RAPID CONVERSION OF SOMATOSTATIN TO ACTIVE METABOLITE IN HUMAN PLASMA

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Received 11 March 1981

1. Introduction

Immunoreactive somatostatin occurs naturally in many parts of the body and is known to inhibit the secretion of a large number of peptide hormones [1]. Whether or not its physiological actions are entirely local is an open question [2] but it has been demonstrated by radioimmunoassay that biologically active concentrations [3] are present in normal human plasma after a meal [4]. Somatostatin is known to be converted rapidly to a closely related product, [des-Ala^1]-somatostatin, in rat plasma in vitro and in vivo [5]. We can now report that this conversion also occurs in human plasma and that the product is equipotent with somatostatin in several biological assay systems.

2. Experimental

2.1. *Extraction and purification of peptides*

Following incubation in plasma, peptides were extracted and purified using Sephisorb ODS–silica as described in [5]. Extraction consists of adsorption of hydrophobic peptides onto a small bed of ODS-silica in a disposable syringe, followed by elution with methanol/water/trifluoroacetic acid (80:19:1, by vol.). The eluate is diluted with water and loaded onto a 4 × 250 mm partisil–ODS high-pressure liquid chromatography (HPLC) column. The column was eluted with a linear gradient (100 ml total vol.) from water/trifluoroacetic acid (99:1, by vol.) to acetonitrile/water/trifluoroacetic acid (80:19:1, by vol.). The eluate was monitored for absorbance at 280 nm and peaks were film dried and subjected to amino acid analysis after acid hydrolysis.

2.2. *Determination of the potency of [des-Ala^1]-somatostatin*

[des-Ala^1]-Somatostatin was synthesised by conventional fragment condensation [6]. Its potency was compared with somatostatin: in vivo by measuring the serum insulin decrease in the mouse [7] and the plasma insulin and glucagon decrease in the rat [8]; in vitro by measuring inhibition of growth hormone release by rat pituitary cells in culture [9]; and in vitro by measuring inhibition of insulin and glucagon release from the perfused rat pancreas [10].

3. Experimental and results

Cyclic somatostatin (synthesised by conventional fragment condensation) was incubated with fresh heparinised human plasma and peptides were subsequently extracted using ODS–silica and purified by HPLC. The amino acid composition of peptide contained in the single major peak (see table 1) indicated that after 2 min 40% conversion to [des-Ala^1]-somatostatin had taken place.

Inhibition of the release of insulin, glucagon and growth hormone by [des-Ala^1]-somatostatin was

 Elsevier/North-Holland Biomedical Press

Table 1
Amino acid composition of product isolated from human plasma following in vitro incubation with somatostatin for 2 min at 37°C

	Amino acid molar ratios		
	Theoretical	Control extract	Plasma extract
Asp	(1)	1.04	1.05
Thr, Ser [a]	(3)	2.95	2.70
Gly	(1)	1.00	1.20
Ala	(1)	1.01	0.62
Cys[b]	(2)	1.36	1.22
Phe	(3)	3.00	3.00
Lys	(2)	2.01	2.04
Trp[b]	(1)	0.38	0.43

[a] Combined estimate due to lack of separation
[b] Partially destroyed during acid hydrolysis

Plasma and a control (pure somatostatin in water) were both extracted, purified by HPLC, dried and analysed after acid hydrolysis [5]

assessed and the results are shown in table 2. [des-Ala[1]]-Somatostatin is equipotent with somatostatin on release of insulin and glucagon in vivo and on release of growth hormone in vitro, and slightly more potent on release of insulin and glucagon in vitro.

4. Discussion

The biological significance of the conversion mechanism is not clear. Rapid conversion in plasma of somatostatin to a fully active metabolite suggests that whereas somatostatin probably acts in its unmodified form at the site of release, it is likely that [des-Ala[1]]-somatostatin is the active form in the circulation.

Several N-terminally directed antisera presently used for the radioimmunoassay of somatostatin (with [^{125}I-Tyr[1]]-somatostatin as tracer) have not been shown to discriminate between somatostatin and [des-Ala[1]]-somatostatin [11]. The development of selective antisera is essential if the physiological role of circulating somatostatin/[des-Ala[1]]-somatostatin is to be evaluated.

[des-Ala[1]]-Somatostatin did not markedly differ from somatostatin in the above biological tests. However, differences in potency may have been masked in some tests in vivo by conversion of somatostatin to [des-Ala[1]]-somatostatin before it could reach the target cell. It is still conceivable that [des-Ala[1]]-somatostatin may be found to differ from somatostatin in its effects on release of peptide hormones other than those tested so far.

Table 2
Biological activity of [des-Ala[1]]-somatostatin in hormone release

Assay	Potency[e] (relative to somatostatin = 1)	
In vivo decrease of:		
serum insulin, mouse[a]	0.97	(0.56–1.66)
plasma insulin, rat[b]	1.14	(0.80–1.67)
plasma glucagon, rat[b]	1.05	(0.58–1.94)
in vitro inhibition of:		
growth hormone release, rat pituitary cells[c]	0.89	(0.53–1.47)
insulin release, rat pancreas[d]	1.7	(1.3 –2.3)
glucagon release, rat pancreas[d]	2.3	(1.7 –3.2)

[a] Determined by radioimmunoassay (RIA) in serum samples (N = 28) collected 10 min after sub-cutaneous injection of peptides [7]
[b] Determined by RIA in plasma samples (N = 18) collected 10 min after sub-cutaneous injection of peptides [8]
[c] Determined in culture media [9] (N = 7–9) by means of the NIAMDD rat GH RIA kit generously provided by the NIAMDD rat pituitary hormone distribution program
[d] Determined by RIA in effluent from isolated rat pancreas perfused with medium containing 20 nmol arginine/l [10]
[e] Mean (95% fiducial limits in brackets) calculated by 4 point assay

Acknowledgement

The authors would like to thank Mr Bruce Evans for carrying out the amino acid analyses.

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