Somatostatin content and release of isolated pancreatic islets from obese-hyperglycemic mice[1]

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Summary. The somatostatin content in pancreatic islets of obese-hyperglycemic mice was much lower than in the islets of normal mice. Also the release of somatostatin was decreased from the islets obtained from the obese-hyperglycemic mice. Tissue culture for 1 week changed neither the content of, nor the amount of somatostatin released from, the pancreatic islets.

It is well known that somatostatin inhibits the release of insulin and glucagon from the islets of Langerhans (review, Luft et al.[2]). Recently a compound cross-reacting with antibodies against somatostatin was found in the A_1-cells of the islets[3,4]. It is, however, an open question whether somatostatin participates in the establishment or maintenance of different diabetic syndromes. In streptozotocin diabetic rats and mice, an increased concentration of somatostatin has been observed in the islets as well as in the whole pancreas[5-7]. In contrast, the diabetic Chinese hamster has a decrease of the somatostatin content of the pancreas[8]. Furthermore there is a remarkable decrease of the somatostatin content of the pancreatic islets and whole pancreas of the obese-hyperglycemic mouse as compared with their lean litter mate[6,7,9].

Our studies of the islets of obese-hyperglycemic mice have now been extended to include also the release of somatostatin. Since the collagenase isolation procedure may damage the A_1-cells with a consequent leak of somatostatin, we used cultured as well as freshly isolated islets.

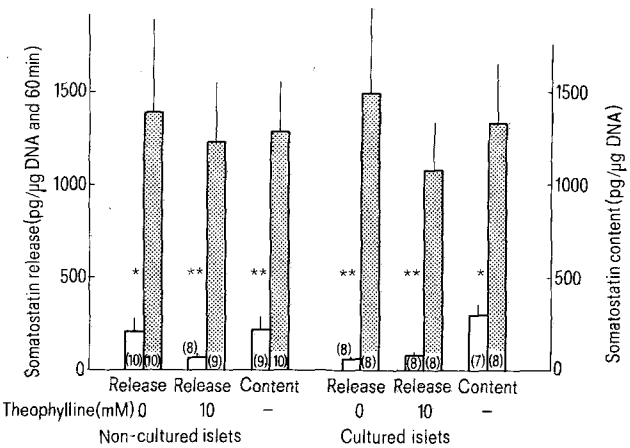
Material and methods. Altogether 31 obese-hyperglycemic mice (gene symbol *ob/ob*) and 65 lean litter mates were used. The animals were starved overnight before being killed by decapitation. Blood glucose concentrations were determined with the method described by Hjelm and de Verdier[10]. Pancreatic islets were isolated by a modified collagenase method[11] using 2.5 mg/ml collagenase and 0.25 mg/ml albumin dissolved in Hanks' solution. Free islets were collected with a braking pipette under a stereomicroscope. Some of the islets were cultured for 7 days[12,13] in a glucose concentration of 5.5 mmoles/l before their somatostatin release was estimated.

Groups of 50 freshly isolated or cultured islets were preincubated 30 min in 500 µl in a bicarbonate buffer[14] supplemented with 25 mmoles/l of HEPES, 1000 U/ml of trasylol and 5.5 mmoles/l glucose. 25 islets were then incubated for 60 min in 500 µl of this medium, the remaining islets being incubated in a similar volume of medium, to which 10 mmoles/l of theophylline had been added. At the end of the incubation period samples of the incubation media were frozen and stored at −20 °C before somatostatin assay. The incubated islets were washed in Hanks' solution and then sonicated for 30 sec in a MSE Ultrasonic Disintegrator, PG 1000 (Crawley, UK) in 250 µl redistilled water. 125 µl of the disintegrated material was used for determination of the DNA-content[15] and 125 µl for somatostatin assay, which was performed by a radioimmunabsorbant technique as recently described by Arimura et al.[16]. The antiserum used, R 141, was a generous gift from Dr R.P. Elde, University of Minnesota, Minneapolis, and has been characterized earlier with respect to reactivity against different parts of the somatostatin molecule[16] as well as its lack of cross-reactivity against other pancreatic hormones[17]. Synthetic ovine somatostatin (Beckman, Geneva) was used for preparation of standards and synthetic Tyr-1-somatostatin (Beckman, Geneva) was used for iodination with the lactoperoxidase technique according to Thorell and Johansson[18]. The sensitivity of the method varied between 2 and 5 ng/l of added somatostatin to assay buffer.

Results. At the time of the experiment, the body weight of the obese mice was about 60 g, whereas the lean litter mates weighed about 25 g. The blood glucose concentration of the obese mice was 10.2±1.4 mmoles/l (n = 16), the corresponding figure for the control animals being 6.4±0.4 mmoles/l (n = 24).

As is illustrated in the figure, both the somatostatin content of, and release from islets of the obese-hyperglycemic mice was lower than that found for the islets of the lean mice. Thus, the somatostatin content of the lean mice islets was about 5 times higher, independently of whether the islets had been cultured or not. It is also evident that culture for 1 week influenced the somatostatin content of neither the *ob/ob* nor the control islets ($p > 0.05$). Furthermore, the somatostatin release from both fresh and cultured islets was between 6 and 30 times higher in the lean than in the obese-hyperglycemic mice. Addition of theophylline to the incubation medium was without effect on somatostatin release.

Discussion. Recent immunocytochemical studies have shown that the pancreatic somatostatin is located in the A_1-cells of the pancreatic islets[3,4,19,20]. Hellman[21] found that in the obese-hyperglycemic mice, the relative proportion of A_1-cells in the islets is only half that in the lean mice; this is consistent with a lower somatostatin concentration in the pancreas and the pancreatic islets of the obese-hyperglycemic mice[6,7]. The present data lend further support to the view of a lower content of somatostatin in the pancreatic islets of the obese-hyperglycemic mice, and furthermore the reduction still persists after culture for 1 week in a medium with a normal glucose concentration. It is noteworthy that the decrease was apparent independently of whether the concentration was calculated per islet or per µg



The somatostatin release and content of isolated pancreatic islets from obese-hyperglycemic mice (unfilled bars) and their lean litter mates (cross-hatched bars). The bars indicate the mean values ± SEM. The significances are indicated with asterisks, * $p < 0.05$, ** $p < 0.01$, and the number of experiments is given at the bottom of each bar.

DNA, since the mean islet mass is much higher in the obese than in the lean mice[22]. The release of somatostatin from both freshly isolated and cultured *ob/ob* islets was also less than in their lean littermates. The lack of effect of adding theophylline to the incubation medium is in contrast to the report of Schauder et al.[23].

From the present findings, it is concluded that the turnover of somatostatin is very high as indicated by the high ratio between the amount of released somatostatin and the somatostatin content in the incubated islets. Furthermore the results obtained in the cultured specimens indicate also that the synthesis of somatostatin occurs in adult mouse pancreas without neural influence, as has been shown earlier for fetal rat pancreas[24]. Somatostatin is known to inhibit the stimulated release of insulin and glucagon, but it has also inhibitory effects on many gastrointestinal functions[25,26]. A relative deficiency of somatostatin-producing cells might thus explain many of the metabolic disorders found in the obese-hyperglycemic mice, and also explain the marked increase in the number of somatostatin cells in animals with experimental diabetes[26]. These questions, however, need further attention.

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Corpus luteum function in ageing inbred mice[1]

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Summary. Impaired breeding performance of aged female mice was associated with reduced numbers of ovulations and increased mortality of embryos. The amounts of progesterone in the sera, corpora lutea and uterine flushings of these animals were similar to those of young animals when measured by radioimmunoassay.

There is substantial evidence to show that the gametogenic potential of the ovaries of mice and some other animals outlasts the ability of the uterus to maintain conceptuses to term[3]. The results of embryo transplantation experiments in which the age of donors and of recipients were variables showed that the latter was the major factor affecting embryo survival and that the majority of ova from old mice were viable in a young uterine environment[4,5]. The relative contribution of extrinsic and intrinsic factors to the decreased gestational potential of the ageing uterus is not clearly established. Morphological studies[6] and hormone supplementation studies[7] implied that the secretory activity of the corpora lutea (CL) might be sub-optimal for uterine function. Other investigators have doubted this conclusion and implied that unknown intrinsic age changes of the uterus are responsible for most pregnancy wastage[8–10]. We have therefore measured the levels of progesterone in ageing pregnant mice in order to directly assess luteal function.

Materials and methods. Young (2–3 month) and aged (8–12 month) virgin CBA/H female mice were paired either with young albino male mice or proven sterile vasectomized animals. They were examined each day for the presence of a copulatory plug (= day 1 of pregnancy/pseudopregnancy). Mated females were allocated randomly to 3 groups which were either autopsied on day 4 or 8 of pregnancy or used for litter size determination at term. On the day of autopsy the animals were anaesthetized with ether and a blood sample was collected from the orbital sinus. They were killed by cervical dislocation before recovering from the anaesthetic. The number of uterine embryos was determined either by flushing them out with saline and counting them in a watch glass (day 4) or by counting the number of implantation swellings (day 8). The most hyperaemic set of CL was counted under the microscope. 2 CL from 1 ovary of each animal were removed by fine dissection for subsequent hormone determination, closely apposed CL being avoided. Histological preparations showed that CL isolated by this technique were intact and generally free of adhering tissue apart from occasional preantral follicles. The undissected ovaries were prepared by routine histological methods and stained with haemalum and eosin. The dis-