectric point (approximately pH 4.1) proved to be the same as that of the peptides obtained from animal adenohypophysis and from urine of proteinuric diabetic patients. The average yield is 0.03–0.04%. This material is designated as human PI.

The oxycellulose powder (16–22% COOH, Eastman Chemical Products, Inc., Kingsport, Tenn.) used in the procedure was kept refrigerated and before being used was washed successively with distilled water, 0.1 *N* HCl and distilled water.

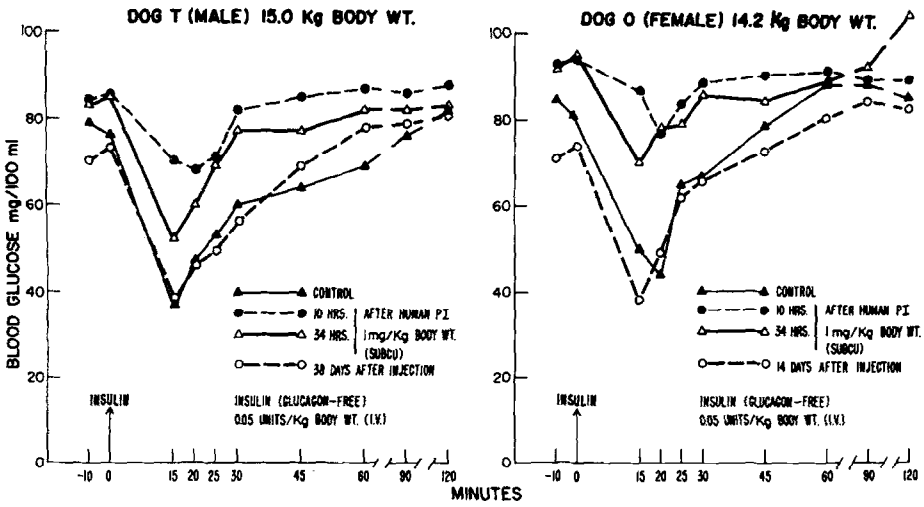

Fig. 2.—Effect of human PI on insulin tolerance in dogs T and O.

A Sephadex G-100 superfine descending column (0.9 × 60 cm) was used for the purification of human PI. The gel was equilibrated and eluted with 0.03 *N* HCl containing 0.2 *M* NaCl. Diabetogenic peptide (20 mg) was dissolved in 0.05 *N* HCl (0.6 ml) and applied to the column. The elution rate was 0.3 ml/20 min. The operating pressure was 20 cm. Protein was determined by the procedure of Lowry.[5] The active polypeptide was recovered either by isoelectric point (pH 4.1) precipitation or by dialysis against distilled water. The procedure for acrylamide gel electrophoresis has been described previously.[2]

Human growth hormone (lot 1-C) was obtained from the National Institute of Arthritis and Metabolic Diseases, National Pituitary Agency of the University of Maryland. This preparation has a biopotency of 1 I.U. per milligram. The hormone was dissolved in 2 ml of distilled water and administered subcutaneously as a single dose. Human growth hormone (Raben #16) has a mean biopotency of 1.1 I.U. per milligram. The protein was dissolved in dilute hydrochloric acid and neutralized with 0.05 *M* NaOH to pH 7.0. Human growth hormone (Merck Sharp and Dohme C-1555) was dissolved in 2 ml distilled water and neutralized with 0.05 *M* NaOH to pH 7.0.

Ovine prolactin (NIH-P-S-7) was obtained from the National Institute of Health, Endocrinology Study Section, Bethesda, Md. This preparation has a mean potency of 24.3 I.U. per milligram. The hormone was dissolved in 2 ml of distilled water and administered subcutaneously as a single dose.

Four trained normal dogs, two males and two females, were employed for biological assay. Maintenance and preparation of dogs for glucose and insulin tolerance tests have been previously described.[4] The polypeptide was dissolved in 1.5 ml 0.05 *N* HCl and neutralized by dropwise addition of 0.05 *N* NaOH to pH 6.7–7.0. The solution was administered subcutaneously as a single dose. No untoward effects were observed. Blood glucose was determined by the Somogyi-Nelson procedure.[6]

Molecular weight estimation was carried out by the dodecyl sulfate-polyacrylamide gel electrophoresis procedure of Weber and Osborn.[7] Pure ovalbumin, chymotrypsinogen A, ribonuclease A and cytochrome C were used as protein standards.

Results

The polypeptide isolated from the human pituitary glands was tested for anti-insulin activity in two normal dogs (O and T). Each animal was given a single subcutaneous injection of 1 mg of the polypeptide per kilogram of

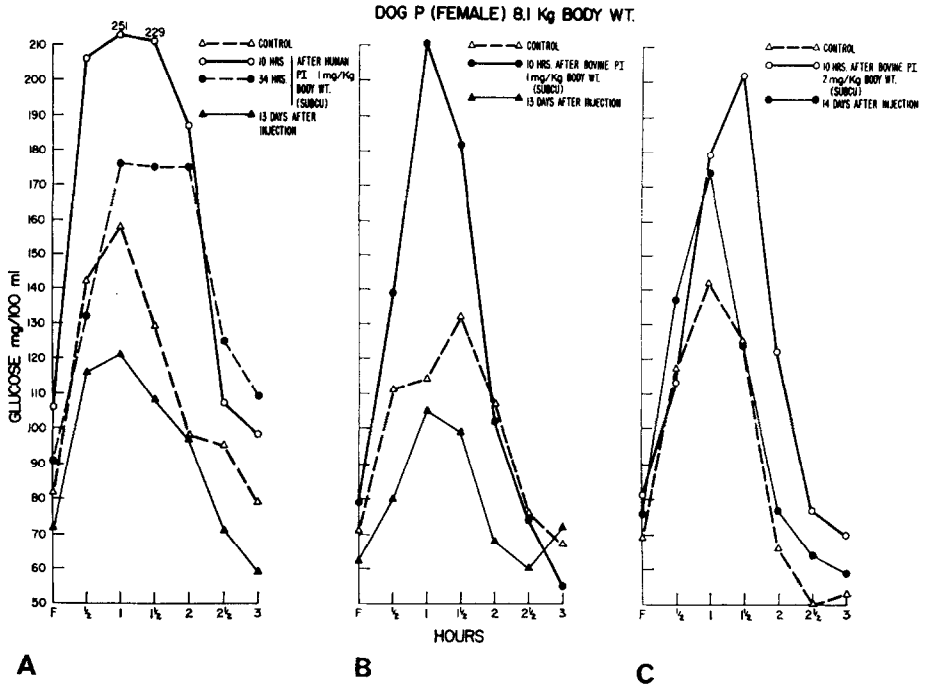

Fig. 3.—Effect of subcutaneous administration of human PI and bovine PI on glucose tolerance of normal dog P.

body weight. Insulin tolerance tests were carried out 10 and 34 hr after polypeptide administration. It is apparent (Fig. 2) that sensitivity to the hypoglycemic effect of exogenous insulin was significantly reduced. Insulin resistance was still evident in both animals 34 hr after polypeptide administration.

The effect on glucose tolerance was studied in dogs P and L. Again a dose of 1 mg of polypeptide per kilogram of body weight was employed. Carbohydrate tolerance was greatly impaired in both animals (Figs. 3A and 4A) at 10 hr and less so at 34 hr. In dog L, 58 hr after polypeptide administration, glucose tolerance had returned to the preinjection level.

For comparative purposes, the same two animals (dogs P and L) were studied using bovine polypeptide. In dog P, two separate experiments with doses of 1 mg and 2 mg of bovine PI per kilogram of body weight were carried out with an interval of 13 days between them. The results are presented in Fig. 3B and 3C. One milligram per kilo of the human peptide appears to have induced the greatest decrease in glucose tolerance. In dog L, 2 mg/kilo of the bovine peptide induced no change in glucose tolerance but the effect of 4 mg/kilo is seen in Fig. 4B. While the number of tests is too small to make a meaningful comparison, it appears that in both animals the human peptide induced the greater loss of glucose tolerance.

The effect of human growth hormone on glucose tolerance was also observed in dogs L and P. The dose was 0.125–1.0 mg of hormone per kilogram of body weight and glucose tolerance test was carried out 10 hr after each injection.

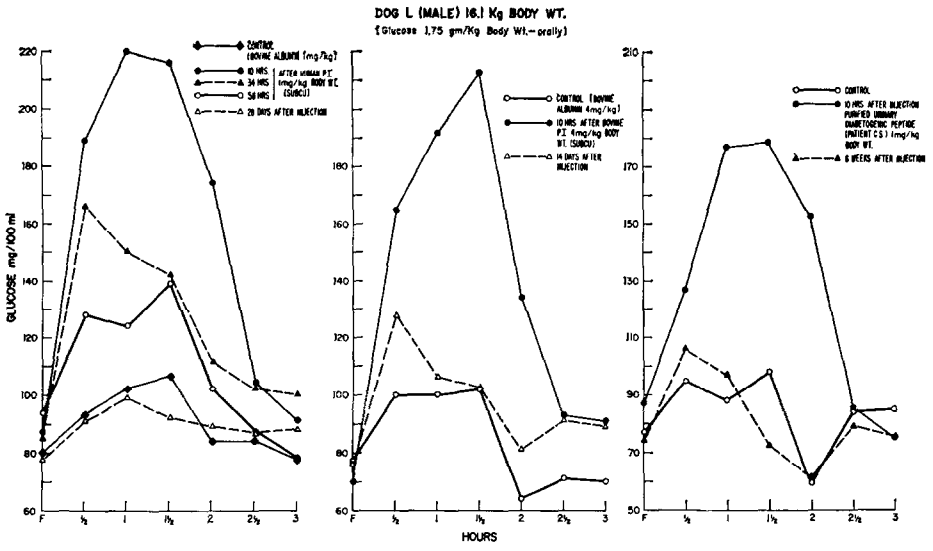

Fig. 4.—Effect of subcutaneous administration of human PI, bovine PI, and purified diabetogenic polypeptide isolated from urine of diabetic patient C.S. on glucose tolerance of normal dog L.

Table 1.—Effect of Human Growth Hormone on Glucose Tolerance

	Dog L, Male, 15.4 kg Body Weight						
Hours after injection	F	½	1	1½	2	2½	3
Control	73	87	79	84	57	73	83
10 (0.125 mg/kg HGH*)	74	86	88	63	69	55	72
10 (0.25 mg/kg HGH*)	72	123	129	132	72	77	82
Control	78	93	88	74	72	82	80
10 (0.5 mg/kg HGH*)	72	122	78	99	69	83	84
10 (1.0 mg/kg HGH*)	86	126	98	96	74	81	84
	Dog P, Female, 7.8 kg Body Weight						
Control	86	152	186	131	80	68	75
10 (0.5 mg/kg HGH †)	77	142	162	107	79	67	70
10 (0.5 mg/kg HGH ‡)	73	158	167	116	72	65	73

* NIH-HGH, lot 1-C.

† NIH-HGH, Raben #16.

‡ Merck Sharp and Dohme, Lot C-1555.

The results are shown in Table 1. It is clear that at these dose levels no significant change in glucose tolerance was observed in both of the animals. Ovine prolactin (0.5 mg/kilo) did not induce any significant change in glucose tolerance in dog P, the results are shown in Table 2. In contrast, administration of 1.0 mg/kilo of human PI in dog L induced a great decrease of glucose tolerance (Fig. 4A). In dog P 0.5 mg/kilo of purified human PI induced significant intolerance to glucose. The results are shown in Table 3. However, 0.25 mg/kg body weight of purified human PI did not provoke any significant alteration of glucose tolerance in this dog.

Table 2.—Effect of Ovine Prolactin on Glucose Tolerance

	Dog P, Female, 7.8 kg Body Weight						
Hours after injection	F	½	1	1½	2	2½	3
Control	72	156	164	92	59	68	66
Control	64	153	157	86	58	70	76
Ovine Prolactin* 10 (0.5 mg/kg)	80	123	158	134	76	69	69

* NIH-P-S-7.

Table 3.—Effect of Human PI on Glucose Tolerance

	Dog P, Female, 7.8 kg Body Weight						
Hours after injection							
Human PI 0.5 mg/kg body weight	F	½	1	1½	2	2½	3
Control	66	124	151	118	66	65	64
10	84	135	211	159	96	74	74
34	87	140	222	164	104	71	88
106	66	118	165	162	86	60	67
14 days	65	138	141	134	81	54	58

The effect of a highly purified polypeptide preparation (isoelectric point approximately pH 4.1) extracted from the urine of a proteinuric diabetic patient (C.S.) was studied on the same animal (dog L). One milligram per kilogram was administered subcutaneously as a single dose (Fig. 4C). Its diabetogenic potency in this animal is clearly greater than that of the bovine peptide which was inactive at 2 mg/kilo.

Figure 5 shows the Sephadex column chromatography profile of the diabetogenic polypeptide from human pituitary glands. The first peak has no diabetogenic effect in dogs. Disc acrylamide gel (7.5%, pH 8.8) electrophoresis of the active fraction is shown in Fig. 6. The diabetogenic activity (0.5 mg/kilo) of this fraction (second peak) is shown in Fig. 7. Larger amounts are not available for testing.

Five separate molecular weight determinations of purified human PI by the method of Weber and Osborn give the following values: 20,500; 22,600; 19,600; 21,000 and 19,500. The molecular weight of the peptide as determined by this method appears to be approximately 20,600. A typical run is shown in Fig. 8.

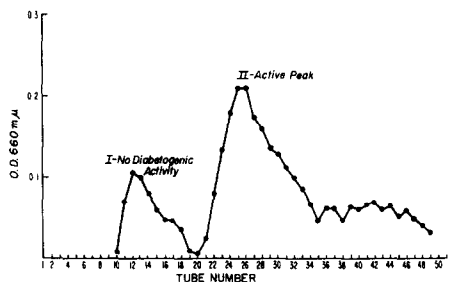

Fig. 5.—Purification of human PI on Sephadex G-100 superfine.

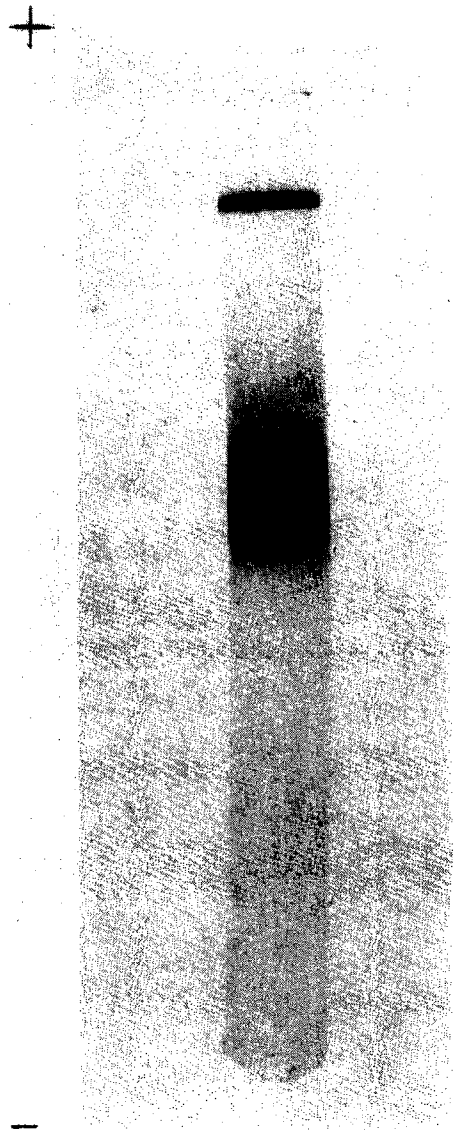

Fig. 6.—Polyacrylamide gel electrophoresis of purified human PI. Band at Bottom of gel represents tracking dye.

Discussion

A diabetogenic polypeptide has been isolated from human pituitary glands employing a slight modification of the procedure with which a similar polypeptide was previously isolated from adenohypophysis of cattle,[3] sheep, and pigs.[4] The physicochemical properties of the substance isolated from the adenohypophysis of all four species are similar to those of the peptides extracted from the urine of patients with lipoatrophic diabetes and from the urine of proteinuric diabetic patients without lipoatrophy.[2] The isoelectric point of the active polypeptide isolated from all these sources is approximately pH 4.1. The possibility that the substance might be somatotropin is unlikely since the

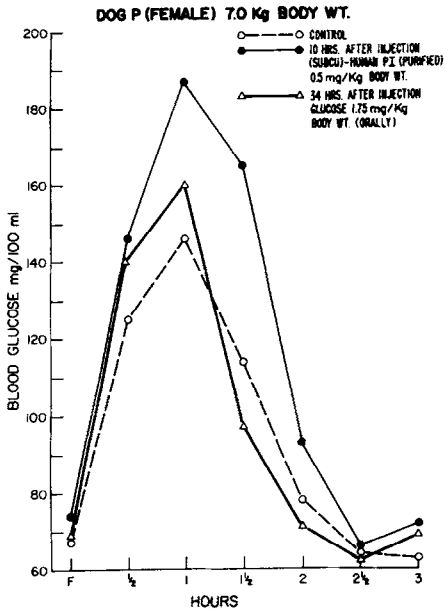

Fig. 7.—Effect of purified human PI on glucose tolerance in dog P.

isoelectric point of human growth hormone is pH 4.9 and all material that precipitated at this pH was removed during our isolation procedure. Nevertheless, our polypeptide could still be contaminated with growth hormone since the disc acrylamide gel electrophoresis pattern indicates a small band before the large one. Antihuman growth hormone serum reacts with our substance to about 10% (performed by Dr. Ralph Knopf of our group) but the possibility that the cause of the glucose intolerance might be due to the presence of somatotropin in our preparation is remote since administration of 0.5–1.0 mg/kilo

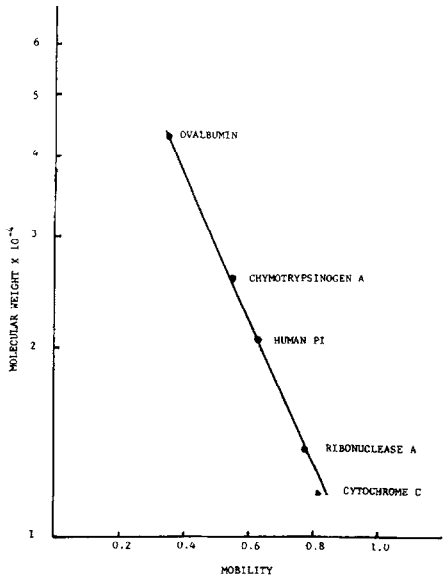

Fig. 8.—Molecular weight determination of human PI. Four marker proteins used were ovalbumin, 45,000; chymotrypsinogen A, 25,000; ribonuclease A, 13,700; and cytochrome C, 11,700.

of human growth hormone does not provoke any significant change in carbohydrate tolerance in the same animals. Whether our human pituitary diabetogenic polypeptide (human PI) has any growth-promoting activity remains to be determined. It should be mentioned, however, that by means of the tibia test, our bovine pituitary diabetogenic polypeptide (bovine PI) exhibits no growth promoting activity.[8] The chance that the peptide might be ACTH is also unlikely since the molecular weight of the two compounds are greatly different. We have shown previously that bovine PI[3] and the diabetogenic polypeptide extracted from the urine of patients with lipoatrophic diabetes exhibit no adrenal stimulating activity in man.[1] The possibility that the peptide might be prolactin cannot be ruled out completely. In one experiment, ovine prolactin (0.5 mg/kilo) did not induce any significant change in glucose tolerance in the same dog (dog P) in which human PI decreased it. By means of the Reece-Turner indermal pigeon crop technique, the diabetogenic polypeptide extracted from ovine adenohypophysis (ovine PI) has approximately 4 I.U. per milligram of prolactin activity.[4] The molecular weight of human PI has now been determined to be 20,600; that of bovine PI is 26,000.[10]

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