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Insulin Secretion and Biosynthesis in Sucrose Fed Rats*

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Summary. Long term feeding of a sucrose rich diet to rats is accompanied by a decreased glucose assimilation rate, despite high plasma insulin levels. Hyperinsulinism is at least partially based on a relative obesity, with increased amounts of abdominal- and retroperitoneal fat tissue, but unchanged total body weight compared to starch fed controls. The secretory pattern of insulin release was studied following glucose, arginine, fructose and sulfonylurea administration in the isolated perfused pancreas of sucrose and isocaloric starch fed rats. In addition, isolated islets of Langerhans were used to demonstrate the effects of glucose on insulin secretion and the incorporation of H-3 leucine into the proinsulin and insulin fraction of islet proteins. Following 11 mM glucose, the dynamics of insulin release in the isolated perfused pancreas of sucrose fed rats is characterized by a markedly elevated, late plateau-like response, usually seen only at higher glucose concentrations. Hyperinsulinism, as compared to starch fed controls, can also be demonstrated following arginine and the sulfonylurea HB-419, whereas fructose has no effect in the presence of low glucose concentrations. During incubation of the pancreatic islets, the hyperinsulinism in sucrose-, compared to starch fed rats, is more pronounced at 11 mM glucose than at 5.5 mM glucose. The incorporation of H-3 leucine into the proinsulin-insulin fraction of islet proteins in sucrose compared to starch fed rats, however, is significantly greater with glucose 5.5 mM than at high glucose level. In sucrose fed rats, secretion and biosynthesis of insulin thus appear to be elevated but closely linked only at physiological glucose concentration.

Key words: Sucrose, increased body fat, isolated perfused pancreas, dynamics of insulin secretion, hyperinsulinism, insulin biosynthesis, isolated islets of Langerhans.

High sucrose concentration in the diet leads to a diabetic glucose tolerance in rats [4], despite elevated levels of glucose-induced insulin secretion [12]. This effect was correlated by some authors with the preferential rate of liver metabolism of fructose compared to glucose [24]. Others, however, considered this as a consequence of the fast postprandial blood sugar rise, resulting in a massive impact of glucose on the carbohydrate regulating mechanisms of the body, in particular the B-cell [3]. Since delayed but increased insulin release and slow glucose assimilation rate are known to be frequently determined by diet composition [8] and obesity [1], we decided to analyze to what extent changes in the diet, in particular a high sucrose content, might affect the body fat content and the quantity of insulin release in rats.

As there is no strict correlation between insulin secretion and biosynthesis [22], further studies, aimed at revealing the connection between both specific processes of the B-cell in sucrose induced diabetic metabolism in rats, were carried out.

Material and Methods

Male Wistar rats (FW-49, Fa. Thomae, Biberach/R) weighing 40–45 g were randomly selected and fed a diet containing, as a percentage of weight, 22% casein, 68% carbohydrate, 2% soya oil and a mixture of mineral salts (6%) and vitamins as given and defined

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by Fa. Altromin in the diet C-1000. Raw corn starch or sucrose were substituted on an isocaloric basis and served as the only carbohydrate component. Body weights were measured weekly.

Following 50 days of the sucrose or starch-rich diets, 10 animals in each group, fasted not longer than 2 hours, were decapitated. The weights of the liver and the total removable fat tissue in the abdominal and retroperitoneal cavities were measured immediately. Samples of liver were taken for lipid analysis. Total liver lipids were extracted in an aliquot of tissue with chloroform/methanol, according to Folch [5].

15 animals of each diet group were fasted for 18 hours and anesthetized by intraperitoneal injection of 50 mg/kg pentobarbital. The pancreas was isolated without any adjacent organs such as stomach or spleen. Arterial flow was achieved by cannulation of the aorta [11]. The perfusate was freely collected from the portal vein, without recirculation, at intervals of 1–2 min. The perfusion fluid consisted of an Umbreit buffer (pH 7.35) supplemented with 1% albumin and equilibrated with 95% O_2 and 5% CO_2. A constant flow rate of 2.5 ml/min was achieved by a pressure of about 40 mm Hg. The temperature was adjusted to 38° C. The stimulating substances – glucose, 2.75 mM; arginine-monohydrochloride, 8.25 mM; fructose, 11 mM; and sulfonylurea HB-419, 1 μg/ml (Fa. Boehringer, Mannheim) were infused for various periods of time.

Insulin secretion was also tested in islets of Langerhans. The pancreas of decapitated rats was removed and cut up with scissors in chilled Krebs-Ringer bicarbonate buffer, pH 7.4. Digestion, using 40 mg collagenase (Serva, Heidelberg, Germany), was carried out under constant stirring at 37° C for 17–25 min [11]. After 3 washings in chilled buffer, intact islets of comparable size were collected. 25 islets were incubated for 3 hours in the presence of glucose 2.75, 5.5 and 11 mM glucose. Insulin secretion was estimated hourly in 2×0.01 ml of the incubation media by radioimmunoassay [18].

To measure insulin biosynthesis, batches of 25 islets were incubated in the presence of L-leucine-4.5-H-3(50 μCi/mole, Radiochemical Center, Amersham) for 3 hours in 1 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) supplemented with bovine serum albumin (2 mg/ml), Trasylol, 1000 KIU/ml (Bayer, Leverkusen) and 17 naturally occuring amino acids (20 μg/ml of each amino acid, leucine excluded). Glucose was present in the incubation media at concentrations of 2.75, 5.5 and 11 mM. Incubations were carried out under a constant gas phase of $O_2:CO_2$ (95 : 5% v/v), using a metabolic shaker, at 37° C. After incubation of 0–3 hours aliquots of the incubation media were taken for double determination of immunologically measurable insulin. After 3 hours ice cold trichloroacetic acid was added to the samples, yielding a final concentration of 10 per cent. Ultrasonic disintegration of the precipitated proteins was carried out for 15 seconds. The islet proteins were separated on a Sephadex G 50 fine column, 1.2×55 cm, which had been equilibrated with 1 mM acetic acid and calibrated with albumin, proinsulin, insulin and leucine. Elution was done with a flow rate of 10 ml per hour. The recovery of the separated islet proteins after chromatography, as judged by added labelled insulin, was over 90%. Fractions of 1 ml were collected. Radioactivity, as well as immunoassayable insulin, was determined in each fraction. The proinsulin and insulin nature of the peaks was further assayed by polyacrylamide gel electrophoresis, pH 8.6, 15% gel.

Following homogenisation, the insulin content of the various batches of islets was determined by radioimmunoassay. No significant differences, however, were found among the diet groups. Thus direct comparison was made between the amount of radioactivity incorporated into the different peaks of the islet proteins as well as between the secretion data from the pancreas of sucrose and starch fed animals. A detailed description of the methods for studying secretion and biosynthesis of insulin, using isolated islets, has been given elsewhere [21, 22].

Student's t test was used for carrying out the calculations of significance.

Results

Feeding of sucrose or isocaloric, starch rich diets over a 50 day period resulted in no significant difference in total body- and liver weight (Table 1). Abdominal and retroperitoneal fat tissue, however, was markedly increased in sucrose fed rats. In addition, the extractable liver fat content was higher following the feeding of sucrose, compared with an isocaloric, starch rich diet.

The perfusion of the isolated rat pancreas was characterized by the well known pattern of a biphasic insulin release, where a high initial insulin output is followed by a short fall and another increase to a steady plateau within 25 min. While there was no significant difference in the altitude of the first peak in the diet groups, the later response was markedly elevated in sucrose fed rats, thus leading to a pronounced hyperinsulinism compared with starch fed animals (Fig. 1).

Hyperinsulinism in sucrose-fed rats is not a typical effect of glucose stimulation per se, but can also be evoked by arginine and sulfonylurea HB-419. In the presence of arginine, superimposed on a low glucose

concentration, insulin release appeared to be significantly greater in sucrose fed rats than in starch fed controls. Fructose had no apparent effect on insulin release, in the presence of glucose 2.75 mM, in sucrose and starch fed rats, while HB-419 stimulated insulin release in both diet groups, more pronounced, however, in sucrose fed rats (Fig. 2).

Following a prolonged period of stimulation by arginine, there was a distinct waning after 20 min stimulation in both diet groups, despite continous arginine infusion up to 44 min (Fig. 3).

Glucose induced hyperinsulinism in sucrose fed rats could also be demonstrated by incubating isolated islets of Langerhans (Fig. 4). While there was no significant difference at a low glucose concentration (2.75 mM), insulin release was markedly elevated during 3 hours of incubation when the islets of sucrose, compared to starch fed rats, were exposed to a glucose concentration of 5.5 and 11 mM.

No difference was found in H-3 leucine incorporation into the proinsulin and insulin fractions between islets from sucrose and starch fed animals at 2.75 mM glucose. At 5.5 mM glucose, incorporation of H-3 leucine into the insulin fraction was significantly higher in islets of sucrose fed rats. At 11 mM glucose, both proinsulin and insulin fractions of the islets from sucrose and starch fed animals were further increased. However, the difference between diet groups in terms of biosynthesis was reduced compared to glucose 5.5 mM (Table 2). The total insulin content of the islets in sucrose and starch fed rats, however, was not significantly different (Table 1).

Table 1. The effect of sucrose- or starch-rich feeding on body weight, fat tissue and insulin content of isolated islets of Langerhans (Mean ± SEM,* = $p < 0.05$)

	Sucrose	Starch
	(N = 10)	(N = 10)
Body weight (g)	301 ± 11	310 ± 12
Liver weight (g)	10.9 ± 1.0	10.1 ± 0.9
Fat tissue (g)	8.9 ± 0.6*	7.1 ± 0.3
Liver fat (% dry wt)	11.2 ± 0.6*	8.0 ± 0.4
Insulin content of 25 islets of Langerhans (mU IMI)	17.6 ± 0.58	16.7 ± 0.46

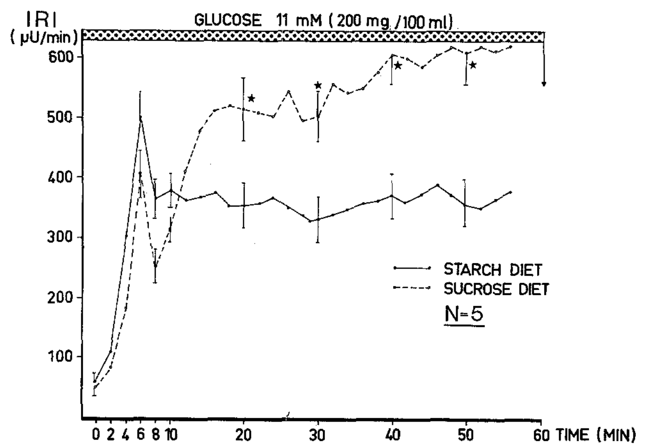

Fig. 1. Glucose-induced insulin release in the isolated perfused pancreas of rats fed a high sucrose or isocaloric starch-rich diet (the vertical bars indicate + SEM;* = significantly different with $p < 0.05$)

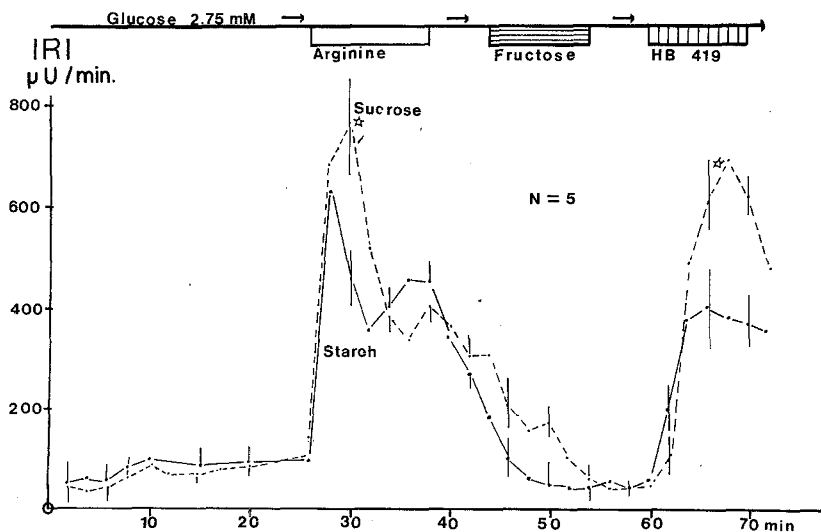

Fig. 2. Insulin release in the isolated perfused pancreas in sucrose or starch fed rats following a continous stimulation with glucose (2.75 mM), and an intermittent stimulation with arginine (8.25 mM), fructose (11 mM) and HB-419 (1 μg/ml). Significantly different values are marked by an asterisk*

Table 2. Radioactivity (total counts per min, mean values ± SEM) incorporated into 3 fractions of islet proteins in sucrose and starch rich fed rats, at glucose concentration of 2.75; 5.5 and 11.0 mM. Significant differences between both diets are indicated by asterisks:* $p < 0.05$

	Sucrose	Starch
Glucose 2.75 mM	(N = 5)	(N = 5)
Other proteins	cpm: 6170 ± 1115	7726 ± 376
Proinsulin	541 ± 109	676 ± 85
Insulin	379 ± 60	392 ± 25
Glucose 5.50 mM	(N = 5)	(N = 5)
Other proteins	cpm: 7938 ± 467	8721 ± 1896
Proinsulin	1918 ± 338	1531 ± 432
Insulin	3153 ± 376	1834 ± 198*
Glucose 11.0 mM	(N = 5)	(N = 5)
Other proteins	cpm: 11602 ± 1897	10542 ± 1417
Proinsulin	3645 ± 360	3087 ± 277*
Insulin	6805 ± 767	5672 ± 319*

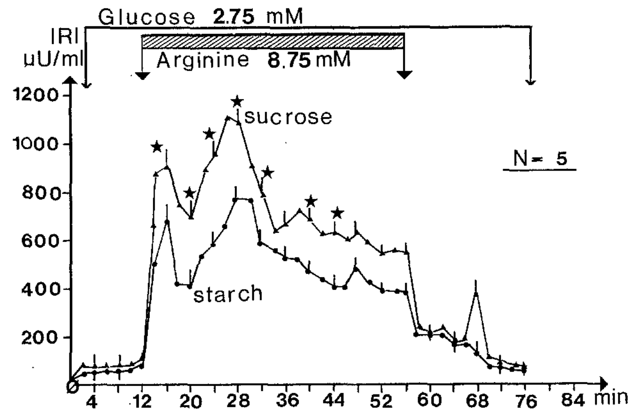

Fig. 3. Insulin release of the isolated perfused pancreas of rats following a sucrose or starch rich diet, stimulated with arginine (8.25 mM) superimposed on a glucose concentration of 2.75 mM (The vertical bars indicate ± SEM, $p < 0.05$)

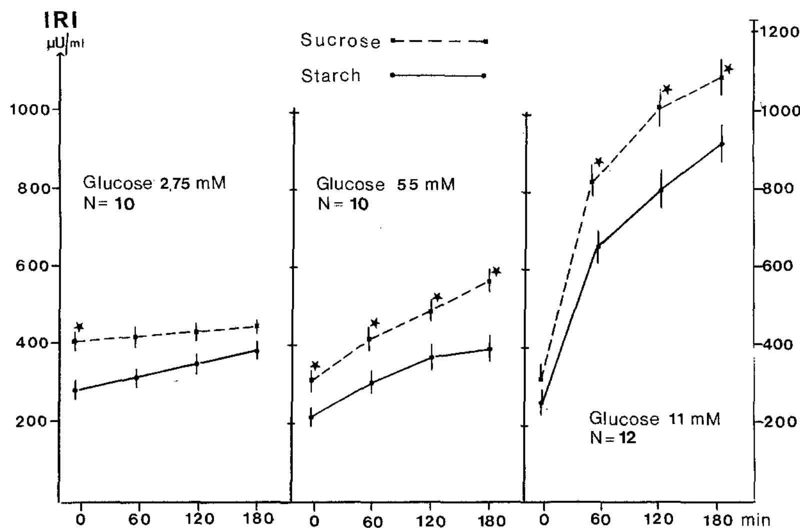

Fig. 4. Insulin release in isolated pancreatic islets from sucrose and starch fed rats (the vertical bars indicate ± SEM,* = $p < 0.05$)

Discussion

The present study demonstrates that sucrose feeding in rats results in a hyperinsulinism of the isolated perfused pancreas and of incubated islets of Langerhans. The exact reason for the hyperreactivity of the pancreatic B-cell is yet unknown. Hyperinsulinism, however, is regularly found in obesity. Sucrose feeding for 50 days is accompanied by a state of relative obesity, where the fat tissue in the abdominal and retroperitoneal cavities and the total carcass fat [2, 17] is significantly enhanced, while the body weight is not markedly increased. A preferential rate of sucrose conversion into abdominal fat [15] and liver lipids was also noted by Cohen and Teitelbaum [4]. In addition to the fact that starch and sucrose are metabolically not identical [20], MacDonald and Turner [16] suggested, that the "rate of presentation" of a carbohydrate, including the speed of chewing, swallowing and digestion, influencing the extent of postprandial hyperglycaemia, might be *the* determining factor in inducing the increased activity of lipogenic enzymes, leading to enhanced fat deposits. The additional fat tissue might effectively contribute to the elevated secretion of insulin in sucrose fed rats by way of a reduction of the number of insulin receptors, as usually found and recently described in acquired obesity [25].

The overall magnitude of insulin response to the same intravenous glycaemic stimulus is markedly con-

ditioned by the antecedent carbohydrate intake. High carbohydrate diet stimulates the islets, in particular the late insulin output [9], significantly more than an ordinary diet. Regarding the biphasic pattern of insulin release, similar effects were seen in both sucrose fed and starch fed rats.

It appears characteristic that the hyperinsulinism is not only found after glucose stimulation, but can also be observed following arginine and sulfonylurea. In sucrose fed rats, arginine-induced insulin release was markedly higher compared to starch fed controls and included the first and second part of the biphasic release pattern in the presence of low glucose levels. Despite continous infusion, waning of arginine effects was striking under both experimental conditions. This appears to be consistent with observations suggesting a difference in the mechanism of action of glucose and arginine on insulin release [7, 14] and biosynthesis [21]. Both substances, however, seem to interact in a common phenomenon which controls insulin release [14].

Although newly synthesized insulin is not directly responsible for the second phase of the isolated perfused pancreas [27], Fujita and coworkers [6] suggested that, following high carbohydrate intake, the late hyperinsulinism is an expression of the hormonal biosynthetic chain in a supernormal state of activity and that insulin-synthesis, storage and release do not exist as functional separate compartments, but as a single process, conditioned by the previous activities. Insulin secretion and biosynthesis, however, do not always appear to be directly linked [22]. Although glucose affects both [10], there is no apparent and detailed information concerning the influence of diet composition on glucose stimulated insulin biosynthesis and its correlation to hypersecretion. Only fasting for 24–72 hours is known to reduce insulin secretion and insulin biosynthesis in the presence of glucose 3 mg/ml [26].

In both sucrose and starch fed animals there is no effect of 2.75 mM glucose on insulin secretion and biosynthesis (Table 2). At 5.5 mM glucose, insulin secretion seems to be more pronounced than biosynthesis in the sucrose fed rats. According to Morris and Korner [19], 5.0 mM glucose appears to be a threshold for the stimulation of insulin biosynthesis, whereas secretion can be induced at lower glucose concentrations. At 11 mM glucose incorporation of tritiated leucine into islet protein is significantly higher than at glucose 5.5 mM. This agrees with the findings of Lin et al. [10], who showed that the biosynthesis of proinsulin and insulin was maximally stimulated only by high glucose concentrations. The difference between sucrose- and starch-fed rats, however, appears to be small. Calculating the specific, glucose-induced insulin synthesis as per cent of total protein synthesis, 5.5 mM glucose caused, in sucrose fed rats, 39% of all protein-incorporated leucine counts to be located in the insulin-proinsulin region, while this figure was only 28% for starch fed controls. At 11 mM glucose, however, there is no gross difference between the two diet groups in terms of biosynthesis. While sucrose fed rats incorporate 47% of all leucine counts into the insulin-proinsulin fraction, 45% are found in the starch fed controls.

Since the blood sugar level is a characteristic factor in regulating insulin biosynthesis, one possible explanation that the differences between both diet groups are only significant at physiological levels, might be that the postprandial blood glucose in starch and sucrose fed rats though different, stays well within physiological limits. In sucrose fed animals, secretion and biosynthesis of insulin thus appears to be elevated and closely linked only at normal blood sugar concentrations.

The mechanism involved in the high insulin output and accelerated insulin biosynthesis in sucrose fed rats is probably mainly based on the fast rise and excessive postprandial hyperglycaemia and supported by the delayed glucose assimilation rate.

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