RELATIVE EFFECTS OF SOMATOSTATIN AND TWO SOMATOSTATIN ANALOGUES ON THE RELEASE OF INSULIN,GLUCAGON AND GROWTH HORMONE

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1. Introduction

It has been amply demonstrated that the growth hormone release-inhibiting hormone (GH-RIH or somatostatin) is also a potent inhibitor of insulin and glucagon release. Furthermore, as shown recently, somatostatin or a somatostatin-like peptide (s) is produced not only in the hypothalamus but, among other tissues, also in the pancreas [1,2], most probably in the α_1-cells of the pancreatic islets [3,4].

It has been suggested by several authors that somatostatin – preferably in long-acting form – might be of value in the treatment of diseases such as acromegaly, diabetes mellitus, spontaneous hyperinsulinism etc. In acromegaly the GH release-inhibiting action and in diabetes mellitus the inhibition of the release of GH as well as glucagon would be of special importance, whereas the insulin inhibiting action would be preferable in hyperinsulinism [5]. For this reason analogues to somatostatin have been synthetized in an attempt to arrive at substances with predominantly one or two of the actions of the original somatostatin. This first report shows that it is possible by modifying the structure of somatostatin to obtain analogues which predominantly inhibit insulin release.

2. Materials and methods

Sprague-Dawley rats, weighing 200–250 g and fasted for 24 h were used for the preparation of the perfused isolated pancreas. The animals were anesthetized by intraperitoneal injection of 50 mg/kg of Pentobarbital, and the pancreas was isolated by a slight modification of the technique of Loubatières et al. [6]. The pancreas was completely separated from adjacent organs, and all vessels were carefully ligated.The gland was perfused with a Krebs-Ringer bicarbonate solution to which was added 0.8 g/l of glucose and 20 g/l of beef albumin. The final solution was adjusted to pH 7.4 with 0.1 N HCl, and was gassed continuously with a mixture of 95% oxygen and 5% carbon dioxide. The perfusate was administered into the coeliac artery, and run into the prepared pancreas by an open circuit 'non-recycling perfusion system'. Total portal effluent was collected over 60 sec. Flow rates were kept approximately at 2.5 ml/min by making minor changes in arterial pressure. The isolated pancreas was always equilibrated with perfusion medium for 40 min before applying the stimulatory concentration of glucose. Somatostatin and somatostatin analogues, dissolved in water, were added to the perfusate and applied by changing the medium reservoir.

L-arginine monochloride was obtained in a 10% aqueous solution (Vitrum AB, Stochkholm) and used at adequate dilutions.

Insulin was determined by a double-antibody radioimmunoassay using insulin reagent kits (Radiochemical Centre, Amersham), and a rat insulin standard. Glucagon was assayed by the charcoal separation radioimmunoassay technique [7], using an antibody specific for pancreatic glucagon (30 K, kindly provided by Dr Roger Unger, Dallas, Texas).

The effect of somatostatin on insulin release was calculated by comparison of the areas under the insulin curves. The initial phase of insulin release was

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Table 1
The sequences of somatostatin and somatostatin analogues

somatostatin (linear)	H–Ala–Gly–Cys(SH)–Lys–Asn–Phe–Phe–Trp–Lys–Thr–Phe–Thr–Ser–Cys(SH)–OH
analogue WY-18 092	H–Cys(SH)–Lys–Asn–Phe–Phe–Trp–Lys–Thr–Phe–Thr–Ser–Cys(SH)–OH
analogue WY-18 166	H–Cys(S)–Lys–Phe–Phe–Trp–Lys–Thr–Phe–Thr–Ser–Cys(S)–OH

taken as the 0–7 min period after the start of the infusion, while the second phase comprised the period between 7–17 min.

Growth hormone release was measured in vitro by means of monolayer cultures of enzymatically dispersed pituitary cells [8] and in vivo 15 min after subcutaneous injection of the peptide and 20 min after intraperitoneal injection of Pentobarbital. The somatostatin analogues were synthetized at the Wyeth laboratories, Philadelphia and their structures are given in table 1.

3. Results

Arginine (5 mg/ml) significantly stimulated insulin and glucagon release from the isolated perfused rat pancreas. A typical biphasic curve was obtained. Somatostatin in a concentration as low as 10 ng/ml, when infused 7 min prior to and then during the infusion of arginine, induced a significant suppression of arginine stimulated insulin and glucagon release (table 2). This effect was more prominent on the primary peak of the insulin and glucagon curve.

The two analogues significantly inhibited arginine enhanced insulin secretion, in this respect being approximately as potent as somatostatin. On the other hand, analogue WY-18 092 had no effect on glucagon secretion, and analogue WY-18 166 had only a slight and not significant inhibitory effect on glucagon release.

The effect of the somatostatin analogues on growth hormone release was tested in vivo and in vitro at the Wyeth laboratories. Both analogues were less potent inhibitors of growth hormone release than somatostatin; the inhibitory activity being about 10% that of somatostatin [9]. In vivo data on the effects of WY-18 166 on growth hormone, glucagon and insulin release are reported by Sarantakis et al. [10].

4. Discussion

We have previously demonstrated that somatostatin inhibits glucose-induced insulin release from the perfused isolated rat pancreas [11]. As shown here, arginine-induced release of insulin – as well as glucagon – is also inhibited by somatostatin. Furthermore, the modification of the structure of somatostatin resulted in dissociation of the effects on insulin and glucagon release. In both analogues dispensing with the free-end Ala^1-Gly^2 of somatostatin was accompanied by decreased or abolished inhibitory activity on glucagon but with preserved inhibitory potency on insulin release. In addition the deletion of Asn^5 (WY-18 166) did not seem to influence the activity on insulin release. The decrease in the effect of des-Ala^1 Gly^2 Asn^5-somatostatin (WY-18 166) on glucagon release was also demonstrated by Sarantakis et al. [10] on rats in vivo.

Both analogues still exerted some inhibitory action on growth hormone release although this seemed to be less pronounced than for somatostatin proper [9,10]. These findings indicate that analogues can be synthetized which preferentially exhibit only one or some of the activities of somatostatin.

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Table 2

Effect of somatostatin and somatostatin analogues on arginine induced insulin release from the isolated perfused rat pancreas[a]

Addition	Amount added per ml	No. of exp.	Insulin release in μU per ml 0–7 min	p^b	7–17 min	p^b	Glucagon release in pg per ml 0–7 min	p^b	7–17 min	p^b
none		8	3232 ± 408		3308 ± 586		8509 ± 1947		15474 ± 5362	
somatostatin	10 ng	8	585 ± 164	<0.001	1173 ± 560	<0.05	2737 ± 543	<0.001	3433 ± 789	<0.02
analogue WY-18 092	10 ng	6	1034 ± 189	<0.001	1335 ± 359	<0.025	11020 ± 2945	NS	11678 ± 3219	NS
analogue WY-18 166	10 ng	6	267 ± 255	<0.001	398 ± 235	<0.01	6115 ± 1527	NS	6593 ± 1634	NS

[a]Mean ± S.E.M.

[b]p-values refer to the significance of difference between control experiments and those with somatostatin or analogues

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