Vagotomy in young obese hyperglycemic mice: effects on syndrome development and islet proliferation

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Edvell, Anders, and Per Lindström. Vagotomy in young obese hyperglycemic mice: effects on syndrome development and islet proliferation. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E1034–E1039, 1998.—Obese hyperglycemic mice have large pancreatic islets and high levels of serum insulin and blood glucose. Vagotomy was performed on 3-wk-old animals to investigate the role of gut cholinergic innervation in young Umeå ob/ob mice. After vagotomy, obesity and hyperglycemia are dissociated. Weight increase in obese vagotomized mice was lower than in sham-operated controls during the 1st wk postoperatively but not thereafter. Blood glucose was lower up to 5 mo after vagotomy, but vagotomized mice showed reduced glucose tolerance. Insulin cell proliferation rate was reduced 2 and 3 wk but not 5 mo after vagotomy. After 5 mo, islet volume was smaller in vagotomized mice. Serum insulin levels were the same in vagotomized animals as in sham-operated controls. The effects of reduced cholinergic innervation are probably caused both by direct effects of denervation and by lowered metabolic demand.

MATERIALS AND METHODS

Animals. Noninbred ob/ob mice from the Umeå colony (Umeå ob/ob) and their lean littermates were used throughout. Although Umeå ob/ob mice are metabolically abnormal, with hyperglycemia and insulinemia, islets from these animals respond adequately to stimulators and inhibitors of insulin release (12). The animals were fed with R3 “rat and mouse breeding food” pellets from Lactamin (Vadstena, Sweden). Water and food were given ad libitum. Both male and female mice were used. The ob/ob trait is recessive; no distinction is made between an/an and an/an ob mice since both groups are phenotypically “lean.” The mice were kept at 22°C. Lights were on between 0600 and 1800.

Study design. Lean and obese littermates were weighed and marked for identification at days 23–25. Blood samples were obtained from the retroorbital plexus. After this the mice were either fed ad libitum or sham-operated, respectively. One group was studied for 12 days. One-half of these animals were fed ad libitum but obtained extra subcutaneous injections of 10% glucose, 0.3 ml three times a day (0900, 1200, and 1400) between 7 and 12 days after vagotomy. The others were only fed ad libitum. A second group of mice was studied for 21 days and a third for 5 mo (150 days). Mice were weighed and blood glucose samples were obtained at days 7, 12, 14, and 21 and at 120 and 150 days (4 and 5 mo) after surgery. Mice were killed by decapitation after 12 or 21 days or after 5 mo. Two hours before being killed, the animals received an intraperitoneal injection of 5-bromo-2’-deoxyuridine, 120 mg/kg body weight (Radiochemical Centre, Amersham, Bucks, UK). The pancreas was removed for further studies as will be described.

Subdiaphragmatic truncal vagotomy. Mice were anesthetized by a combination of fluanisone (2.5 mg/kg), fentanyl (0.05 mg/kg), and propofol (12.5 mg/kg) given intraperitoneally. The subdiaphragmatic truncal vagotomy was performed under a stereomicroscope with a magnification of 20 times, according to Laye et al. (21), with the exception that no ligatures were put around the vagal trunks. Instead a 2-mm section from each trunk was removed to inhibit reinnervation. The mouse was pinned to the operative table with needles to remain in exact location. After that a 10- to 15-mm incision was made cranio-caudally. The skin was dissected from the peritoneum, which was opened with a transversal 10- to 12-mm long incision. The ventricle and esophagus were located, and a stylet was put under the cardia region to lift it up and improve access to the region. With use of the microscope the adventitia surrounding the muscularis esophagus was cut together with the two branches of the vagus nerve. Incisions were sutured with 6.0 catgut. The mice were given 1 ml 0.9% NaCl solution subcutaneously and put in separate cages at 30°C for 2 h. After that the mice were put with their original litters. No attempt was made to perform a more selective vagotomy due to the size of the mice. When killed, vagotomized animals showed dilated gastric ventricles when measured around the widest fundus section. This was not observed in sham-operated mice.

Immunohistochemical staining. Pancreases were removed from 74 animals at 12 days, 21 days, and 20 wk postoperatively. The pancreas was fixed in 10% formaldehyde and dehydrated and embedded in paraffin. The pancreas was then cut transversely from head to tail in slices of 5 μm, with a microtome. Every 20–30th slice was placed on a microscope slide. After removal of paraffin the slides were incubated for
10 min in Tris buffer (60.6 g Tris, 79 g NaCl, 10 liters H2O, and 1.0 M HCl in sufficient amounts to make pH 7.6; Boehringer Mannheim, Mannheim, Germany) supplemented with 30% H2O2 to reduce endogenous peroxidase activity. The slides were then rinsed in Tris buffer. After that the slides were incubated in 1% BSA to reduce background staining. The slides were incubated overnight at room temperature with monoclonal anti-5-bromo-2'-deoxyuridine antibody (Amer sham, UK), diluted 150 µl/ml in Tris, and then rinsed and incubated for 30 min with antimouse IgG antibody diluted 1:25, supplemented with 15% normal rabbit serum. The slides were rinsed and incubated for 30 min with alkaline phosphatase anti-alkaline phosphatase complex diluted 1:50 in Tris buffer. Alkaline phosphatase activity was finally revealed by incubating for 30 min in 5-bromo-chloro-3-indolyl phosphate and nitroblue tetrazolium, supplemented with levamisole diluted 1:25. Chemicals for immunohistochemistry were obtained from Dako. The slides were rinsed in distilled H2O, counterstained for 10 min with calcium red (nuclear fast red), dehydrated, and mounted. With this technique labeled nuclei are stained dark blue and nonlabeled nuclei are stained red. More than 90% of the endocrine cells in the pancreas from Ob/ob mice are B-cells (11).

Measurements of labeling index and islet volume. Slides prepared and stained according to the immunohistochemical method described previously were examined under a light microscope. An oil-immersion lens with magnification ×1,000 was used. At least 500 islet cells were counted per pancreas, in most cases >1,000 cells. None of the 12 experimental groups included <2,000 counted cells. Islet volume was estimated in pancreases from vagotomized and sham-operated animals 5 mo after operation. Sections of pancreas 400 µm apart were analyzed for islet area using a Reichert Polyvar 2 (Leica, Vienna, Austria) connected to image-processing software (Pixeltools, Perceptics, Knoxville, TN). Islet volume was then calculated as described by Tejning (28) and Hellerström (14). The smallest islet area included was 220 µm2.

Pair-feeding experiments. To evaluate the effect of different food intake in vagotomized and sham-operated animals, a pair-feeding experiment was performed. Seven vagotomized and seven sham-operated animals were given the same amount of food during the first 2 wk postoperatively. The amount of food was adjusted by measuring the spontaneous food intake in vagotomized animals over a 2-wk period after operation. This amount was then given to a second group of sham-operated and vagotomized mice. During days 1–3 the mice received 3 g of pelleted food per mouse daily, during days 4–6 they received 4 g per mouse daily, and during days 7–14 they received 5 g per mouse daily. Water was supplied ad libitum. After 7 and 14 days blood glucose and weight were measured. At the end of the experiment the mice were killed and the islet cell proliferation rate was measured as previously described.

Glucose tolerance test. A glucose tolerance test was performed in eight vagotomized and eight sham-operated obese animals 14 days after surgery. Blood glucose was measured immediately before and at 30, 60, 120, and 240 min after intraperitoneal injection of an isosmotic (317 mM) glucose solution (500 mg/kg body wt).

Blood glucose and serum insulin analysis. All blood samples were obtained from the retroorbital plexa between 1000 and 1300, with a capillary blood-collecting tube (Kebolaboratory, Stockholm, Sweden). Unless otherwise stated, in animals receiving glucose injections the last injection had been given at midnight the previous day. To analyze blood glucose an Accutrend alpha (Boehringer Mannheim) was used. All serum insulin samples were assayed by RIA using crystalline mouse insulin as standard. Free and antibody-bound insulin was separated by precipitation with ethanol (13). 125I-labeled insulin was supplied by Eurodiagnostica (Malmö, Sweden).

RESULTS

Blood glucose levels in vagotomized young obese mice. Mice were vagotomized at days 23–25. At this age young obese hyperglycemic mice have significantly higher blood glucose values compared with their lean littermates (8.6 ± 0.5 vs. 6.6 ± 0.25 mM, P < 0.005, n = 22 and 19). Seven days after surgery blood glucose levels in sham-operated obese mice had risen to 11.1 ± 0.7 mM (n = 10, P < 0.05) compared with the preoperative level of 8.6 mM. In vagotomized obese mice the blood glucose level was 7.6 ± 0.6 mM (n = 19) 7 days postoperatively (P < 0.02) compared with 11.1 mM. Blood glucose levels in vagotomized obese mice were lower than in sham-operated obese mice throughout the 5-mo observation period (Fig. 1) and were not different from those observed in lean mice. Blood glucose levels in lean vagotomized mice were at no time different from those in lean sham-operated mice.

To test if blood glucose and islet cell proliferation rate in vagotomized mice could be raised in response to increased metabolic demand, vagotomized obese mice were injected with 0.3 ml 10% glucose solution three times daily for 5 days. Blood glucose was measured in the morning before the first injection and was higher in glucose-injected mice compared with vagotomized mice not injected with glucose (10.5 ± 1.2 vs. 8.1 ± 0.6 mM, n = 10 and 8, P = 0.07). An increase in blood glucose was observed also in lean vagotomized injected mice after 5 days of glucose injections (8.8 ± 0.6 vs. 7.1 ± 0.4 mM, P < 0.05, n = 9). Blood glucose levels in obese vagotomized animals receiving glucose injections were not different from those in sham-operated obese mice (10.5 ± 1.2 mM, n = 10, compared with 10.3 ± 1.0 mM, n = 18). When vagotomized and sham-operated mice were pair fed, there was a lower blood glucose level in
vagotomized mice (6.3 ± 0.4 mM, n = 10) compared with sham-operated animals (8.5 ± 0.4 mM, n = 7, P = 0.005). The blood glucose level in sham-operated animals fed ad libitum was 10.3 ± 0.6 mM (n = 18, P = 0.09) compared with pair-fed obese sham-operated mice.

Weight increase after vagotomy. Figure 2 shows the increase in body weight during the first 3 wk postoperatively. During the 1st wk a larger weight increase was seen in sham-operated obese mice, but from 7 days on the weight increase was the same in vagotomized and sham-operated mice. A decreased weight gain during the 1st wk after vagotomy was seen also in lean mice (Fig. 2). When vagotomized and sham-operated obese animals were given the same amount of food, there was still a difference in weight increase during the 1st wk postoperatively (4.5 ± 0.5 and 6.6 ± 0.3 g, respectively, n = 7). There was no difference in weight increase between vagotomized obese and sham-operated lean littermates during the 1st wk postoperatively. As for obese mice there was no difference in weight increase between vagotomized and sham-operated lean animals during the 2nd wk. Glucose injections had no effect on weight gain (not shown). After 4 mo there was no longer any difference in weight between sham-operated (48.3 ± 1.5 g, n = 4) and vagotomized obese mice (45.8 ± 1.0 g, n = 5).

Islet cell proliferation rate after vagotomy. By 14 and 21 days postoperatively there was a reduced islet cell proliferation rate in obese vagotomized mice, compared with islets from sham-operated obese animals (Fig. 3). The proliferation rate was reduced with time during the observation period, and after 150 days the proliferation rate was the same in vagotomized animals and controls (P < 0.05 when compared with data 14 days after vagotomy). No differences in islet cell proliferation rate could be detected between lean vagotomized and lean sham-operated animals at any time (0.9 ± 0.1 vs. 1.1 ± 0.2%, n = 2 and 4, respectively, in lean mice 2 wk after vagotomy and 1.0 ± 0.3 vs. 0.8 ± 0.15%, n = 7 and 3, 3 wk after vagotomy).

Figure 4 shows that when obese vagotomized mice had been injected three times daily with glucose there was an increase in mitotic index (islet cell proliferation rate). Glucose injections had no effect in sham-operated animals. When pair fed, vagotomized mice showed a reduced islet cell proliferation rate compared with sham-operated mice, 1.3 ± 0.3%, n = 5 vs. 2.6 ± 0.3%, n = 7, P < 0.02. There was no difference in islet proliferation rate between sham-operated animals that had been pair fed or fed ad libitum. (cf. Fig. 3).

There was no strict correlation between blood glucose or serum insulin values on the one hand and islet cell proliferation on the other hand when mean values for groups of animals were observed over a 5-mo period. There was also no correlation between blood glucose values and islet cell mitotic index in individual obese animals at any time point. However, 2 and 3 wk
postoperatively there is a correlation (correlation coefficient 0.47, n = 19, P < 0.05) between serum insulin and islet proliferation index in individual vagotomized and sham-operated obese mice.

Islet volume. Pancreas sections were taken at regular intervals from vagotomized and sham-operated animals to get an estimate of the islet volume 5 mo after operation. In pancreases from five vagotomized obese mice 13 ± 4% of the islets had an area smaller than 2,000 µm² and 6 ± 1.7% had an area larger than 30,000 µm². Corresponding figures in eight sham-operated animals were 8.8 ± 3.1% small islets (P = 0.08) and 15.5 ± 5.5% large islets. The total islet volume was larger in sham-operated mice (1.2 ± 0.3 mm³ vs. 0.6 ± 0.2 mm³, P = 0.06, Student's t-test).

Glucose tolerance test. Figure 5 shows that there was reduced glucose tolerance in response to intraperitoneally administered glucose in vagotomized animals (P < 0.02), with an elevated blood glucose level present for >2 h.

Serum insulin in vagotomized animals. Two weeks after operation, when the animals were ~40 days old, serum insulin in obese vagotomized animals was not different from sham-operated controls (Fig. 6). One week later the serum insulin had risen in both groups. Glucose injections had no effect on serum insulin levels when measured 8–10 h after the last injection (not shown). Serum insulin was lower in lean mice compared with obese in all litters.

DISCUSSION

The early stages of the ob/ob syndrome are not as well explored as the later stages, but still a number of early signs of the syndrome have been recognized, such as an abnormal thermoregulation (18), congenital hypothyroidism (29), lowered oxygen consumption (10), and increased eating (31). Earlier studies have also found increased serum insulin at days 17–20 (8), marked degranulation in islets together with a reduced insulin content in the pancreas from 5-wk-old obese animals (26) and an increase in serum glucose in obese animals at ~3 wk of age (8). These changes are thought to be secondary to increased functional demand. In adult animals, islets from obese mice are both enlarged and increased in number (11, 15). It is probable that the
increased islet mass reflects both B-cell proliferation and recruitment from ductal stem cells.

Little has been done to characterize islet function and morphology in ob/ob mice at ages <2 mo, although such knowledge is important if one wants to understand the mechanisms regulating islet growth. To our knowledge this is the first study on the effects of vagotomy in the obese hyperglycemic syndrome.

Vagotomies have been performed for decades, primarily on humans suffering from peptic ulcers, because of the inhibitory effect of vagotomy on HCl secretion (7). After vagotomy there is increased cell proliferation of gastrin- and somatostatin-producing cells, probably due to compensatory mechanisms when the peptic juice becomes less acid (24). There is also a decreased insulin response to glucose after vagotomy (9). This is supported by this study where we find a reduced glucose tolerance after vagotomy. Conflicting reports have been presented as to whether vagotomy stimulates proliferation of exocrine pancreas tissue (5, 30).

We now demonstrate a decreased proliferation rate in pancreatic islet cells 2 and 3 wk after vagotomy in obese hyperglycemic mice. A reduced islet mass was observed 150 days after surgery despite similar weight gain as in sham-operated animals. Also in the sham-operated group, the total islet mass was smaller than what has been reported previously in adult ob/ob mice (14). No difference in islet proliferation was observed between vagotomized and sham-operated lean mice.

Islet cell proliferation was lower in sham-operated mice 5 mo after surgery when compared with 14 days after surgery, although blood glucose values were not changed. A reduction with age despite persisting hyperglycemia is in accord with findings of others (14, 27) and supports the hypothesis that B-cell proliferation at a young age is critical for later islet mass.

Vagotomy in obese hyperglycemic mice caused a persistent decrease in blood glucose levels for up to 150 days after operation. Earlier reports have demonstrated that glucose stimulates islet cell proliferation (1). After vagotomy the motility of the gastric ventricle is reduced and the ventricle is dilated (20). The obese vagotomized animals showed dilated gastric ventricles. This could limit the maximum food intake in vagotomized animals and explain the lower blood glucose values and the decrease in weight gain observed during the 1st wk. When vagotomized and sham-operated mice were pair fed, significantly lower blood glucose values and islet proliferation rate were found in the vagotomized group, suggesting that vagotomy has an effect on blood sugar and islet proliferation not dependent on food intake. A lower blood glucose level could then be part of the cause of the decreased islet cell proliferation rate in vagotomized animals. In line with this we find that blood glucose values and proliferation index are the same in vagotomized obese mice as in lean mice.

An elevated islet mitotic index is evident before the rise in blood glucose in obese mice (Edvell, unpublished data). In this study we find a correlation between serum insulin and islet cell proliferation rate in obese animals but no correlation between islet proliferation rate and blood glucose levels. These observations suggest that stimuli other than a rise in blood sugar per se stimulate islet proliferation.

Gut motility is improved within 2 wk (30) but not to normal values. After 1 wk no difference in weight gain could any longer be seen between sham-operated and vagotomized animals. Although it would appear that food absorption is again sufficient, islet cell proliferation is lower in vagotomized ob/ob mice 2 and 3 wk after operation. We also observed a decreased islet mass during the 5-mo observation period in vagotomized obese mice. It may be that food absorption is still retarded because of persistent impaired gut motility. This could lead to a smaller increase in blood glucose after eating and to a reduced demand for insulin and lower islet cell proliferation. The findings show that obesity and hyperglycemia are not necessarily linked in the ob/ob syndrome.

When glucose was injected to stimulate B-cell function, a rise in islet proliferation rate was evident in obese vagotomized mice, suggesting that vagotomy does not make islet cells insensitive to factors stimulating proliferation. The islet labeling index was lower in vagotomized glucose-injected obese mice compared with sham-operated mice despite similar blood glucose levels. These findings suggest that vagal innervation can have a modulating effect on islet cell proliferation rate independent also of blood glucose levels. One possible explanation for this could be the reduced blood flow in the islets after vagotomy (17). However, reduced blood flow and reduced proliferation rate can be manifestations of the same underlying cause.

One possible explanation for the effects of a vagotomy in the obese hyperglycemic mice is the existence of a pancreatic-neural feedback loop. When blood glucose and body fat are running low in the body, the levels of leptin will be lower, which triggers the hypothalamus in turn to prepare the pancreas and gastrointestinal tract for receiving food via release of mediators, such as neuropeptide Y and/or other regulatory peptides. In ob/ob mice this effect might be enhanced because of the defective leptin inhibitory signal. Vagotomy could reduce or abolish the efferent signal to the pancreas in such a neural feedback loop.

Vagotomized obese mice showed reduced glucose tolerance but lower resting blood glucose values, and cholinergic innervation is probably important in several aspects of metabolic regulation in these animals. Chen et al. (6) showed that islets from young obese mice release more insulin than islets from their lean littermates at different glucose concentrations and that this effect may be mediated by increased pancreatic cholinergic stimulation. Vagotomy could thus lead to a larger decrease in β-cell response in obese animals compared with lean littermates.

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