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Research Article

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ABSTRACT The existence of a relationship between the ventromedial hypothalamic area (VMH) and the activity of the endocrine pancreas has been shown previously. This relationship has been further tested and extended in the present study, using isolated perfused pancreases from rats previously lesioned (4–7 d) in the VMH. It was found that in isolated pancreases obtained from rats fed ad lib. for 4 d after VMH lesions (i.e., that were hyperphagic), the typical biphasic pattern of insulin secretion was observed following glucose stimulation (20 mM) and that the total insulin output was much greater than that of controls. The increased insulin output was not a result of hyperphagia because similar results were obtained using pancreases obtained from VMH-lesioned rats in which a food restriction matching exactly that of control rats was started either immediately or 3 d after the lesions. Pancreases from such food-restricted VMH-lesioned rats oversecreted insulin, when compared with controls fed the same amount, from 7 mM of glucose concentration in perfusion medium onwards. After the addition of arginine (10 mM), the total output of glucagon by pancreases from food-restricted VMH-lesioned rats was twice that of controls. Qualitatively, the arginine-induced glucagon secretion by pancreases from food-restricted VMH-lesioned rats retained its biphasic pattern. Similarly, epinephrine (0.1 μM) elicited a greater glucagon release by pancreases from food-restricted VMH-lesioned rats when compared with controls. These data further support the concept of a link (as yet undefined) between the hypothalamus and the endocrine pancreas, as lesions of the VMH area resulted in abnormal secretion not only of insulin, but of glucagon as well.

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INTRODUCTION

Chemical or electrolytic lesions of the ventromedial hypothalamus (VMH)[1] have been shown to produce obesity in several species (1–4). Although the VMH syndrome is often accompanied by hyperphagia (5), lesions effective in causing obesity in the absence of overeating have suggested that factors other than hyperphagia also contributed to the occurrence of this type of obesity (6, 7).

Insufficient growth hormone production (6, 8, 9), as well as hypoactivity and pituitary insufficiency leading to hypothyroidism have been demonstrated in VMH-lesioned animals (10, 11). However, among the observed endocrine changes, hyperinsulinemia has been found to be the most prominent one (12, 13). Thus, VMH lesions failed to induce weight gain in diabetic animals (14, 15), suggesting the absolute requirement of intact B cells in the establishment and maintenance of the obesity syndrome (15). The observation that hypothalamic obesity could not be produced in rats in which B cells had been destroyed by streptozotocin administration and replaced by fetal pancreas transplants devoid of intact innervation (16), supported the concept of a direct neural influence on the B-cell function. Analogous conclusions were drawn on the basis of chronic experiments carried out in VMH-lesioned rats with superimposed vagotomy (17–19). More recently it was shown that bilateral subdiaphragmatic vagotomy performed 50 min after VMH lesions, immediately and completely reversed the observed glucose-induced hyperinsulinemia (20). It is well established that the stimulation of the vagus nerve increases both insulin and glucagon output by the pan-

[1] *Abbreviations used in this paper*: IRG, immunoreactive glucagon; IRI, immunoreactive insulin; VMH, ventromedial hypothalamus.

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creas (21–23). Thus, based on the hypothesis of a vagally-mediated hyperinsulinemia in VMH-lesioned animals, one would anticipate changes not only in insulin secretion but in glucagon secretion as well. A combined oversecretion of insulin and glucagon in VMH-lesioned animals would help to explain the recent observations that perfused livers from these animals have increased deamination of amino acids, once taken up, and a diversion of the deaminated intermediates toward lipid synthesis pathways concomitantly with an increase in urea production (24–26).

The present studies were undertaken to further substantiate the concept of the relationship between the hypothalamus and the secretory activity of the endocrine pancreas, using isolated perfused pancreases from normal and previously VMH-lesioned rats, and measuring not only insulin secretion but that of glucagon as well.

METHODS

Animals. 8–10 wk-old female Wistar rats, weighing between 200 and 240 g and bred in our laboratories, were used. They were placed in animal quarters with a constant temperature (23°C) and a fixed 12-h light cycle. They were fed with standard laboratory chow (UAR Laboratories, Villemoisson, Epinay/Orge, France).

Electrolytic lesion of the hypothalamus. Bilateral stereotaxic lesions of the VMH (stereotaxic apparatus, David Kopf Instruments, Tujunga, Calif., model 900) were made electrolytically in rats anesthetized with ketamine hydrochloride (80 mg/kg body wt). The stereotaxic coordinates used were selected according to De Groot (27): anterior-posterior (AP)+6.0, lateral ±0.6–0.8, and ventral −2.8. Anodal DC (1 mA for 30 s) was passed through epoxy-coated stainless steel electrodes (0.4 mm Diam with bared tip of 0.2 mm). Unoperated rats were used as controls for the following reasons: (*a*) no difference in glucose-induced insulin secretion was found between perfused pancreases from unoperated or sham-operated rats (i.e., rats treated as the lesioned animals except that no current was passed through the electrodes); (*b*) it was observed that sham operation actually decreased food intake on the day after the operation (28).

Feeding conditions of rats. Perfusion of pancreases from control and VMH-lesioned rats was carried out using three different feeding conditions.

A. In the first, pancreases were isolated 4 d after VMH-lesions from 9-wk-old rats fed ad lib. As these rats became hyperphagic after VMH-lesioning and were heavier than controls (mean increase over controls: 30±6 g, n = 5), they were compared with 10 wk-old controls, so that the body weights of both groups were almost identical (controls: 226±5 g, n = 14; VMH-lesioned rats: 235±7 g, n = 5; NS).

B. In the second experimental condition, 8 wk-old rats were used. As shown by Fig. 1A, VMH-lesioned rats were first fed ad lib. for 3 d to detect successful lesions of the VMH area by the occurrence of hyperphagia and increased body weight (mean excess in body weight over controls after 3 d: 44±5 g, n = 7). Subsequently (Fig. 1A), control and VMH-lesioned rats were fed for 4 d the same amount of food using an automatic food distributor that delivered weight-standardized pellets (3 g) every 6 h. Total food intake (i.e., 12 g/d) was calculated to be less, by 25%, than the usual

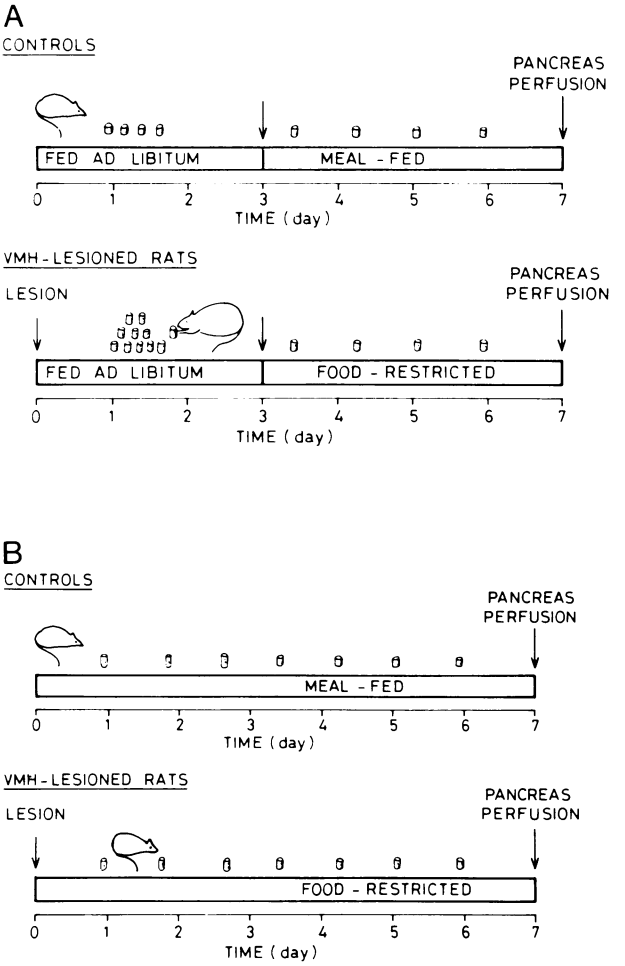

FIGURE 1 Experimental designs used for preventing hyperphagia of VMH-lesioned rats before pancreas perfusion. (A) Prevention of hyperphagia after 3 d. (B) Immediate prevention of hyperphagia. See Methods for details.

food intake of normal rats at this age. This made the avidity for food at distribution time similar (although not identical) in control and VMH-lesioned rats. At the end of this 7-d period, the body weights of VMH-lesioned animals were significantly greater than those of controls as was total carcass lipid content (Table I); moreover, basal insulinemia measured 4 d after lesioning was greater in VMH-lesioned rats than in controls. VMH-lesioned animals that did not increase their body weight by at least 30 g during the first 3 d of hyperphagia (control rats increased their body weight by 6–9 g during the same period), and which had basal plasma insulin level < 6 ng/ml, were discarded.

C. In the third experimental condition, 8 wk-old rats were also used. To avoid the initial phase of hyperphagia, VMH-lesioned animals were immediately placed in the automatic food distributor for a 7-d period (Fig. 1B). At the end of this period, and as shown by Table I, the body weights of VMH-lesioned animals were identical to those of controls, although total carcass lipid content was significantly increased in VMH-lesioned rats when compared with controls. Because diagnosis of successful lesions of the VMH area could no

TABLE I
Basic Characteristics of VMH-lesioned Rats

	Controls		VMH-lesioned	VMH-lesioned
	Feeding conditions: Fig. 1A	Feeding conditions: Fig. 1B	Feeding conditions: Fig. 1A	Feeding conditions: Fig. 1B
Body weight (g)	204±4	207±5	233±4*	206±5‡
Total carcass lipids (g/100 g)	9.0±0.6	8.6±1.0	12.6±1.4§	10.8±0.4§
Basal insulinemia (*ng/ml*)	2.7±0.3	2.5±0.4	10.6±1.2*	5.2±0.6‖
Urea (*mg/100 ml*)	—	43±2	—	58±2*
Glucose (*mg/100 ml*)	—	130±6	—	113±4¶

Basal insulinemia, plasma urea, and glucose levels were measured on the 4th d after VMH lesions, 4 h after the last meal given by the automatic food distributor. Each figure is the mean of 12–16 values±SEM.
Statistical analysis: controls vs. VMH-lesioned:
* $P < 0.0005$; ‡ = NS; § $P < 0.025$; ‖ $P < 0.005$; ¶ $P < 0.01$.

longer be made on the basis of initial hyperphagia and increased body weight, three other criteria were used. On the 4th d after the VMH lesions, i.e., 3 d before pancreas perfusion, blood was taken from a tail vein for plasma insulin, urea, and glucose measurements. As seen in Table I, adequate lesions of the VMH could be determined, in such animals that were never hyperphagic, by the observations of consistent increases in both plasma insulin and urea levels (the latter observation has been previously reported [26, 29, 30]), and decrease in glycemia. The first two criteria were most reliable and were used to decide which VMH-lesioned rats could be used for pancreas perfusion. VMH-lesioned rats with basal insulinemia < 3.5 ng/ml and plasma urea levels < 49 mg/100 ml were discarded, the rejection percentage being ~40% of total lesioned rats.

For the three different experimental conditions A to C, the percentage of rejection of VMH-lesioned rats was similar, i.e., 40%. The subsequent histological examination of brains, carried out in all animals, revealed that in the rejected ones, the VMH lesions were usually not complete and asymmetrical with respect to the third ventricle. In rare instances, (~2% of the total rejected rats) rejected VMH-lesioned rats had adequate and complete VMH lesions. This is in keeping with the recent observation that successful and unsuccessful VMH lesions (in terms of their capacity to produce hyperinsulinemia) are not necessarily different in size and location but could depend upon damaging critical fibers that may or may not pass directly through the ventromedial nucleus (20).

Pancreas perfusion and perfusion medium. Rats were anesthetized with thiopentone sodium (80 mg/kg body wt), and the pancreas was isolated and perfused according to the method of Grodsky (31) as modified by Assan et al. (32) using a non-recirculating medium gassed with O_2/CO_2 (95:5). The perfusion medium was a Krebs-Ringer bicarbonate buffer containing 0.4% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.). When glucagon secretion was measured, the perfusion medium contained 0.25% human serum albumin (Red Cross, Berne, Switzerland), and Iniprol (2,000 antiprotease U/ml, Laboratoire Choay, Paris, France). The flow rate was measured regularly and maintained at 3 ml/min. Unlabeled secretagogues dissolved in perfusion medium were infused into the circuit at a rate of 0.5 ml/min (Braun infusion pump, B. Braun Apparatebau, Melsungen, Germany) 15 cm away from the pancreas to allow adequate mixing with the perfusion medium. Total flow was therefore 3.5 ml/min. In all experiments, pancreases were perfused for 35 min before addition of the secretagogue, with a stimulation period lasting for 20 min. For the kinetic studies of hormone release, perfusion samples were collected at various time intervals from the cannula leaving the portal vein, cooled on ice, and stored at −20°C. The secretagogues used for insulin or glucagon secretion were either glucose (20 mM) or arginine (10 mM). The rate of the hormone release was calculated by multiplying the hormonal concentration of the respective samples by the flow rate. In some cases, when patterns of hormonal secretion were not evaluated, the whole effluent was collected during a 20-min stimulation period to measure total hormone output. In these experiments the secretatogues were glucose (3–19 mM) for insulin release, and epinephrine (0.1 μM) for that of glucagon. The functional integrity of the pancreases was assessed by: (*a*) the constancy of perfusion pressure over the whole experiment time (~80 mm Hg); (*b*) adequate O_2 consumption (80–140 mm Hg); (*c*) presence of duodenal peristaltic activity; (*d*) presence (not shown in the figures) of a rebound hormonal secretion following a bolus of secretagogue at the end of the stimulation period; (*e*) even distribution of trypan blue dye infused into the preparation at the end of the experiment. Pancreases that did not meet these five criteria were discarded and the rejection percentage was about 10%. Results have been expressed per total pancreases, as pancreases from both groups had identical wet weights (controls: 0.87±0.03 g; VMH-lesioned: 0.83±0.03, $n = 14$, NS). The Student t test for unpaired data was used throughout the study for comparison of mean values.

Immunoassays. Aliquots of medium were analyzed for

insulin according to Herbert et al. (33), using rat insulin as standard, and for glucagon according to Unger et al. (34), using the 30 K antibody specific for pancreatic glucagon and porcine-glucagon standards.

Biochemical measurements. Plasma glucose levels were measured by the glucose oxidase method (35) and plasma urea by the urease technique (36). The total lipid content of the carcasses was measured, after homogenization of the animals, according to the method of Folch et al. (37).

Chemicals. All organic and inorganic chemicals were of analytical grade and purchased from Merck AG (Darmstadt, Germany) or from Sigma Chemical Co.

RESULTS

Insulin secretion. As illustrated by Fig. 2, basal insulin release in the presence of 5 mM glucose was significantly higher in pancreases from VMH-lesioned rats fed ad lib. for 4 d than in controls. An increase of the glucose concentration in the perfusion medium to 20 mM markedly stimulated insulin secretion in control pancreases with the typical biphasic pattern. Insulin secretion decreased when glucose concentration was reduced from 20 to 5 mM. In pancreases from VMH-lesioned rats fed ad lib. for 4 d, the pattern of insulin secretion was qualitatively similar to that of controls after the addition of 20 mM glucose, but the total insulin output was much greater and remained at a higher level than in controls when glucose concentration in the perfusate was lowered to 5 mM.

Because the excessive insulin secretion observed in pancreases from VMH-lesioned rats fed ad lib. might be the result of hyperphagia, the latter was prevented by using the first experimental design schematized by Fig. 1A (i.e., 4 d of food restriction). Under these conditions, basal insulin secretion (5 mM glucose) by pancreases from VMH-lesioned rats was identical to controls, as shown by Fig. 3. Increasing the glucose concentration from 5 to 20 mM again caused greater insulin output by pancreases from VMH-lesioned rats when compared with controls, the biphasic pattern of the secretion of the hormone being preserved. By using animals with the same feeding conditions before perfusion (Fig. 1A), we tested insulin secretion elicited by various glucose concentrations. In these experiments, the total amount of insulin released during a 20-min perfusion period was measured. As shown in Fig. 4 total insulin output was similar in both groups at 3 mM glucose but, from 7 mM onward, pancreases from VMH-lesioned rats consistently released more insulin than controls.

To further substantiate the observation that increased output of insulin by pancreases from VMH-lesioned rats was not a result of the 3-d initial hyperphagia (Fig. 1A), the experimental protocol was modified so that food restriction started immediately after the VMH lesions (Fig. 1B). Successful VMH lesions were diagnosed as described in Methods. As illustrated by Fig. 5, insulin secretion by perfused pancreases from VMH-lesioned rats that were never hyperphagic remained qualitatively normal (i.e., biphasic) but, following a glucose challenge, was still quantitatively greater than that of controls. Table II summarizes the calculated net insulin secretion above base line of all experiments. Regardless of the feeding paradigm used, pancreases from VMH-lesioned rats always responded

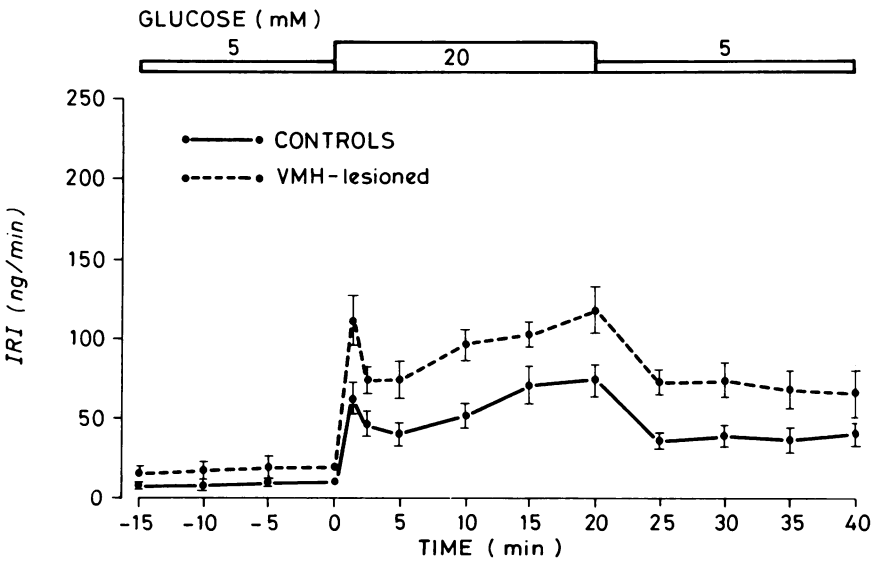

FIGURE 2 Immunoreactive insulin (IRI) release by perfused pancreases from 4 d ad lib.-fed control and VMH-lesioned rats. Perfusion medium was a Krebs-Ringer bicarbonate buffer with bovine serum albumin (0.4%) and glucose as indicated. Each point, mean±SEM of 14 (controls) and 5 (VMH-lesioned) experiments. For statistical analysis of total IRI output, see Table II.

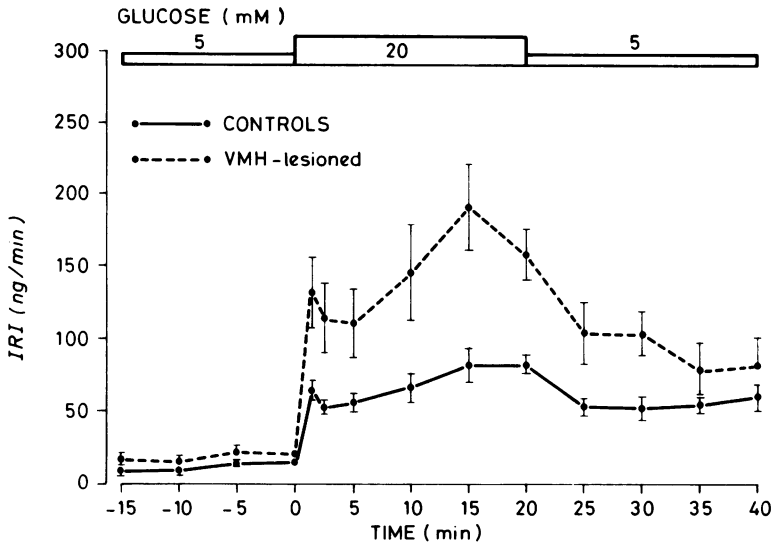

FIGURE 3 IRI release by perfused pancreases from fed controls and VMH-lesioned rats fed a restricted diet for 4 d matching that of controls (see experimental design, Fig. 1A). Perfusion medium as in Fig. 2. Each point, mean±SEM of six (controls) and seven (VMH-lesioned) experiments. For statistical analysis of total IRI output, see Table II.

to a glucose challenge by an insulin secretion that was markedly greater than that of controls.

Glucagon secretion. The first experiments were carried out using the experimental design shown by Fig. 1A (i.e., 4-d food-restricted VMH-lesioned rats). As shown in Fig. 6, during the prestimulation period, basal immunoreactive glucagon (IRG) secretion by pancreases from VMH-lesioned rats was greater than that of controls. The addition of arginine (10 mM) to control pancreases or to pancreases from VMH-lesioned rats produced the typical biphasic pattern of glucagon release. Although the first phase glucagon release was identical in both groups, the second phase was clearly

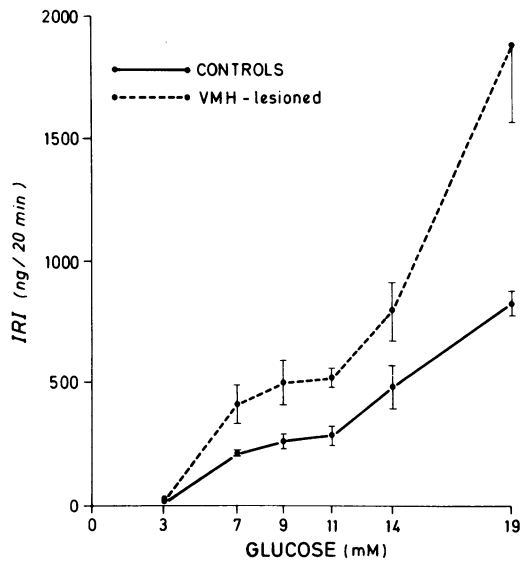

FIGURE 4 IRI release as a function of glucose concentration in the medium by perfused pancreases from fed controls and VMH-lesioned rats fed a restricted diet for 4 d matching that of controls (see experimental design, Fig. 1A). Perfusion medium was as in Fig. 2. Total insulin output was measured by collecting the whole effluent during a 20-min perfusion in the presence of glucose at the indicated concentrations. Each point, mean±SEM of four to five experiments. All differences were statistically significant at at least $P < 0.05$ from 7 mM onward.

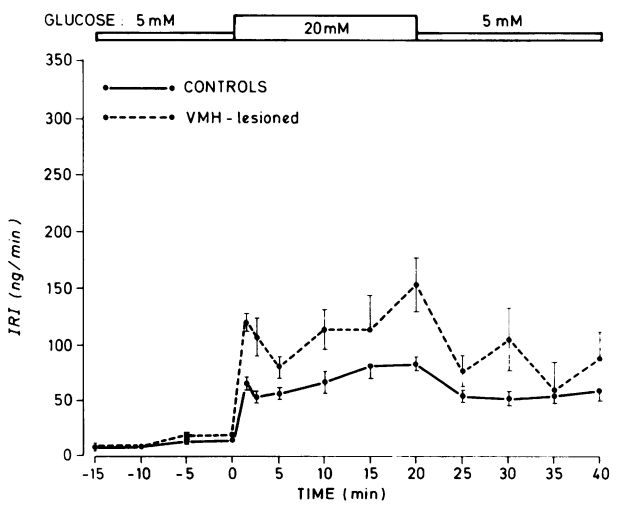

FIGURE 5 IRI release by perfused pancreases from immediately food-restricted control and VMH-lesioned rats (see experimental design, Fig. 1B). Perfusion medium was as in Fig. 2. Each point, mean±SEM of six (controls) and five (VMH-lesioned) experiments. For statistical analysis of total IRI output, see Table II.

TABLE II

Calculated Net IRI Secretion Minus Base Line by Perfused Pancreases from Control and VMH-lesioned Rats Tested under Varying Feeding Conditions

Animals	Feeding conditions	In vitro added glucose	IRI release (surface area minus base line)	No. of experiments
		mM	*ng*	
Controls	Ad lib.	20	862±146	14
VMH-lesioned	Ad lib. for 4 d after lesion		1,545±205 *	5
Controls	Ad lib. for 3 d, food-restricted for 4 d‡	20	937±100	6
VMH-lesioned			2,476±420 §	7
Controls	Food-restricted for 7 d‖	20	937±100	7
VMH-lesioned			1,850±300 §	5

* $P < 0.0125$.
‡ See Fig. 1A.
§ $P < 0.005$.
‖ See Fig. 1B.

increased in pancreases from VMH-lesioned rats when compared with controls. The effect of the high arginine concentration was not the result of changes in osmolarity of the perfusion medium, as proposed elsewhere (38), because increasing osmolarity by the addition of 20 mM saccharose completely failed to alter the secretion of glucagon by perfused pancreases of both groups (data not shown). The increased glucagon output observed in pancreases from VMH-lesioned rats fed according to Fig. 1A was not restricted to arginine stimulation and could also be observed with epinephrine, as shown by Fig. 7. In other experiments, VMH-lesioned rats were immediately food-restricted (Fig. 1B) and their pancreases perfused again in the presence of epinephrine. From Fig. 7 it is clear that total glucagon secretion over a 20-min period in the presence of epinephrine was also greater in pancreases from immediately food-restricted VMH-lesioned rats than in controls, indicating that such finding could be obtained with either one of the two feeding paradigms.

DISCUSSION

These experiments, using an in vitro system, confirm and extend experiments carried out in our own (20, 28, 30) and in other laboratories (12–15, 39) suggesting a functional relationship between some hypothalamic area and the activity of the endocrine pancreas.

Isolated perfused pancreases obtained from VMH-lesioned rats that were either fed ad lib. or food-restricted to an amount of food matching that of controls either after 3 d of hyperphagia or immediately, always responded to a glucose challenge by an oversecretion of insulin without alteration of the biphasic pattern of the hormone release. Furthermore, total insulin secretion during a 20-min stimulation with a glucose concentration in the perfusate above 7 mM was always higher in pancreases from VMH-lesioned rats than in control pancreases.

Another important finding in this study was that glucagon was also oversecreted by pancreases from VMH-lesioned rats. Indeed, arginine stimulation resulted in a twofold increase in total glucagon output in perfused pancreases from VMH-lesioned rats that had been food-restricted after 3 d of hyperphagia when compared with their respective controls. In addition, pancreases from VMH-lesioned rats that were either food-restricted after 3 d of hyperphagia or immediately food-restricted (Fig. 1A, 1B) overresponded identically to an epinephrine stimulation. This indicated that both experimental protocols were valid and that the initial hyperphagia was of little subsequent consequence.

Reports on peripheral plasma glucagon levels in VMH-lesioned rats are scant and contradictory because either a decrease (40) or an increase (30) in basal levels has been reported. Furthermore, little data are available on the hepatic clearance of glucagon in normal rats (41) and none in VMH-lesioned animals. Our perfusion data are compatible with what may happen in the portal vein in vivo. Indeed, preliminary observations of an increase in portal levels of both insulin and glucagon in anesthetized VMH-lesioned rats when compared with controls have been reported.[2]

[2] Bobbioni, E., and C. Coscelli. Insulin and glucagon response to arginine in portal blood of VMH rats. *Horm. and Metab. Res.* In press.

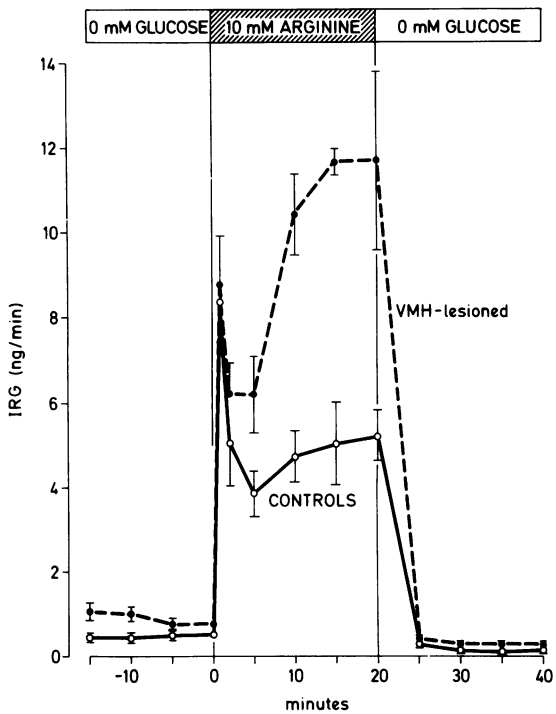

FIGURE 6 IRG release by perfused pancreases from fed controls and VMH-lesioned rats fed a restricted diet for 4 d matching that of controls (see experimental design, Fig. 1A). Perfusion medium was a Krebs-Ringer bicarbonate buffer with human serum albumin (0.25%) plus Iniprol (2,000 antiprotease U/ml), and arginine as indicated. Basal IRG secretion (0 glucose) was significantly different between the two groups ($P < 0.025$). First IRG peak: control vs. VMH-lesioned: NS. Calculated net IRG secretion above base line was 86±12 (controls) and 172±5 ng (VMH-lesioned), significant at $P < 0.0005$. Each point, mean±SEM of six (controls) and eight (VMH-lesioned) experiments.

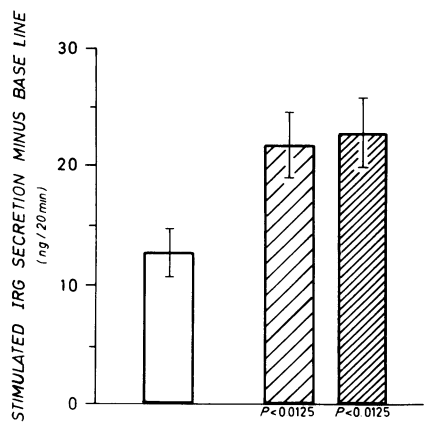

FIGURE 7 Epinephrine-stimulated IRG release by perfused pancreases from fed controls, VMH-lesioned rats, food-restricted as indicated. Perfusion medium was as in Fig. 6. Glucagon output was measured by collecting the whole effluent during a 20-min perfusion in the presence of epinephrine (0.1 μM) plus glucose (2.3 mM). Basal IRG secretion was 0.73±0.05 ng/min (controls), 0.83±0.12 ng/min (VMH-lesioned fed as indicated in Fig. 1A), and 0.98±0.13 ng/min (VMH-lesioned fed as indicated in Fig. 1B). Intergroup basal IRG secretion: NS. Each bar, mean±SEM of 9 (controls), 11 (VMH-lesioned, Fig. 1A), and 8 (VMH-lesioned, Fig. 1B) experiments. P values indicated: control vs. VMH-lesioned rats. □, controls; ▨, VMH-lesioned (ad lib. 3 d, food-restricted 4 d); ▨, VMH-lesioned and food-restricted, 7 d.

It has also been suggested that livers from VMH-lesioned rats could be overstimulated by both insulin and glucagon (26).

The true nature of the changes observed in pancreases from VMH-lesioned rats is still ill-defined. As mentioned in the introduction, it has been strongly suggested that the vagus nerve was involved in the development of the VMH syndrome. It could therefore be postulated that a trophic action of the vagus nerve on the endocrine pancreas would prevail in VMH-lesioned rats that would result, with time, in an increase of the size and/or number of the islets. This is in keeping with the observation of increased islet size in food-restricted VMH-lesioned rats (42). Such increased islet size (or number) could, in particular, be responsible for the changes observed in insulin and glucagon secretion. It is also possible that in VMH-lesioned rats the various endocrine cells of the pancreas would be altered in their respective putative interactions. Indeed, it has been shown recently that, when compared with controls, pancreases from VMH-lesioned rats secreted more insulin and glucagon but less somatostatin in response to an arginine stimulation. These changes were returned to normal upon addition of the cholinergic inhibitor, atropine (43). Thus it is mechanistically conceivable that the somatostatin-containing cell (D-cell) responsiveness of pancreases from VMH-lesioned rats would modulate the release of insulin and glucagon, and that the changes observed in the secretory activity of these pancreases would be related to an increased cholinergic activity.

A final possibility would be the involvement of various humoral factors that would chronically stimulate the A and B cells from VMH-lesioned rats, an effect that would persist in isolated perfused pancreases. Increased gastrointestinal secretion could occur in VMH-lesioned rats either due to the reported increased gastric retention in the VMH syndrome (3) or via a direct effect of the vagus nerve. Some gastrointestinal hormones are known to stimulate insulin release, e.g., gastric inhibitory polypeptide (44) which, interestingly, has also been found in pancreatic A cells (45). The existence of a factor arising from the ventrolateral area of the hypothalamus which triggers insulin output (46–48) when added to isolated islets should also be mentioned. It is not known as yet whether such a factor would be released and play a role in the changes observed in pancreases from VMH-lesioned rats.

In conclusion, the present study showed that VMH lesions brought about an increase of both insulin and glucagon secretion, changes that were unrelated to hyperphagia as the latter was prevented. Although the precise mechanism(s) by which VMH lesions produce these changes are, as mentioned above, still unsettled, these observations add additional evidences for the existence of a link between the hypothalamus and the function of the endocrine pancreas, implicating at least the A and B cells.

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