Somatostatin in the Pancreas and Hypothalamus of Obese Mice

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Summary. The pancreatic content of somatostatin, insulin, and glucagon and the hypothalamic content of somatostatin were examined in *ob/ob* mice at various ages and in goldthiogluco-obese mice. The total pancreatic content of somatostatin was increased in *ob/ob* mice compared to controls: 92 ng vs 75 ng (a 22% increase) at 2 months of age; 208 ng vs 131 ng (a 60% increase) at 6 months of age; and 184 ng vs 118 ng (a 60% increase) at 8 months of age. The total pancreatic content of glucagon in *ob/ob* mice was already enhanced by 70% over controls at 2 months of age (301 ng vs 173 ng) and did not increase further at later stages, whereas that of insulin progressively rose with age. In goldthiogluco-obese mice the pancreatic content of insulin was also increased but to a lesser extent than in *ob/ob* mice; the pancreatic levels of somatostatin and glucagon were unaltered. In both *ob/ob* mice (regardless of age) and goldthiogluco-obese mice, there was no significant change in the hypothalamic content of somatostatin compared with that of lean controls.

Key words: Somatostatin, insulin, glucagon, endocrine pancreas, hypothalamus, obesity, *ob/ob* mouse, goldthiogluco-obese mouse.

Somatostatin (or a somatostatin-like material) which was first isolated from bovine hypothalamus [1], has subsequently been localized in the pancreas and throughout the gastrointestinal tract [2–6]. Interrelationships between somatostatin, insulin, and glucagon have been suggested by the observation that somatostatin inhibits insulin and glucagon release [6], whereas glucagon appears to stimulate somatostatin release [7, 8]. Since somatostatin is present in the D

cells of the islets of Langerhans a local action of somatostatin in the regulation of endocrine pancreatic functions may be suggested.

Recent studies have shown that obese hyperinsulinaemic mice [9, 10] and diabetic hypoinsulinaemic rodents [11–13] exhibit alterations (compared to control animals) in the somatostatin content of their pancreases; however, variable patterns of changes were observed, which appeared to depend, partly at least, on the animal model investigated. Furthermore, studies in obese mice were performed only at one stage of the development of the obese hyperglycaemic syndrome.

The present work was aimed at investigating the somatostatin content of the pancreas and hypothalamus at various stages of the development of obesity and hyperinsulinism in the genetically obese *ob/ob* mouse. We have also examined the somatostatin levels in the pancreas and hypothalamus of mice with acquired, goldthiogluco-induced obesity. In both animal models, the pancreatic content of insulin and glucagon was determined in parallel.

Materials and Methods

Lean and ob/ob Mice

Male obese (C57BL/6J-*ob/ob*) mice and their lean controls (C57BL/6J-+/+) were purchased from the Jackson Laboratory (Bar Harbor, Maine, USA). They were sacrificed at 2, 6 or 8 months of age.

Lean and Goldthiogluco-Obese Mice

Male Swiss albino mice were injected IP at 3 weeks of age either with saline (0.154 mol/l) (lean controls) or with goldthiogluco (GTG). A first dose of 200 mg GTG/kg body weight was injected to fed animals and 72 h later a second dose of 400 mg/kg body

weight was injected after 6 h of fasting. Mice were weighed monthly and obese mice were used after obesity had reached a plateau, i. e., at 8 months of age [14].

Animals were maintained in a constant-temperature (23 °C) animal room with a fixed 12 h artificial light (0800–2000 h) cycle. All mice had free access to laboratory chow (UAR, Villemoisson, Epinay/Orge, France) containing 21% protein, 4% fat and 51% carbohydrate, until the time of sacrifice (0900–1200 h).

Preparation of Tissue and Blood Samples

Animals were anaesthetised with sodium pentobarbital (100 mg/kg body weight, IP). Blood samples, obtained from the inferior vena cava, were collected into heparinized microfuge tubes, centrifuged at 4 °C and the plasma was stored at – 20 °C until assayed. Hypothalami and pancreases were rapidly dissected out, frozen in liquid nitrogen and weighed.

Extraction of Tissues and Hormone Assays

The frozen pieces of hypothalamus and pancreas were homogenized (Polytron Kinematica) at 0 °C in acetic acid (0.2 mol/l) containing Trasylol (1000 KIU/ml). Extracts were immersed in a boiling water bath for 5 min [9], except an aliquot of the pancreatic extract which was kept at 0 °C (for insulin determination). After ice-cooling, all samples were centrifuged at 800 xg for 30 min at 4 °C. Aliquots of the supernatants were stored at – 70 °C until assayed.

Prior to the assays, extracts were neutralized with NaOH (2 mol/l), and diluted with the appropriate buffer for each assay. Pancreatic insulin was measured by radioimmunoassay using a guinea-pig anti-porcine insulin serum, mouse insulin as unlabelled standard, and porcine insulin for iodination (^{125}I) by a modified chloramine T method [15]. Glucagon was radioimmunoassayed using a rabbit anti-glucagon serum and highly purified porcine glucagon as ^{125}I -hormone and unlabelled standard. Separation of antibody-bound and free ^{125}I -insulin or free ^{125}I -glucagon was accomplished by adsorption of free hormone on magnesium silicate [16]. Somatostatin was radioimmunoassayed using a sheep antisomatostatin serum, cyclic somatostatin as unlabelled standard, and Tyr¹-somatostatin for iodination; the latter was performed by a modification of the chloramine T method [17]. The ^{125}I -Tyr¹-somatostatin was purified by ion exchange chromatography on carboxymethyl cellulose [18]. The anti-somatostatin serum used has been shown to be directed against the Lys⁴-Thr¹⁰ sequence of the peptide [17]; and the absence of cross reaction with other polypeptides has been established [17]. The sensitivity and intra-assay coefficients of variation were as follows: 7.5 pg and 8.1% for insulin; 20 pg and 7.8% for glucagon; 8 pg and 7.5% for somatostatin.

Plasma glucose was determined by the glucose oxidase method and plasma insulin was radioimmunoassayed as described above.

Data are presented as means $\pm$ SEM; statistical analysis was performed by Student's t test for unpaired comparisons [20].

Materials

The following were gifts: anti-somatostatin serum ("Barbar", P. Brazeau, San Diego, Ca, USA); anti-glucagon serum (GM₃, G. Rosselin, Paris, France); anti-insulin serum (P. H. Wright, Indianapolis, Ind., USA); mouse insulin, porcine insulin, and porcine glucagon (J. Schlichtkrull, Novo Research Institute, Copenhagen, Denmark; and G. Jouve, Novo France, Paris). Cyclic somatostatin and Tyr¹-somatostatin were purchased from Beckman Bioproducts; carboxymethyl cellulose (CM52), from What-

man; Na ^{125}I , from Commissariat à l'Energie Atomique (CEA, Saclay, France); Trasylol (aprotinin) (Zymofren), from Specia (Paris, France).

Results

Characteristics of Lean and Obese Mice

Characteristics of the animals used are indicated in Table 1. At 2 months of age *ob/ob* mice were 60% heavier than their lean controls; at 6 or 8 months of age they were 100% heavier than the controls. By contrast, 8 month-old GTG-obese mice were only 25–30% heavier than their lean controls. All obese mice were hyperinsulinaemic, but to a variable degree. In *ob/ob* mice, insulin levels which had already increased about 7-fold over controls at 2 months of age increased further until they were 70 times control levels at 6–8 months of age. In 8 month-old GTG-obese mice, insulin levels were increased by only about 3-fold. In the fed state, all obese mice were normoglycaemic, except the 6 month-old *ob/ob* mice which were hyperglycaemic. In each group, hypothalamic and pancreatic weights of obese and control mice were similar, despite differences in body weight and insulinaemia.

Hypothalamic Somatostatin

The total somatostatin content in the hypothalamus of *ob/ob* mice did not differ significantly from that of control mice, regardless of the stage of evolution of the obesity (Table 2). Similarly, the somatostatin content in the hypothalamus of GTG-obese mice was not significantly different from that of control animals. Similar results were obtained when expressed as concentrations of somatostatin per unit of wet weight or protein weight (not shown). It should be noted that the hypothalamic content of somatostatin varied between the two types of mice used as controls.

Pancreatic Insulin, Glucagon and Somatostatin

Insulin, glucagon and somatostatin contents of pancreases from obese and control mice are shown in Fig. 1. The total insulin content was significantly greater in *ob/ob* mice than in their lean controls, this difference being greater in the older animals, due to a progressive increase in the insulin content of pancreases from *ob/ob* mice. The amount of insulin was also significantly higher (50% above control) in the pancreases of GTG-obese mice than in those of lean animals.

The amount of glucagon was also markedly

Table 1. Characteristics of lean and obese mice

			Age months	Body weight g	Plasma		Wet weight	
					Glucose mmol/l	Insulin ng/ml	Pancreas mg	Hypothalamus mg
Control	(11)	2		25.8 ± 0.7	9.7 ± 0.2	1.8 ± 0.3	257 ± 10	9.6 ± 0.4
<i>ob/ob</i>	(11)	2		41.2 ± 0.5 ^b	9.3 ± 1.0	12.7 ± 1.5 ^b	273 ± 12	9.9 ± 0.3
Control	(20)	6		30.0 ± 0.4	7.4 ± 0.3	2.1 ± 0.2	296 ± 8	11.1 ± 0.3
<i>ob/ob</i>	(20)	6		61.5 ± 3.0 ^b	11.9 ± 1.0 ^b	141.0 ± 22.0 ^b	304 ± 10	11.4 ± 0.3
Control	(14)	8		31.6 ± 0.5	8.3 ± 0.3	1.3 ± 0.3	330 ± 13	9.6 ± 0.3
<i>ob/ob</i>	(13)	8		61.0 ± 1.1 ^b	10.1 ± 1.3	92.7 ± 18.1 ^b	341 ± 15	9.3 ± 0.2
Control	(12)	8		41.6 ± 0.7	9.1 ± 0.4	2.0 ± 0.2	414 ± 20	8.6 ± 0.3
GTG	(19)	8		52.9 ± 0.6 ^b	8.8 ± 0.2	6.3 ± 1.0 ^a	406 ± 13	8.7 ± 0.3

Blood and tissue samples were obtained as described in Materials and Methods. Values are means ± SEM (with the number of animals in parentheses). ^a Values significantly different from controls ($p < 0.02$); ^b Values significantly different from controls ($p < 0.001$)

Table 2. Somatostatin contents in hypothalamus of *ob/ob* mice, GTG-obese mice, and their respective lean controls

		Age months	Somatostatin ng/hypothalamus
Control	(11)	2	35.5 ± 2.1
<i>ob/ob</i>	(11)	2	44.3 ± 4.1
Control	(20)	6	37.2 ± 1.5
<i>ob/ob</i>	(20)	6	41.5 ± 2.0
Control	(14)	8	46.8 ± 2.9
<i>ob/ob</i>	(13)	8	43.4 ± 2.0
Control	(12)	8	23.5 ± 2.1
GTG	(19)	8	21.5 ± 1.5

Values are means ± SEM (with the number of animals in parentheses). Differences between obese and their controls were statistically not significant

increased in the pancreases of *ob/ob* mice. This increase could already be observed in 2 month-old mice and was of a similar magnitude in the three age groups examined, in contrast with the progressive increase seen for insulin. In GTG-obese mice, however, the amount of pancreatic glucagon was not significantly different from that of control lean mice.

The amount of somatostatin was higher in the pancreases of *ob/ob* mice than in those of control lean mice. This increase was already significant at 2 months of age (22% increase above controls), and reached 60% above controls at 6 and 8 months of age. In GTG-obese mice, the somatostatin content was not significantly different from that of control lean mice (Fig. 1).

Since pancreas wet weight did not differ significantly between obese mice and their lean controls

Fig. 1. Insulin, glucagon and somatostatin contents in pancreases of *ob/ob* mice, GTG-obese mice, and their respective lean controls. Values are means ± SEM of 11 to 20 animals (see Table 1). * $p < 0.05$; ** $p < 0.02$; *** $p < 0.001$. □ = Control; ▨ = *ob/ob*; ▤ = GTG

(Table 1), similar results were obtained when expressed as peptide concentration per organ weight unit. When expressed per unit of protein weight (not presented) the results were qualitatively similar to those shown in Fig. 1.

Discussion

This study has shown that somatostatin, insulin and glucagon are present in increased amounts in pancreases of *ob/ob* mice. As observed for insulin, the total pancreatic content of somatostatin was already increased at a relatively early stage (2 month-old *ob/ob* mice) and further increased during the development of obesity between 2 and 6 months of age. These findings are at variance with a report by Patel et al. [9] who observed that both total insulin and somatostatin pancreatic concentrations were decreased in 11–12 week-old *ob/ob* mice compared to lean controls; other studies, however, have shown that, as observed here, total pancreatic insulin is increased rather than decreased in adult *ob/ob* mice [21–23]. Our results also indicate a marked increase in total pancreatic glucagon in *ob/ob* mice. In contrast to that observed for insulin and somatostatin, this increase appears to be already maximal at 2 months of age.

It is noteworthy that, in GTG-obese mice, no significant alteration in the total pancreatic content of somatostatin and glucagon could be detected, whereas a slight but significant increase in total pancreatic content of insulin was observed. Although the GTG-obese mouse exhibits a variety of alterations that are qualitatively similar to those observed in the *ob/ob* mouse [14], increases in body weight and plasma insulin in GTG-obese mice are of a lesser degree than those observed in *ob/ob* mice. Thus, the discrepancy observed between the two animal models with regard to glucagon and somatostatin pancreatic contents may suggest that alterations in these two variables can only be detected when obesity and/or hyperinsulinism are prominent, as observed in *ob/ob* mice even at an early stage (2 months of age) of development of the obese syndrome. It has also been reported that pancreatic polypeptide is increased in the pancreas of *ob/ob* mice [23]. It thus appears that all four pancreatic peptides are increased in the *ob/ob* mouse. These results do not permit a clear-cut delineation of the relationship among the four pancreatic peptides with regard to a local regulation of their function.

The present study failed to demonstrate a significant alteration in the total content of somatostatin in the hypothalamus of both *ob/ob* and GTG-obese mice, compared to their respective lean controls. The hypothalamic concentrations of somatostatin in 8 week-old *ob/ob* mice has been reported to be slightly increased [10]. We have also observed a slight, but not statistically significant, increase in the hypothalamic content of somatostatin in 2 month-old *ob/ob* mice. However, this trend was not maintained in

older animals (Table 2). This observation makes it unlikely that changes in hypothalamic somatostatin play an important role in the development of obesity in these two animal models, as well as in the alterations of their endocrine pancreatic functions.

Addendum. After this study had been submitted for publication, Makino et al. reported [Endocrinology **101**, 243–247 (1979)] that pancreatic somatostatin content was increased in *ob/ob* mice (5–7 months of age), whereas hypothalamic somatostatin content was unchanged. Their findings are thus in agreement with those of this study.

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