HETEROGENEITY OF PANCREATIC POLYPEPTIDE IMMUNOREACTIVITY IN HUMAN PLASMA

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1. Introduction

Chance et al. [1] have recently isolated a nearly identical polypeptide from bovine, ovine, porcine and human pancreata. These pancreatic polypeptides represent the mammalian counterpart of avian pancreatic polypeptide (APP) [2,3].

Human pancreatic polypeptide (HPP) contains 36 amino acids and has an approximate molecular weight of 4200 [1]. Immunoassayable HPP is present in blood and its levels increase in response to food ingestion and insulin-induced hypoglycemia [4,5].

Since a considerable number of peptide hormones appear to circulate in blood in multiple immunoreactive forms [6], this work was undertaken to examine the behaviour of plasma immunoreactive HPP on molecular sieve chromatography. We have fractionated the HPP immunoreactivity present in plasma of normal subjects, both in the basal state and after stimulation of HPP release by insulin-induced hypoglycemia.

2. Methods and materials

Blood samples were obtained from five healthy, young volunteers through an indwelling butterfly needle inserted in an antecubital vein. To stimulate HPP secretion regular pork insulin (Actrapid) was injected i.v. at a dose of 0.1 U/kg. The extractions were made in the basal state (after an overnight fast) and at 40, 50 and 60 min following administration of insulin. Blood specimens were placed in chilled tubes containing EDTA and immediately centrifuged at 4°C. The plasma was stored at −20°C until used.

Circulating HPP was fractionated by molecular sieve chromatography on Bio Gel P-30 or Sephadex G-100 columns (1 × 50 cm). The columns were equilibrated and developed in the same diluent that was used in the radioimmunoassay. These procedures have been described in detail elsewhere [7].

HPP in plasma and eluates was measured by radioimmunoassay employing rabbit anti-HPP serum (Lot # 615-1054B-248-19), HPP (Lot # 615-1054B-200) as standard, and bovine pancreatic polypeptide (BPP) (Lot # 615-D63-188-9) for iodination, which was performed with ^{125}I by the technique of Greenwood et al. [8]. All these substances were kindly donated by Dr Ronald E. Chance (Lilly Research Laboratories, Eli Lilly Co. Indianapolis, USA). ^{125}I-BPP was purified on Bio Gel P-30, and the monoiodinated component was subsequently separated by polyacrylamide disc-gel electrophoresis. ^{125}I-BPP was also used as tracer in the gel filtration studies. Glycine buffer (0.2 M), containing 0.25% human serum albumin and 1% normal sheep serum (pH 8.8), served as the assay diluent. Full details of our assay will be described in a later report.

3. Results

Filtration on Bio Gel P-30 of basal plasma from each of the five subjects revealed the presence of three discrete fractions of immunoreactive HPP, as expressed in table 1 and illustrated in the represen-

Table 1
HPP-immunoreactive components on Bio Gel P-30 filtration of plasma (3 ml) from normal subjects

Subject no.		Total HPP loaded (pg)	HPP-immunoreactive components		
			Fraction I (pg)	Fraction II (pg)	Fraction III (pg)
1	Basal	193	37	36	45
	Post-insulin	2448	83	1779	18
2	Basal	240	60	24	69
	Post-insulin	2430	72	1194	57
3	Basal	369	156	12	189
	Post-insulin	3582	237	3939	183
4	Basal	414	48	69	62
	Post-insulin	3450	81	2310	72
5	Basal	834	126	132	18
	Post-insulin	4800	92	1731	33

tative elution profile depicted in fig.1 (upper panel). Between 27% and 46% of the recovered immunoreactivity eluted in the void volume of the column, concurrently with plasma proteins (fraction I). The second peak appeared in the same effluent position as that of the ^{125}I-PP marker and accounted for 4–48% of the toatal immunoreactivity (fraction II). The slowest migrating component (fraction III) was eluted slightly earlier than the iodide marker and comprised 7–53% of the immunoreactivity of the eluates.

Plasma samples obtained after insulin-induced hypoglycemia contained from 6 to 12-fold higher concentrations of HPP than basal specimens. When these samples were filtered on Bio Gel P-30, immunoreactive HPP appeared to be distributed in the same three fractions that have been described above. However, the increase in total loaded immunoreactivity was recovered mainly in the area of fraction II, which, in this condition, represented more than 90% of the total (table 1; fig.1, lower panel).

Finally, to obtain additional information on the gel filtration behaviour of fraction I, basal plasma was subjected to chromatography on Sephadex G-100 (fig.2). On this gel, 20% of the recovered immunoreactivity eluted in a position which coincided with

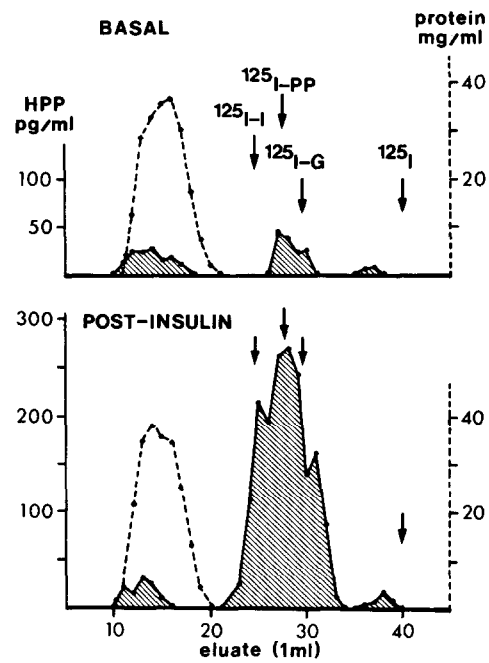

Fig.1. Chromatographic pattern on Bio Gel P-30 of the HPP immunoreactivity present in the plasma of a normal subject. The arrows indicate the elution volume of the radioactive markers (^{125}I-insulin: ^{125}I-I; ^{125}I-pancreatic polypeptide: ^{125}I-PP; ^{125}I-glucagon: ^{125}I-G; ^{125}I). The shaded area represents the immunoreactive HPP, and the broken line the protein content of the eluates.

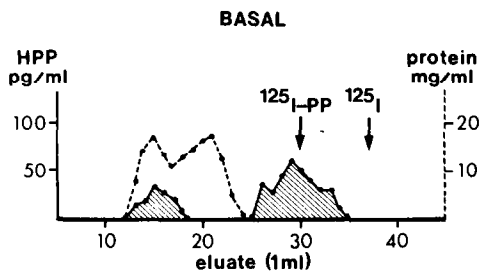

Fig.2. Chromatographic pattern on Sephadex G-100 of the HPP immunoreactivity present in the plasma of a normal subject. The arrows indicate the elution volume of the radioactive markers (^{125}I-pancreatic polypeptide: ^{125}I-PP; ^{125}I). The shaded area represents the immunoreactive HPP, and the broken line the protein content of the eluates.

plasma globulins and the remaining 80% migrated in the zone of the ^{125}I-PP marker. While the first peak seemed to correspond to fraction I, the second component appeared to represent the areas of fractions II and III which could not be separated by this type of gel.

4. Discussion

Our results indicate that, at least, three HPP immunoreactive fractions are present in plasma of normal subjects. In the basal state, each component represents roughly one-third of the total immunoreactivity. The elution position of fraction II allows to assume that this material is identical to HPP as isolated from the pancreas (mol. wt 4200) [1]. Furthermore, an increase in this fraction accounts for most of the immunoreactive HPP found after stimulation of HPP secretion by insulin-induced hypoglycemia.

The early-eluting component of immunoreactive HPP (fraction I) migrates with plasma globulins on Sephadex G-100. Therefore, if it represents a discrete molecular species its size would be greater than 100 000 daltons. On the other hand, the molecular weight of the smallest component (fraction III) seems to be about 1500. The areas of fractions I and III were not obviously modified after stimulation of HPP secretion.

The present study does not permit us to define further the nature of fractions I and III, although it would be tempting to speculate about their possible implications in the processes of HPP biosynthesis (fraction I) and degradation (fraction III). Very little information is available concerning the chromatographic pattern of HPP in its tissue of origin. Adrian et al. [9] found no evidence of size heterogeneity of immunoreactive HPP in pancreatic extracts. If this finding is confirmed, it would favour the possibility that fraction I contained HPP in association with plasma proteins.

Finally, the occurrence of fractions I and III in blood, independently of their significance, should be considered when determining basal HPP concentrations, since both components account for more than half of the measured immunoreactivity.

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References

[1] Chance, R. E., Lin, T. M., Johnson, M. G., Moon, N. E., Evans, D. C., Jones, W. E. and Koffenberger, J. E., Jr. (1975) in: Program of the 57th Annual Meeting of the Endocrine Society, New York, p. 183 (Abstract No. 265).
[2] Kimmel, J. R., Pollock, H. G. and Hazelwood, R. L. (1968) Endocrinology 83, 1323–1330.
[3] Kimmel, J. R., Hayden, L. J. and Pollock, H. G. (1975) J. Biol. Chem. 250, 9369–9376.
[4] Schwartz, T. W., Rehfeld, J. F., Stadil, F., Larson, L.-I., Chance, R. E. and Moon, N. (1976) Lancet 1, 1102–1105.

[5] Adrian, T. E., Bloom, S. R., Besterman, H. S., Barnes, A. J., Cooke, T. J. C., Russell, R. C. G. and Faber, R. G. (1977) Lancet 1, 161–163.
[6] Melani, F. (1974) Hormone Metab. Res. 6, 1–8.
[7] Valverde, I., Villanueva, M. L., Lozano, I. and Marco, J. (1974) J. Clin. Endocrinol. Metab. 39, 1090–1098.
[8] Greenwood, F. C., Hunter, W. M. and Glover, J. S. (1963) Biochem. J. 89, 114–123.
[9] Adrian, T. E., Bloom, S. R., Bryant, M. G., Polak, J. M., Heitz, Ph. and Barnes, A. J. (1976) Gut 17, 940–944.