Br. J. Pharmac. (1978), **64**, 311–314.

EFFECTS OF SOMATOSTATIN ON GASTRIC ACID SECRETION AND ON LIPID AND CARBOHYDRATE METABOLISM IN THE RAT

WILLEM G. LINSCHEER & KRISHAN L. RAHEJA

Department of Medicine and Pharmacology, Veterans Administration Hospital and SUNY Upstate Medical Center, Syracuse, N.Y. 13210, U.S.A.

1 Studies were conducted to examine the effects of somatostatin on pentagastrin-induced gastric acid secretion, and on bile, plasma and liver lipid composition in the rat.

2 In addition, liver glycogen and plasma glucose concentrations were measured.

3 Somatostatin was administered intravenously as a bolus (100 μg) at the start of the experiment followed by a continuous infusion (50 μg/h) for 2 h.

4 Controls were infused with isotonic saline (1 ml/h) throughout the experiment. Gastric secretions and bile were collected during the infusions.

5 Somatostatin significantly decreased the volume of gastric secretion as well as total gastric acid output but it had no effect on bile flow or bile lipid composition.

6 Plasma and liver lipids were not affected. Plasma glucose concentrations were significantly elevated but liver glycogen was not changed.

7 Our results suggest that somatostatin probably decreases glucose uptake by peripheral tissue. The effect of somatostatin on bile composition and glucose levels in the rat is different from observations reported in other species.

Introduction

Somatostatin (growth hormone-release-inhibiting hormone, GHRIH) present in hypothalamus, pancreas and stomach has been shown to elicit a multitude of biological effects. It inhibits growth hormone (Ferland, Labrie, Jobin, Arimura & Schally, 1976), thyrotrophin (Vale, Rivier, Brazeau & Guillemin, 1974; Ferland *et al.*, 1976), and prolactin (Vale *et al.*, 1974) release from the pituitary. Also pancreatic insulin and glucagon release (Brown, Rivier & Vale, 1976) and secretion of gastric acid (Lippman & Borella, 1976) are inhibited. Recently it has been reported by Lin, Spray & Tust (1977) that somatostatin has profound effects on bile flow and bile lipid composition in the dog. However, Raptis, Schlegel & Pfeiffer (1978) have reported that somatostatin had no influence on the volume, bilirubin, and particularly bicarbonate contents of biliary secretion in man. The effect of somatostatin on biliary lipids in the rat has not been reported. In this paper we describe the effect of somatostatin on bile flow, on bile, plasma and liver lipids and on liver glycogen and plasma glucose levels in the rat. Since the inhibition of gastric acid secretions by somatostatin has been demonstrated in the rat (Lippman & Borella, 1976), in other animal species (Barros, Bloom & Baron, 1975; Gomez-Pan, Reed, Albinus, Besser, Coy, Kastin & Schally, 1975) and in man (Raptis, Dollinger, VonBerger, Schlegel, Schroden & Pfeiffer, 1975), we used this criterion to establish the effectiveness of the dose level of somatostatin used in this study.

Methods

Adult Wistar rats (mean body weight 350 g) were selected for study and housed individually in raised wire screen cages in an animal laboratory with controlled temperature and 12:12 h light dark cycle. Rats were divided into 2 groups at random and given Purina chow and tap water *ad libitum*. Food was withdrawn 24 h before the experiment but water was available. Rats were anaesthetized with pentobarbitone (5 mg/100 mg body weight, i.p.) and both the femoral vein and common bile duct were cannulated with polythene tubing. The stomach was also cannulated through the duodenum for collection of gastric secretions. Body temperature was maintained by placing rats on heating pads and by recording body temperature periodically. Bile was collected in tared test tubes placed on ice for 2 h during two 1 h collections,

while either 0.9% w/v NaCl solution (saline, controls) or somatostatin dissolved in saline (experimental group) was infused through the femoral vein. Gastric secretion was simultaneously collected by continuous gravity drainage for 2 h in tared test tubes. A total of 200 μg somatostatin per rat was administered intravenously (batch No. F17-74B-38 from Eli Lilly Company, Indianapolis, Ind.); 100 μg was given as a bolus at the start of infusion, and the rest as a continuous intravenous infusion (50 μg ml^{-1} h^{-1}) for 2 h. Pentagastrin (5 μg, Peptavlon, Ayerst Laboratories, New York, N.Y.) was administered subcutaneously 10 min after the start of the saline or somatostatin infusion. After bile and gastric secretion collections for a period of 2 h, rats were bled by cardiac puncture; heparinized syringes were used. The oesophagus was ligated and stomach rinsed with distilled water for total recovery of gastric secretion. The rats were then killed and livers were removed and frozen on dry ice in tared foils. Volumes of bile and gastric secretion were recorded.

Similar experiments were done in the absence of pentagastrin in order to rule out the possibility that pentagastrin may change the effect of somatostatin on liver and plasma lipids and carbohydrates. In addition the effect of somatostatin was studied in the non-fasting rats. Liver and plasma were analyzed for cholesterol, triglycerides and phospholipids by the methods of Zlatkis, Zak & Zilversmit (1953), Van Handle & Zilversmit (1957) and Bartlett (1959) respectively; bile for cholesterol and phospholipids by the methods used for plasma, and total bile acids by the method of Iwata & Yamasaki (1964). Plasma glucose was determined by the glucose oxidase method with Worthington Glucostat reagents. Liver glycogen was determined by digesting samples of liver in 30% KOH in test tubes immersed in a boiling water bath. Glycogen was precipitated twice with 95% ethanol, heated in 2 N H_2SO_4, neutralized with 2 N NaOH and the glucose liberated by acid hydrolysis was then measured by the glucose oxidase method.

The gastric secretions collected during the 2 h experiment and stomach washings were combined and titrated with 0.01 N NaOH to pH 7.0 to determine the total gastric acid output and expressed as μmol h^{-1} 100 g^{-1} body weight. The volume of bile and gastric secretions are expressed as ml h^{-1} 100 g^{-1} body weight. Data were analyzed by Students' *t* test.

Results

Somatostatin significantly decreased gastric secretions and gastric acid production in response to pentagastrin (Table 1). Gastric secretion and gastric acid output in the somatostatin-treated rats were 38 and 46% respectively of the control values. While somatostatin significantly decreased gastric acid secretion, it had no effect on either bile flow or bile lipid composition (Table 2). The bile lipids (cholesterol, bile acids and phospholipids) were significantly lower in the second hour of collection than in the first hour in both the control and somatostatin-treated rats due to the interruption of the enterohepatic circulation.

The effect of somatostatin on liver and plasma lipids and on plasma glucose is shown in Table 3. It had no effect on liver weight, on hepatic or plasma cholesterol, triglyceride and phospholipid concentrations. Liver glycogen levels in both the control and treated rats fasted for 24 h were not different and had almost disappeared (control 0.44 ± 0.17 mg/g (mean ± s.e. mean) and somatostatin-treated 0.54 ± 0.44 mg/g). The corresponding values in the non-fasted rats were 11.2 ± 3.2 and 17.5 ± 3.5 mg/g respectively. Plasma glucose concentrations were significantly elevated by somatostatin.

Plasma glucose levels were also significantly elevated when somatostatin along with pentagastrin was administered to non-fasted rats (somatostatin 216 ± 19 mg/dl vs control 149 ± 20) or when somatostatin was administered alone in non-fasted rats (somatostatin 234 ± 38 mg/dl vs Control 142 ± 9). Plasma, liver and biliary lipids were not changed by somatostatin under these conditions.

Table 1 Effect of somatostatin on gastric secretion and gastric acid output in response to pentagastrin in the rat[1]

	Gastric secretion (ml h^{-1} 100 g^{-1} body wt.)	*Gastric acid output (μmol h^{-1} 100 g^{-1} body wt.)*
Control (10)	0.156 ± 0.016**	9.40 ± 1.72*
Somatostatin (10)	0.060 ± 0.010	4.34 ± 0.58

[1] Somatostatin was infused intravenously, 100 μg as a bolus and then 50 μg ml^{-1} h^{-1} was infused continuously for 2 h. Pentagastrin (5 μg) was given subcutaneously 10 min after start of saline or somatostatin administration. Values are mean ± s.e. for number of rats given in parentheses.
* $P < 0.05$; ** $P < 0.01$.

Discussion

In order to demonstrate the effectiveness of somatostatin in the rat, we have confirmed its effect on pentagastrin-stimulated gastric acid output as described previously (Lippman & Borella, 1976). Lin *et al.* (1977) found that somatostatin administration significantly decreased bile flow and concentrations of bile acids in the dog. Although somatostatin significantly decreased gastric acid output in our studies, we did not observe any effect on bile volume or bile lipid composition. Since we used a much higher dose level of somatostatin in the rat compared to the dose used by Lin *et al.* (1977) in the dog, the results suggest species differences in response to somatostatin. Moreover, Raptis *et al.* (1978) also observed no effect of somatostatin on bile volume in man.

Although plasma insulin and glucagon (the pancreatic hormones which affect lipid metabolism) were not determined in this study, the absence of changes in plasma and liver lipids due to somatostatin administration suggests that either the ratio of these hormones was not significantly altered or the 2 h experimental time period was too short to show an effect on lipid metabolism.

However, plasma glucose levels were significantly increased by somatostatin in the rat. Glucose levels could be increased either by increased gluconeogenesis or decreased glucose utilization. Increased hepatic glucose output or decreased extra hepatic uptake was ruled out by Cherrington Caldwell, Dietz, Exton & Crofford (1977) in *in vitro* studies. Sukurai, Dobbs & Unger (1975) observed in dogs that plasma glucose levels were initially lower after an oral glucose load when somatostatin was simultaneously infused than when glucose was administered alone. However, 30 min after the glucose load, plasma levels remained higher in the somatostatin-treated dogs as compared

Table 2 Effects of somatostatin on bile volume and bile lipid composition in the rat[1]

	Control		*Somatostatin*	
	Collection I	*Collection II*	*Collection I*	*Collection II*
Bile volume (ml h^{-1} 100 g^{-1} body wt.)	0.327 ± 0.016	0.344 ± 0.015	0.338 ± 0.027	0.321 ± 0.018
Bile cholesterol (μmol/ml)	0.521 ± 0.031*	0.409 ± 0.039	0.548 ± 0.037*	0.408 ± 0.039
Bile acids (μmol/ml)	29.04 ± 1.22**	24.8 ± 2.19	29.50 ± 1.50*	22.89 ± 1.84
Bile phospholipids (μmol/ml)	5.48 ± 0.39**	3.99 ± 0.27	5.90 ± 0.41*	4.37 ± 0.39

[1] Values are means ± s.e. for 10 observations in control and 11 observations in somatostatin group. The administration schedule of somatostatin and pentagastrin is given in Table 1. Statistical comparisons are made between Collections I and II within each group.
* $P < 0.05$; ** $P < 0.01$.

Table 3 Effects of somatostatin on plasma glucose and on plasma and liver lipids in the rat

	Control	*Somatostatin*
Body weight (g)	350.4 ± 13.3	342.2 ± 14.1
Liver weight (% body wt)	2.97 ± 0.11	3.29 ± 0.11
Plasma glucose (mg/dl)	111.8 ± 14.6	155.2 ± 11.1*
Plasma cholesterol (mg/dl)	34.8 ± 3.3	41.0 ± 4.4
Plasma triglycerides (mg/dl)	80.1 ± 13.4	67.0 ± 11.5
Plasma phospholipids (mg/dl)	110.8 ± 14.1	130.2 ± 12.1
Liver cholesterol (mg/g)	2.03 ± 0.19	2.09 ± 0.20
Liver triglycerides (mg/g)	5.37 ± 0.70	5.90 ± 0.58
Liver phospholipids (mg/g)	47.02 ± 1.47	50.78 ± 1.67
Liver glycogen (mg/g)	0.44 ± 0.17	0.54 ± 0.44

Values are means ± s.e. for 9 to 11 observations. Experimental conditions are described under Table 1.
* $P < 0.05$.

to controls. In our studies in the non-fasting rats plasma glucose levels were consistently higher in the somatostatin-treated group while liver glycogen concentrations were similar (17.5 ± 3.5 vs 11.2 ± 3.2 mg/g) in the treated and non-treated groups. When the rats were fasted, liver glycogen in both groups was practically depleted but plasma glucose was still significantly elevated in somatostatin-treated rats, which support the hypothesis that the hyperglycaemic effect of somatostatin is not mediated by increased glycogenolysis but by the mechanism of the decreased uptake by the peripheral tissues. The theoretical possibility that pentagastrin may alter the effects of somatostatin on plasma glucose level was ruled out by similar observations in rats receiving only somatostatin (somatostatin 234 ± 38 mg/dl vs control 142 ± 9).

We conclude from our experiments that somatostatin in the rat inhibits pentagastrin-stimulated gastric acid output and increases the plasma glucose level, most probably by decreased utilization of glucose by peripheral tissues.

The authors acknowledge the expert technical assistance of Marla F. Share and are grateful to Dr T. M. Lin of the Eli Lilly Company, Indianapolis, Indiana, for supplying somatostatin. This study was supported in part by VA Medical Research Service and research grant AM 18496 from the National Institutes of Health, Public Health Service, U.S.A.

References

BARROS, A.A.J., BLOOM, S.R. & BARON, J.H. (1975). Direct inhibition of gastric acid by growth-hormone release-inhibiting hormone in dogs. *Lancet*, **i**, 886–887.

BARTLETT, G.R. (1959). Phosphorus assay in column chromatography. *J. biol. Chem.*, **234**, 466–468.

BROWN, M., RIVIER, J. & VALE, W. (1976). Biological activity of somatostatin and somatostatin analogs on inhibition of arginine-induced insulin and glucagon release in the rat. *Endocrinology*, **98**, 336–343.

CHERRINGTON, A.D., CALDWELL, M.D., DIETZ, M.R., EXTON, J.H. & CROFFORD, O.B. (1977). The effect of somatostatin on glucose uptake and production of rat tissues in vitro. *Diabetes*, **26**, 740–748.

FERLAND, L., LABRIE, F., JOBIN, M., ARIMURA, A. & SCHALLY, AV. (1976). Physiological role of somatostatin in the control of growth hormone and thyrotropin secretion. *Biochem. biophys. Res. Commun.*, **68**, 149–156.

GOMEZ-PAN, A., REED, J.D., ALBINUS, M., BESSER, C.M., COY, D.H., KASTIN, A.J. & SCHALLY, A.V. (1975). Direct inhibition of gastric acid and pepsin secretion by growth-hormone release-inhibiting hormone in cats. *Lancet*, **i**, 888–890.

IWATA, T. & YAMASAKI, K. (1964). Enzymatic determination and thin layer chromatography of bile acids in blood. *J. Biochem. (Tokyo)*, **56**, 424–431.

LIN, T.M., SPRAY, G.F. & TUST, R.H. (1977). Action of somatostatin (SS) on choledochal spincter (CS) gallbladder (GB) and bile flow (BF) in dogs. *Fedn Proc.*, **36**, 557.

LIPPMAN, W. & BORELLA, L.E. (1976). Inhibition of gastric acid secretion by somatostatin and analogs in the rat. *Pharmac. Res. Commun.*, **8**, 445–454.

RAPTIS, S., DOLLINGER, H.C., VONBERGER, L., SCHLEGEL, W., SCHRODEN, K.E. & PFEIFFER, E.F. (1975). Effects of somatostatin on gastric secretion and gastric release in man. *Digestion*, **13**, 15–26.

RAPTIS, S., SCHLEGEL, W. & PFEIFFER, E.F. (1978). 70. Effects of somatostatin on gut and pancreas. In *Gut Hormones*. Bloom, S.R. pp. 446–451. New York, N.Y.: Churchill Levingstone, Longman Inc.

SUKURAI, H., DOBBS, R.E. & UNGER, R.H. (1975). The effect of somatostatin on the response of GLI to intraduodenal administration of glucose, protein and fat. *Diabetologia*, **11**, 427–430.

VALE, W., RIVIER, C., BRAZEAU, P. & GUILLEMIN, R. (1974). Effects of somatostatin on secretion of thyrotropin and prolactin. *Endocrinology*, **95**, 968–977.

VAN HANDEL, E. & ZILVERSMIT, D.B. (1957). Micromethod for direct determination of serum triglycerides. *J. lab. Clin. Med.*, **50**, 152–157.

ZLATKIS, A., ZAK, B. & ZILVERSMIT, D.B. (1953). A new method for the direct determination of serum cholesterol. *J. lab. Clin. Med.*, **41**, 486–492.

(Received April 18, 1978.
Revised June 9, 1978.)