Vol. 89, No. 3, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 13, 1979 Pages 913-918

SUPPRESSION OF SOMATOSTATIN LEVELS IN THE HEPATIC PORTAL AND SYSTEMIC PLASMA OF THE RAT BY SYNTHETIC HUMAN PANCREATIC POLYPEPTIDE

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Received June 29,1979

SUMMARY

Intravenous administration of synthetic human pancreatic polypeptide (hPP) with 36 amino acid residues significantly decreased immunoreactive somatostatin levels in both hepatic portal and systemic plasma in anesthetized rats. The effect of hPP on plasma somatostatin levels is acute. The results suggest that hPP may have a role in controlling somatostatin secretion from the gut and pancreas.

Homologous pancreatic polypeptides (36 amino acid residues) with hormonal properties were isolated from avian and mammalian pancreas by Kimmel et.al. (1,2,3) and by Chance et.al. (4,5). The search for the physiological stimuli which affect pancreatic polypeptide (PP) secretion has been facilitated by antisera generated against aPP (6), and hPP (7). These antisera have been used for immunocytochemical studies and for radioimmunoassay (RIA) (8,9,5). Basal levels of plasma hPP increase with age (10). Ingestion of protein meal, fat, or intravenous administration of amino acids increase plasma PP levels (10). On the other hand, infusion of somatostatin suppresses the postprandial rise of plasma hPP (10). Although relationships between somatostatin and other pancreatic and gut hormones are actively studied, the effect of PP on the secretion of somatostatin has not been reported. These and related studies have been largely hampered by lack of ample quantities of pure PP. Recently, the total synthesis of pure hPP_{1-36} has been achieved in high yield by a solid phase method in our laboratory which has enabled us to investigate its physiological function.

Abbreviations: PP,pancreatic polypeptide; hPP, human pancreatic polypeptide; aPP, avian pancreatic polypeptide; RIA, radioimmunoassay; B.W., body weight; KIU, Kallikrein Inactivator Units; iv, intravenous; ip, intraperitoneal.

0006-291X/79/150913-06$01.00/0

The structure of hPP is as follows:

Ala-Pro-Leu-Glu-Pro-Val-Tyr-Pro-Gly-Asp-Asn-Ala-Thr-Pro-Glu-Gln-Met-Ala-Gln-Tyr-Ala-Ala-Asp-Leu-Arg-Arg-Tyr-Ile-Asn-Met-Leu-Thr-Arg-Pro-Arg-Tyr-NH_2

Furthermore, we have recently overcome the difficulty of radioimmunoassay for rat plasma somatostatin (11). This paper reports the first observation that hPP, unlike other gut hormones, suppresses secretion of somatostatin from the gut and pancreas.

METHODS

Young adult male rats of CD strain from Charles River Co., weighing 240-300 g, were used throughout the experiments. They were housed in animal quarters equipped with controlled light (on at 0500, off at 1900) and temperature (24 ± 2°C). The animals were fed Purina rat diet ad libitum (Exp.1) or fasted overnight before the experiment (Exp.2). All rats were allowed access to water ad libitum. Rats were anesthetized with sodium pentobarbital (Nembutal), 5mg/100g body weight ip. Fifteen min after Nembutal, 0.5 ml 0.9% saline or hPP dissolved in saline was injected into the jugular vein over a period of 0.5 - 1 min. In Experiment 2, hPP was given iv as a bolus followed by an infusion over a 5 min period. Two or 5 min after the initiation of injection, 2 ml of blood was withdrawn from the jugular vein. The animal was then laparotomized and 2 ml of blood was collected from the hepatic portal vein. Blood collection from the jugular and the hepatic vein was completed within 2 minutes. Blood was placed in a chilled test tube containing 5 mg EDTA and 1000 KIU Trasylol (FBA Pharmaceuticals, New York). Plasma was separated by centrifugation at 2000 rpm at 4°C. To 0.5 ml plasma was added 0.5 ml 2M acetic acid, and then 4 ml cold acetone was added drop-wise to the acidified plasma while the test tube was being vortexed. The mixture was sonicated for 30 sec and centrifuged at 3000 rpm for 10 min at 4°C. The supernatant was decanted into a 16 X 100 mm gelatin-coated tube. The precipitate was washed with 1 ml 80% acetone and the tube centrifuged. This supernatant was added to the gelatin-coated tube and washed with 3 ml of an organic solvent (ethyl acetate: ether = 3:1). After a brief centrifugation at 2000 rpm, the upper organic layer was aspirated and the aqueous acetone layer was dried by nitrogen gas at 37-40°C. The dried residue was dissolved in 0.5 ml diluent for RIA, and 0.2 ml was assayed for somatostatin by RIA using rabbit antiserum to somatostatin (R101) as described elsewhere (11).

hPP was synthesized by a stepwise solid phase method with modifications similar to those recently described for the synthesis of somatostatin and other peptides (12-14). Details of the synthesis and purification of hPP will be reported elsewhere.

Mean plasma somatostatin levels of each treatment group were calculated and compared to each other using Duncan's new multiple range test (15).

RESULTS AND DISCUSSION

The extraction method for plasma somatostatin has been found to eliminate almost all interfering substances in the rat plasma including binding proteins and degrading enzymes. This method can be also applied to measurement

TABLE 1. Effect of administration of synthetic hPP on plasma somatostatin levels in rats.

	Substance injected	Somatostatin levels (pg/ml) mean ± SE Systemic plasma		Portal plasma	
Exp. 1.	Saline	10.4 ± 1.36	(5)	72.0 ± 10.81[a]	(5)
	hPP	6.6 ± 0.93[b]	(5)	27.8 ± 9.08[c]	(5)
Exp. 2.	Saline	5.4 ± 1.8	(5)	73.3 ± 14.1[d]	(4)
	hPP	2.9 ± 0.38	(4)	29.5 ± 2.10[e]	(4)

In Exp.1., 10 μg hPP/100 g B.W. was injected iv over 1 min. Blood was collected 2 min after the initiation of injection.

In Exp.2., 5 μg hPP/100 g B.W. was injected iv as a bolus followed by iv infusion of 5 μg/100 g for a 5 min period.

a: vs systemic plasma saline group, $P<0.01$; b: vs corresponding saline group, $P < 0.05$; c: vs corresponding saline group, $P<0.02$; vs corresponding systemic plasma group, $P < 0.05$;
d: vs corresponding systemic blood, $P<0.01$; e: vs corresponding systemic blood, $P<0.01$; vs corresponding saline group, $P<0.01$.

Numbers in parenthesis indicate number of rats.

of somatostatin in whole blood with a slight modification (16). The recovery of added somatostatin tetradecapeptide in doses of 20, 100, and 500 pg per ml averaged 99%. The assay sensitivity of RIA as determined by the amount for the lower limit of 95% confidence limits of the buffer control is 0.5-1 pg/tube, or 2.5-5 pg/ml plasma when 200 μl plasma is used.

Mean control somatostatin levels in acetone extracts of systemic plasma were 10.4 and 5.4 pg/ml in Exp. 1 and Exp. 2, respectively. Those in the hepatic portal plasma were 72.0 and 73.3 pg/ml, respectively; these values were approximately 7 to 14 times greater than the levels in the systemic plasma, indicating that the gut and the pancreas are the major source of circulating somatostatin (Table 1).

Intravenous administration of 10 μg synthetic hPP resulted in approximately 60% reduction of somatostatin in hepatic portal plasma and about 40%

reduction in the systemic plasma in both experiments (Table 1). However, in Experiment 2 where control systemic plasma levels of somatostatin were only about half of those in Experiment 1, the reduction in somatostatin was not significant. Infusion over one min (Exp. 1) and a bolus injection followed by a five-minute infusion of hPP (Exp. 2) both induced similar suppressive effects on plasma somatostatin.

The results clearly show that iv-administered 10 μg synthetic hPP with 36 amino acid residues reduced somatostatin levels in both the systemic and hepatic portal plasma in the rat. Since the half-life of somatostatin immunoreactivity in vivo is extremely short, approximately 2 min (17), the plasma somatostatin levels may reflect moment to moment changes in secretory rates of the hormone in normal animals. Therefore, the acute reduction of plasma somatostatin by hPP observed 2-5 min following administration may indicate prompt suppression of secretion of somatostatin.

Various gut hormones and pancreatic glucagon stimulate release of somatostatin (18). hPP is the only hormone so far reported which clearly suppresses somatostatin release. The dose used in this study may be pharmacological. Therefore, the suppressive action may not necessarily be of physiological significance.

Basal levels of hPP in systemic plasma in young people as determined by RIA are less than 100 pg/ml (10), and increase above 500 pg/ml after protein meals and fat (10). The concentration in the hepetic portal blood or in the local circulation where hPP is secreted is likely to be at least several times greater than the concentration in the systemic blood. To attain such high levels in a local tissue by systemic administration, a considerably large dose of hPP may have to be given. Accordingly, 10 μg hPP/100 g body weight may not be excessive in this regard.

Although several studies (10, 19-21) suggest possible influence of hPP on gastrointestinal functions, clear-cut physiological actions of PP

still remain elusive. On the other hand, somatostatin is known to suppress release of various gut and pancreatic hormones, including PP. Administration of antiserum to somatostatin in dogs increased plasma PP levels (22), suggesting that inhibitory control of PP secretion by somatostatin takes place under physiological conditions. Similar to somatostatin producing D-cells, PP-cells (F-cells) are localized in the peripheral part of pancreatic islets and also scattered throughout the exocrine parenchyma (23). It is possible that D-cells and F-cells control the secretory activity of each other in a paracrine fashion.

Recently we observed that in a patient with a PP-cell tumor who showed extremely high circulating PP levels, plasma somatostatin was undetectable, but rose to 8pg/ml, which is in a low normal range, after removal of the tumor. This finding supports the view that suppression of somatostatin release by hPP has physiological significance (unpublished study with Dr. S. R. Friesen).

ACKNOWLEDGEMENTS

This study was supported in part by NIH grants AM 09094 (AA) and AM 22156 (CAM), and the Veterans Administration.

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