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Prolonged Serum Insulin Decreasing Effects of Two Synthetic Somatostatin Analogues Studied in vivo by a New Animal Method

Preliminary Communication

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ABSTRACT

A newly developed in vivo method, using the ob–ob strain of obese–hyperglycaemic mice with permanently very high serum insulin values, makes it possible to detect more prolonged serum insulin lowering properties than in normal animals. Two newly synthesized analogues of somatostatin, D-alanine-somatostatin and des-alanine-des glycine-des-amino-somatostatin produced a more prolonged and greater decrease in the serum insulin values of ob–ob mice than did somatostatin. Our new in vivo method makes it possible to investigate the duration of insulin suppression of new derivatives.

INTRODUCTION

Somatostatin research has undergone an explosive development in the last few years and an abundant literature has resulted. Several extensive and detailed reviews have been published (6, 8, 23).

Animal experiments. Insulin release inhibiting effects of somatostatin have been studied in isolated rat pancreas (3, 5, 12) and in perfused canine pancreas (11). Glucose, triglycerides and casein administered intraduodenally in conscious dogs cause an increasing glycagon-like immunoreactivity (GLI) in serum. Somatostatin infusion inhibits such GLI increases (19). Somatostatin has been detected in the hypothalamus, posterior pituitary, pancreatic islets (A_1 or D-cells), stomach and intestine in rats with an immunofluorescence technique (10). All these experiments indicate the complex biological effects of somatostatin and several interactions with other hormone systems.

Human experiments. Somatostatin inhibits the arginine-dependent growth hormone release and its basal secretion in the normal male (21). Moreover, somatostatin suppresses the release of glucagon, insulin and growth hormone in normal, diabetic, acromegalic and in hypopituitary patients (14). In normal and acromegalic patients somatostatin infusion inhibits the sleep-related peak of growth hormone release (15). TRH-elicited TSH release is also inhibited by somatostatin (16, 22, 25). Serum glucose and insulin values are depressed by somatostatin injections (1) and somatostatin also lowers the serum insulin values stimulated by glucagon and tolbutamide injections (4). The basal and foot-stimulated secretions of gastrin and gastric acid are also decreased by somatostatin (2).

The aim of our studies was to develop a suitable in vivo method for following the time course after a single injection of somatostatin into the animal. Similar experiments with insulin lowering effects were made in humans given somatostatin infusions (1, 13, 14, 17). In all these studies somatostatin elicited a short insulin inhibition followed by a rebound effect. The biological half-life of somatostatin is very short: 4–6 min (8). Our in vivo animal method, using ob–ob mice with very high serum insulin values demonstrated a dose-dependent somatostatin effect with a decrease in the serum insulin values which lasted for only a short time (9). In this preliminary communication two newly synthesized somatostatin derivatives are described which exhibit markedly prolonged serum insulin depressing effects.

MATERIAL AND METHODS

Male 5–7 months old obese–hyperglycaemic mice, with high serum insulin values (150–250 ng/ml), were selected,

Table I. *The amino acid sequence of the original somatostatin (1) and of two recently synthesised (2, 3) somatostatin analogues*

1. Original GH-RIH:
   H-ALA-GLY-CYS-LYS—ASN-PHE-PHE-TRP-LYS-THR-PHE-THR-SER-CYS-OH
2. Somatostatin analogue synthesised by changing the first amino acid to D-alanin:
   H-D-ALA-GLY-CYS-LYS-ASN-PHE-PHE-TRP-LYS-THR-PHE-THR-SER-CYS-OH
3. Somatostatin analogue produced newly by omission of the two first amino acids and the amino group of the third (des-ALA-des-GLY-desamino polypeptide):
   SCH_2CH_2CO-LYS-ASN-PHE-PHE-TRP-LYS-THR-PHE-THR-SER-CYS-OH

6 animals per group. All animals had body weights between 48 and 55 g.

Somatostatin was synthesized by a step-wise fragmentary condensation technique (9). The two analogues were synthesized by a classical step-wise fragmentary condensation technique at the Department of Peptide Chemistry, Ferring AB, Malmö, Sweden. They were purified by ion-exchange chromatography on carboxymethyl cellulose (Whatman CM 23).

Analytical data

D-Ala¹-somatostatin: Homogeneous in three different TLC-systems. $[\alpha]_D^{24}$: −41°, *c* 0.48 in 1% acetic acid. Amino acid analysis: Ala 0.99, Gly 1.00, Cys 2.0, Lys 2.11, Asp 1.00, Phe 2.85, Thr 1.92, Ser 0.94.

Des-Ala¹-des-Gly²-desamino-somatostatin: Homogeneous in three different TLC-systems. $[\alpha]_D^{22}$: −47°, *c* 0.42 in 1% acetic acid. Amino acid analysis: Lys 2.04, Asp 1.00, Phe 2.88, Thr 1.94, Ser 0.93.

Immuno-reactive serum insulin was determined by double-antibody radioimmuno-assay (7), using a kit obtained from the Radiochemical Centre, Amersham, England. Crystalline mouse insulin (24 IU/mg) was used as standard. The animals were conscious and unanesthetised during the experiments. Blood samples were obtained by puncture of the orbital venous plexus with a thin-walled Pasteur pipette before and after the injection of control saline, somatostatin, or synthesized analogues. In this preliminary report the lowest effective dosage of the drugs, found in an earlier study (9), was used: 100 μg/kg. The solutions (0.1 ml) were injected rapidly into a tail vein. Blood samples were taken from all animals at zero, 8, 16, 32, 64 and 128 min after the start. The small blood samples were collected and stored as described previously (9).

RESULTS

The chemical structure of somatostatin and the synthetic analogues are given in Table I. The first analogue (2) has D-alanine in the first position of the chain in contrast to L-alanine as in somatostatin itself. The second analogue (3) lacks the first two amino acids, on the *N*-terminal end of the chain alanine and glycine, together with the amino group of the third, with an unchanged Cys–Cys bridge.

The serum insulin values, initially and at several times up to 128 min after drug administration, are given in ng/ml in Fig. 1. Control saline injections did not alter the insulin values. Somatostatin injection resulted in rapidly decreasing serum insulin value, which returned to the normal initial value of about 300 ng/ml after 30 min. Both synthetic analogues also caused and markedly and rapidly reduced the

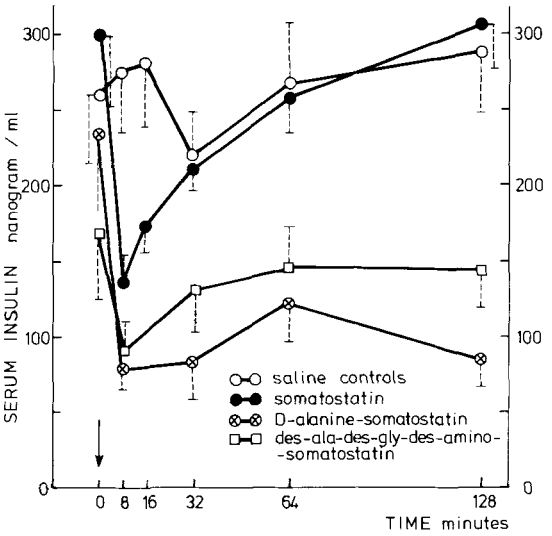

Fig. 1. Serum insulin values at different times after injection of the drugs in vivo. Six animals per group. The controls received physiological saline. All drugs were injected at zero time i.v. in single doses of 100 μg/kg.

insulin values. In contrast to the fairly rapid recovery after somatostatin, both the synthetic derivatives produced a prolonged depression of the insulin value, which lasted throughout the period of observation, i.e. 128 minutes.

DISCUSSION

The great difficulty in the therapeutic use of somatostatin in endocrine disorders has been the lack of a long-acting derivative. Several research groups have synthesized and tested a large number of chemically different derivatives (18, 20, 23). Some attempts were made to shorten the tetradecapeptide chain by excluding one or several amino acids. Another structure-activity relation investigated, was whether cleavage of the disulphide link in the polypeptide resulted in alterations of somatostatin activities. Another possibility was to analyse the separation of the activities of somatostatin derivatives inhibiting growth hormone and influencing insulin and glucagon. The evaluation of the different activities was made by studying the hormone effects in vitro and in some experiments in vivo. In these studies it was found that many shorter amino acid analogues had lower activities than somatostatin in several types of tests. As far as we know, no new method has been described, which permits tests of somatostatin and different structural analogues, by single injections of the agent in vivo with subsequent monitoring of the serum hormone values at different times after injection. Our previous study (9) showed a statistically significant dose–response effect in vivo for serum insulin-inhibiting effects with different doses of somatostatin injected into obese–hyperglycaemic mice.

In our system, somatostatin had a very short-lived serum insulin diminishing effect in the dosage used. In the present investigation of the two somatostatin analogues, both were injected in relatively low doses into each group of animals. It was of great interest to note that both D-ala-somatostatin and des-ala-des-gly-desamino-somatostatin had markedly prolonged serum insulin depressant effects. These prolonged insulin inhibiting effects in vivo were obtained in a complex hormonal environment. Some hours after the experiment the mice seemed to have recovered completely. Experiments with lean litter-mates of the ob–ob mice, which have very low serum insulin values, showed that they were completely uninfluenced by somatostatin.

The synthesis of the two new derivatives tested was modelled on earlier findings by Vávra et al. (24). They modified vasopressin by replacing L-arginine in position 8 by D-arginine and replacing the terminal amino group by β-mercaptopropionic acid, the antidiuretic effect. This resulted in a marked prolongation of the anti-diuretic effect, which was tested in both animals and in patients with diabetes insipidus.

The results of this study also demonstrate the usefulness of the "ob–ob in vivo"-method for testing the duration of the insulin suppressive effect of somatostatin derivatives, which can perhaps give a possibility of finding a new therapeutic agent.

REFERENCES

1. Alberti, K. G. M. M., Juel Christensen, N., Engkjær Christensen, S., Prange Hansen, Aa., Iversen, J., Lundbæk, K., Sayer-Hansen, K. & Ørskov, H.: Inhibition of insulin secretion by somatostatin. Lancet *II:* 1299, 1973.
2. Bloom, S. R., Mortimer, C. H., Thorner, M. O., Besser, G. M., Hall, R., Gomez-Pan, A., Roy, V. M., Russel, R. C. G., Coy, D. H., Kastin, A. J. & Schally, A. V.: Inhibition of gastrin and gastric-acid secretion by growth-hormone release inhibiting hormone. Lancet *ii:* 1106, 1974.
3. Efendić, S. & Luft, R.: Studies on the inhibitory effect of somatostatin on glucose induced insulin release in the isolated perfused rat pancreas. Acta Endocr (Kbh.), *78:* 510, 1975.
4. Gerich, J. E., Lorenzi, M., Schneider, V. & Forsham, P. H.: Effect of somatostatin on plasma glucose and insulin response to glucagon and tolbutamide in man. J Clin Endocr Metab *39* 1057, 1974.
5. Gerich, J. E., Lovinger, R. & Grodsky, G. M.: Inhibition by somatostatin of glucagon and insulin release from the perfused rat pancreas in response to arginine, isoproterenol and theophylline. Evidence for a preferential effect of glucagon secretion. Endocrinology *96:* 749, 1975.
6. Guillemin, R. & Gerich, J. E.: Somatostatin: physiological and clinical significance. Ann Rev Med *27:* 379, 1976.
7. Hales, C. N. & Randle, B. J.: Immuno-assay of insulin with anti-body precipitate. Biochem J *88:* 137, 1963.
8. Hansen, A. P. & Lundbaek, K.: Somatostatin: A review of its effects, especially in human beings. Diabete Metabolisme (Paris) *2:* 203, 1976.
9. Herbai, G., Westman-Naeser, S., Carlsson L., Melin, P. Mulder †, J.: The dose dependent serum insulin lowering effect of somatostatin (GH-RIF) in vivo in the obese-hyperglycaemic (ob–ob) syndrome. Ups J Med Sci *82:* 255, 1977.

10. Hökfelt, T., Efendić, S., Hellerström, C., Johansson, O., Luft, R. & Arimura, A.: Cellular localization of somatostatin in endocrine-like cells and neurons of the rat with special references to the A_1-cells of the pancreatic islets and to the hypothalamus. Acta Endocr (Kbh) Suppl. 200, *80:* 5, 1975.
11. Iversen, J.: Inhibition of pancreatic glucagon release by somatostatin: in vitro. Scand J Clin Lab Invest *33:* 125, 1974.
12. Johnson, D. G., Ensinck, J. W., Koerker, D., Palmer, J. & Goodner, C. J.: Inhibition of glucagon and insulin secretion by somatostatin in the rat pancreas perfused in situ. Endocrinology *96:* 370, 1975.
13. Leblanc, J. R., Anderson, M. B., Siegel, M. B. & Yen, S. S. C.: Inhibitory action of somatostatin on pancreatic α and β cell function. J Clin Endocrinol Metab *40:* 568, 1975.
14. Mortimer, C. H., Turnbridge, W. M. G., Carr, D., Yeomans, L., Lind, T., Coy, D. H., Bloom, S. R., Kastin, A., Mallinson, C. N., Besser, G. M., Schally, A. V. & Hall, R.: Effects of growth-hormone release-inhibiting hormone on circulating glucagon, insulin, and growth hormone in normal, diabetic, acromegalic, and hypopituitary patients. Lancet *i:* 697, 1974.
15. Parker, D. C., Rossman, L. G., Siler, T. M., Rivier, J., Yen, S. S. C. & Guillemin, R.: Inhibition of the sleep-related peak in physiologic human growth hormone release by somatostatin. J Clin Endocr Metab *38:* 496, 1974.
16. Patel, Y. C., Alford, F. P. & Burger, H. G.: The 25-hour plasma thyrotrophin profile. Clin Sci *43:* 71, 1972.
17. Peracchi, M., Reschini, E., Cantalamessa, L., Giustina, G., Cavagnini, F., Pinto, M. & Bulgheroni, P.: Effect of somatostatin on blood glucose, plasma growth hormone, insulin, and free fatty acids in normal subjects and acromegalic patients. Metabolism *23:* 1009, 1974.
18. Rivier, J., Brazeau, P., Vale, W. & Guillemin, R.: Somatostatin analogs. Relative importance of the disulfide bridge and of the Ala-Gly side chain for biological activity. J Med Chemistry *18:* 123, 1975.
19. Sakurai, H., Dobbs, R. E. & Unger, R. H.: The effect of somatostatin on the response of GLI to the intraduodenal administration of glucose, protein, and fat. Diabetologia *11:* 427, 1975.
20. Sarantakis, D., McKinley, W. A., Jaunakais, I., Clark, D. & Grant, N. H.: Structure activity studies on somatostatin. Clin Endocrin *5*, Suppl.: 275s, 1976.
21. Siler, T. M., VandenBerg, G., Yen, S. S. C., Brazeau, P., Vale, W. & Guillemin, R.: Inhibition of growth hormone release in humans by somatostatin. J Clin Endocr Metab *37:* 632, 1973.
22. Siler, T. M., Yen, S. S. C., Vale, W. & Guillemin, R.: Inhibition by somatostatin on the release of TSH induced in man by thyrotropin-releasing factor. J Clin Endocrinol Metab *38:* 742, 1974.
23. Vale, W., Brazeau, P., Rivier, C., Brown, M., Boss, B., Rivier, J., Burgus, R., Ling, N. & Guillemin, R.: Somatostatin. Recent Progr Hormone Res *31:* 365, 1975.
24. Vávra, I., Machová, A., Holeček, V., Cort, J. H., Zaoral, M. & Šorm, F.: Effect of a synthetic analogue of vasopressin in animals and in patients with diabetes insipidus. Lancet *i:* 948, 1968.
25. Weeke, J., Hansen, A. P. & Lundbaek, K.: The inhibition by somatostatin of the thyrotropin response to thyrotropin-releasing hormone in normal subjects. Scand J Clin Lab Invest *33:* 101, 1974.

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