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Enhanced Insulin Secretion in vitro as a Consequence of Ventromedial Hypothalamic Lesions in the Rat

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INTRODUCTION

Destruction of the ventromedial hypothalamic area (VMH) causes extensive metabolic alterations resulting in obesity (5). Besides affecting lipid metabolism electrolytical lesions of VMH in rats also causes alterations of glucose and nitrogen handling leading to changes of corresponding plasma levels (1, 22). Among the endocrine changes observed hyperinsulinemia has been found to be the most prominent one (8,13,26). Studies of the in situ or in vitro perfused pancreas investigated after VMH-lesions have demonstrated an enhanced maximal insulin secretion in the presence of glucose (4,18,28). Our experiments were carried out to investigate alterations of the B-cell sensitivity in the early phase after VMH-lesions and to clarify more in detail the origin of frequently observed hyperinsulinemia in the presence of normal plasma glucose levels (17,22,26). To avoid any effects of hyperphagia the experiments were done in VMH-lesioned rats with food restriction matching the food intake of control rats.

METHODS

Female Wistar rats (body weight: 194 $\pm$ 2 g) were used after an adaptation period of 2 weeks in individual cages under constant conditions (temperature 22°C, 12 h light-dark cycle, relative humidity 60 %). The animals were fed with standard laboratory chow (Futtermittelwerk Altglienecke, GDR) and the measured mean food intake during this period (16 g per animal in 24 h) was used for food distribution after the VMH-lesions.

Bilateral stereotaxic lesions of the VMH were made electrolytically (Anodal DC 5 mA for 10-25 sec) in fed anaesthetized

rat by using the following coordinates: 1.5 mm dorsal the bregma, 0.5 mm lateral the sutura saggitalis, 9 mm deep (30). The size of the coagulation was located and measured in 30 /um thick serial sections after staining with gallocyanine. Sham-operated animals were treated identically except that the electrodes were not lowered into the brain. The operations were done between 8.30 and 10.30 a.m., the blood was sampled at 8.00 a.m. into heparinized capillaries and the plasma was used for determinations of glucose (Beckman-Autoanalyzer), insulin (10), triglycerides (6) and urea nitrogen (7).

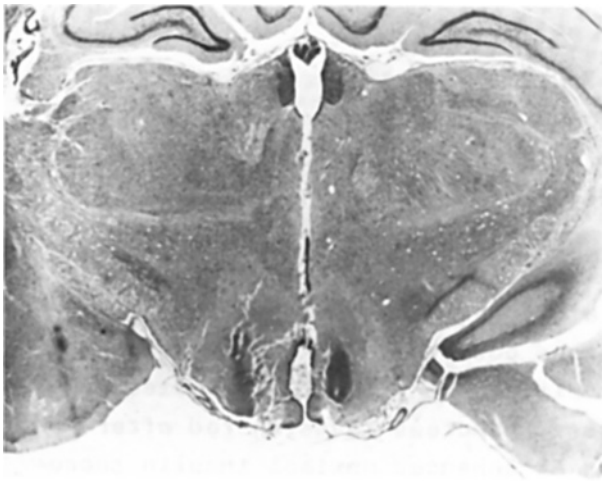

Fig.1 Photomicrograph of frontal section, showing a typical VMH-lesion (x 10).

The methods for pancreas perfusion (3), islet's preparation and incubation (12), determination insulin content (12), glucose utilization (15), and carcass analysis (14) were published elsewhere. Briefly, the in vitro-experiments were done in Krebs-Ringer-bicarbonate buffer after an adaptation period of 30 min in the presence of substimulatory glucose concentration at 37°C. After that the buffer was supplemented with the secretagogues as indicated in the legends to the figures and tables. When pancreatic islets were investigated the tissue was used for the determination of dry weight (11) and the results were expressed per /ug dry weight.

The statistical analysis was carried out by means of Students t-test.

RESULTS AND DISCUSSION

The morphological control of the VMH-lesions (Fig.1) resulted in a fronto-occipital extension of 600 ± 65 /um (right) and 540 ± 90 /um (left) in successfully treated animals. In about 20 % of the rats structures other than or in addition to the VMH were

lesioned and these animals were discarded from the study.

Under conditions of an identical food intake (controls: 15.6 g/24 h, VMH-lesioned: 16.0 g/24 h) we observed within the first 6 days post operationem no differences of weight gain or plasma glucose concentrations (Fig. 2), whereas the peripheral plasma insulin levels were enhanced already 2 days p.o. at a time of decreased urea and triglyceride levels (Fig. 2). These parameters were found to be enhanced from the 4th day onwards when compared with the controls (Fig. 2).

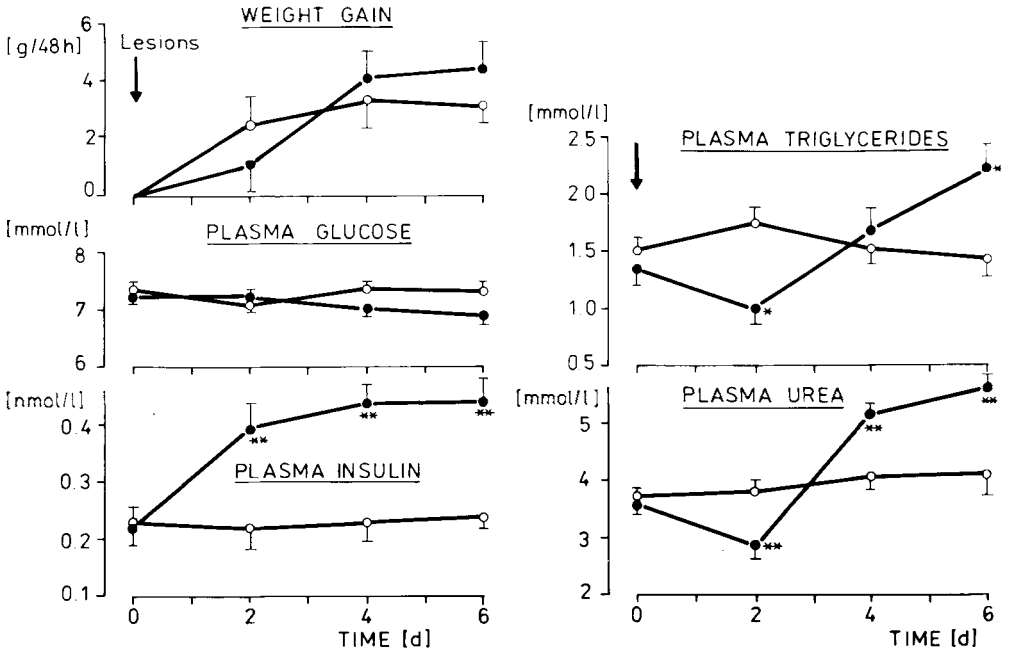

Fig. 2 Clinical characterization of control (o—o, n = 8) and lesioned (●—●, n = 9) rats in the early phase after electrolytical destruction (↓) of VMH.
* P< 0.05, ** P< 0.01 in comparison to controls.

These observations confirm and extend results reported in other laboratories, demonstrating an enhanced plasma insulin level within 24 h after VMH-lesions (9,23) an increased triglyceride and urea level measured 48 h or later postoperatively (17, 22). Taking our feeding schedule in consideration it was not surprising to observe a decrease of plasma triglycerides and urea

levels, since in the early postoperative state changes of these and other plasma levels (including glucose and insulin) are markedly modified by feeding conditions (23).

Nevertheless, under our conditions we found 48 h after the lesion an enhanced plasma insulin level despite normal glucose concentrations and on the basis of these observations we selected this time for investigation of the secretory response of pancreatic B-cells in vitro.

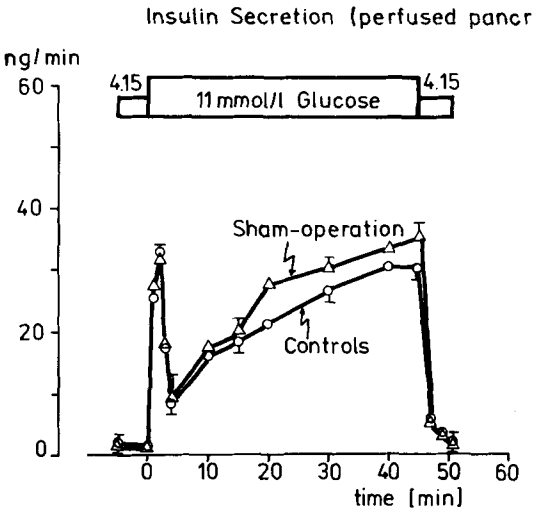

Fig. 3
Glucose-induced insulin secretion of perfused rat pancreas, obtained from control (o—o, n = 15) or sham operated (Δ—Δ , n = 4) animals.

Sham-operation of age- and weight-matched rats did not alter the pancreatic insulin secretion of perfused pancreas (Fig. 3) or of isolated islets (data not shown). Similar results were also found in other groups (28). Therefore nonoperated rat served as controls in the further experimental series.

The enhanced plasma insulin level did not result in any changes of carcass composition (Table 1) or modification of islet's insulin content 2 days after lesions (Table 1).

The insulin secretion in the presence of substimulatory glucose concentrations was not modified in tissue preparation of VMH-lesioned rats (Fig. 4, 5). An enhancement of glucose concentration to 11 mmol/l resulted in the typical biphasic insulin release with a significantly enhanced first and second peak of insulin secretion ($P < 0.01$)(Fig. 4). The dose response-curve of glucose stimulated insulin secretion is characterized by a significantly enhanced hormone release in the presence of glucose concentrations above 6 mmol/l, resulting in a shift of the curve to the left in connection with an enhanced maximal secretion

	Controls (n = 12)	VMH-Lesions (n = 9)
Body Weight(g)	196 ± 4	193 ± 2
Carcass Lipids (g/100g b.w.)	13.3 ± 0.9	12.7 ± 0.6
Carcass Protein (g/100g b.w.)	20.9 ± 0.7	23.1 ± 0.9
Carcass Water (g/100g b.w.)	62.8 ± 0.8	61.8 ± 0.9
Insulin Content (ng/µg d.w.)	70.5 ± 6.3	77.1 ± 6.5

Table 1

Carcass Composition and Insulin Content of Pancreatic Islets 48 h post operationem.

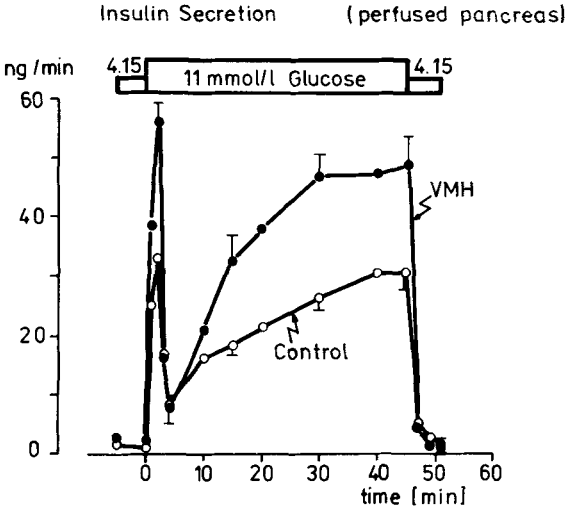

Fig. 4

Insulin secretion by perfused pancreases from control (o—o, n = 15) and 48 h VMH-lesioned (●—●, n = 4) rats. The glucose-induced hormone release is significantly enhanced ($P < 0.01$) in the VMH- experiments at 2, 15, 20, 30, 40, 45 min.

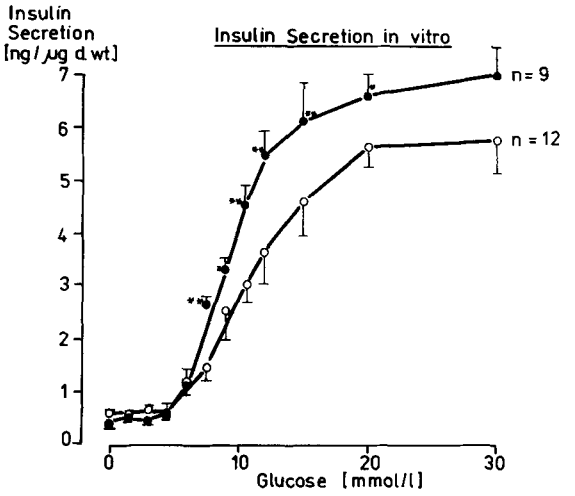

Fig. 5

Glucose-dose-response curve of insulin secretion by pancreatic islets, isolated from control (o—o) or lesioned (●—●) rats (incubation time: 60 min).
* $P < 0.05$,
** $P < 0.01$ in comparison to controls.

rate (Fig. 5). In the presence of atropine the glucose-induced insulin secretion is inhibited (Table 2). The lesion of the VMH-area is also connected with an enhanced secretory response to theophylline (Table 2) but not with that of glyceraldehyde (Table 2).

Secretagogues	Glucose (mmol/l)	Control (n=9)	VMH-Lesion (n = 6)	P
Theophylline (5 mmol/l)	20	+6.4 ± 2.3	+ 12.2 ± 2.0	< 0.05
Glyceraldehyde (10 mmol/l)	1.5	+1.5 ± 0.4	+ 1.5 ± 0.2	n.s.
Atropine (1 µmol/l)	20	-1.5 ± 0.7	- 3.7 ± 0.5	< 0.05

Table 2: Effects of modifiers of insulin secretion (ng insulin in 60 min/µg d.w.) calculated as difference to the corresponding glucose concentration in vitro by means of isolated pancreatic islets.

Furthermore we failed to observe any difference of glucose utilization in islets isolated from VMH-lesioned rats when compared with islets from nonoperated animals (Fig. 6).

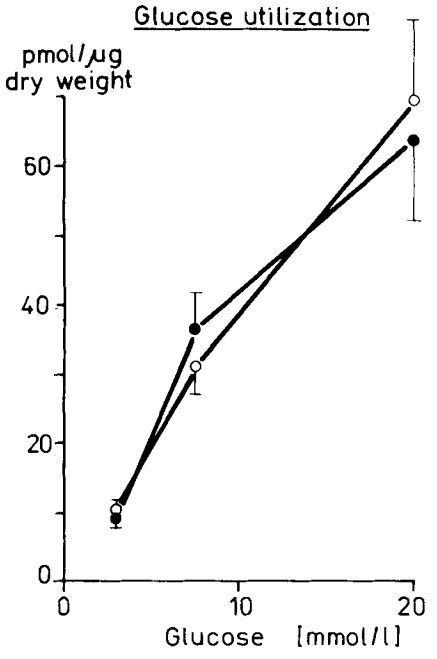

Fig. 6
Glucose utilization of pancreatic islets from untreated (o—o, n = 12) or lesioned (•—•, n = 9) rats within an incubation period of 60 min.

The results obtained demonstrated at first an enhanced insulin secretion at glucose concentration more or less identical to those found postprandial in VMH-lesioned rats. The enlarged secretory response already could be observed two days post operationem under conditions of a restricted food intake and

by using denervated in vitro preparations. Earlier reports had already described an increased glucose-induced insulin output in isolated systems, prepared 7 or more days after the lesions (4,18,28). Our results also demonstrated that the enhanced secretory response is not specific for glucose as a stimulus since the effect of theophylline was also enhanced in islets obtained from lesioned rats. The increased sensitivity of islets from VMH-lesioned rats to secretagogues is neither connected with a modified insulin content or glucose utilization of pancreatic islets, nor found in the presence of glyceraldehyde. Such an increase of the B-cell sensitivity to glucose may well explain the observed enhanced peripheral plasma insulin levels in VMH-lesioned rats with a glycemia of about 7.5 mmol/l (Fig.2, Fig.5).

The observation that the development of hyperinsulinemia due to VMH-lesion could be abolished by a superimposed vagotomie (2, 19,27), supported the concept that the VMH-alteration of pancreatic B-cells is vagally mediated. It is well established that the stimulation of the vagus nerve (20) or the application of cholinergic transmitters (21,24,25) increases insulin secretion in the presence of glucose concentrations near to the stimulus-secretion threshold. The similarities of the B-cell secretion due to VMH-lesions and in response to cholinergic drugs let us propose alterations of the islet's transmitter systems in the operated animals. It is, however, remarkable that the in vitro-systems used maintain their memory for at least 150 min. The glucose stimulated insulin release is only partly inhibited by atropine (29, Table 2) in islets from untreated animals, whereas the enhanced secretory rate of the treated tissue is completely sensitive to atropine. This observation and that of a similar glucose-utilization in both types of islets, the missed effect of glyceraldehyde, which is believed to mediated it secretory effect via glycolysis (16), can be taken as pieces of evidence that glucose may also modify the B-cell secretion via a regulation of nerval structures.

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