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Effects of Streptozotocin on Liver Composition and Blood Glucose, Ketone Bodies and Insulin in the Fed and Fasted Male Rat[1]

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Abstract. Three days after a single injection of streptozotocin rats showed hyperglycemia, hyperketonemia and hypoinsulinemia. Body and liver weights were reduced and the concentration of DNA-P, phospholipid-P, proteins and acetyl-CoA in the liver was augmented, while the concentration of glycogen and citric acid in these animals compared with the controls which did not receive the drug was decreased. After 48 h starvation, blood glucose remained higher in the streptozotocin-treated animals, while circulating ketones and insulin were not different from those in the controls. With the exception of body and liver weights, which were lower, and of liver DNA-P, which was higher than when fed, neither of the other parameters studied in the streptozotocin treated animals changed with fasting, while the response in the controls was normal. The incapacity of increasing the postprandial insulin secretion in these animals may contribute to the metabolic alterations found in the fed state.

Key Words
Streptozotocin
Diabetes
Glycemia
Ketonemia
Insulinemia
Liver
Acetyl-CoA
Citric acid

Introduction

While a great deal of attention has been paid to the metabolic alterations in the diabetic subject, there is only sparce information about the fine regulatory mechanisms which are working in such a situation. Hepatic gluconeogenesis, ketogenesis and lipogenesis are the most altered pathways in diabetes. These pathways may be influenced by the regulatory effects which the content of acetyl-CoA and citric acid [17, 20] can exert upon

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certain enzymes. The present study was designated to examine these interrelationships in rats made diabetic by the administration of streptozotocin. This N-nitrosourea derivative of glucosamine exerts its cytotoxic action on pancreatic β-cells [22, 26], while the α-cells and the exocrine pancreatic tissue remain intact [13, 21] and does not show any evidence of tissue damage throughout.

Most of the studies published so far on the metabolic effects of streptozotocin have been done on starved animals, but adequate characterization of these interrelationships required observation in both fed and fasted states. As great alterations are observed in the size and content of the hepatocytes during starvation in normal conditions [10, 11], the study was extended to determine liver cellularity and intrinsic composition in fed and 48-hour starved rats.

Methods

Animals. Male Wistar rats fed on a standard pellet diet (fat, 3.8%; carbohydrate, 49.5%; protein, 21.4%) were housed two per cage and divided into two groups. A group was made insulin-defficient by a single intraperitoneal injection of streptozotocin (kindly supplied by Dr. W. E. DULIN, The Upjohn Co., Mich.) (65 mg/kg) in 0.5 ml citrate buffer (0.01 M) (pH 4.5) within 5 min after it was put in solution. Another group was injected with 0.5 ml of the same buffer and taken as controls. Fasting animals were given unrestricted access to drinking water. The animals were killed by decapitation and without anesthesia 72 h after the injection between 10 a.m. and noon. Blood was collected from the neck of the animals into heparinized chilled beakers and a piece of liver was removed and, in less than 10 sec after decapitation, was placed in liquid N_2.

Blood components. An aliquot of blood was immediately deproteinized [25] and analyzed for glucose [12] and total ketone bodies [2]. Immunoreactive insulin was measured in the plasma [8] by means of the radio-insulin kit from the Radiochemical Center, Amersham. Rat insulin (Novo, Copenhagen) was used as standard.

Analysis of liver. Aliquots of the liquid N_2 frozen liver were analyzed for protein [18], glycogen [7], phospholipid phosphorus [5], DNA-phosphorus [24], total fatty acids [3], acetyl-CoA [9] and citric acid [19], as described elsewhere [1].

Results

At the onset of the experiment rats of the same weight were divided into two groups (178.5 ± 5.6 and 167.6 ± 6.5 g body weight, NS), the first

group received 65 mg/kg of streptozotocin and the second group received the buffer and were taken as controls. After 3 days the animals were sacrificed; by this time the controls had increased their body weight while the rats treated with streptozotocin remained at the same weight as at the time of injection (table I), the difference between the groups being statistically significant. This might be partially due to the reduced food intake of the streptozotocin-treated rats, although on the third day after the treatment, the amount eaten by both groups was not significantly different (25.5 ± 1.5 vs. 22.8 ± 1.2 g of food eaten/rat/day, NS, of 16 controls vs. 8 rats treated with streptozotocin). When the animals were fasted for 48 h before the sacrifice, their weights were the same in both groups (table I) which means that fasting produces a smaller fall in the body weight of the streptozotocin-treated animals than in the controls. Liver weight followed a similar pattern as body weight (table I): it was smaller in the streptozotocin-treated animals and when the rats were fasted for 48 h the difference between the groups disappeared because of the smaller decrease in this group versus the controls. When the weights of the livers were calculated per 100 g body weight there was no difference between the fed groups while in the fasted streptozotocin-treated rats they were heavier than in their respective controls ($p<0.01$).

Differences in liver weight prompted a study of some index of cellularity, thus DNA-phosphorus was determined (table I). The concentration of DNA-phosphorus was higher in the streptozotocin-treated animals than in the controls but the differences disappeared when calculated per whole liver, suggesting a preservating of the total amount of cells. The concentration of DNA-phosphorus increased with fasting in both groups, as on other occasions [1, 10, 11], and the differences between them were maintained as when fed. Phospholipid-phosphorus and proteins per gram of liver were higher in the fed streptozotocin-treated animals than in the controls. After 48 h of starvation the phospholipid-phosphorus and proteins per gram of liver in the streptozotocin group were the same as when fed, while in the controls they increased in such a way that the differences between the two groups disappeared with fasting (table I). Liver total fatty acids followed a similar pattern, being slightly higher – but not statistically significant – in the streptozotocin-treated animals than in the controls when both were fed. Fasting made liver total fatty acids increase in the controls, and this parameter did not change in the streptozotocin-treated animals in such a way that the difference with the controls disappeared (table I), as is the case with the other parameters studied. Liver glycogen was 5.8 times lower in the rats treated with streptozotocin than in the controls, when both were fed. 48 h

Table I. Effect of streptozotocin injection on body and liver weights and liver composition in fed and 48-hour starved rats[1]

	Controls			Streptozotocin		
	fed	p	48-hour starved	fed	p	48-hour starved
Body weight, g	202.7 ±11.4	<0.01	158.0 ± 4.4	170.1 ± 4.4	<0.05	153.0 ±5.8
p′				<0.02		NS
Liver weight, g	8.15± 0.51	<0.001	4.95± 0.11	6.65± 0.40	<0.02	5.33±0.27
p′				<0.05		NS
Liver weight/100 g body weight, g	4.04± 0.12	<0.001	3.14±0.08	3.89± 0.15	NS	3.54±0.09
p′				NS		<0.01
Liver DNA-phosphorus, μg/g	182.8 ± 6.8	<0.01	234.4 ±13.3	208.5 ± 9.6	<0.001	273.5 ± 8.0
p′				<0.05		<0.01
Liver phospholipid-phosphorus, mg/g	1.27± 0.04	<0.01	1.49± 0.05	1.45± 0.03	NS	1.48±0.03
p′				<0.01		NS
Liver total fatty acids, μmol/g	161.7 ± 7.0	<0.05	196.1 ±12.5	183.9 ±12.6	NS	181.4 ±8.8
p′				NS		NS
Liver proteins, mg/g	152.0 ± 3.7	<0.001	182.8 ± 4.1	191.6 ± 4.4	NS	181.9 ±2.4
p′				<0.001		NS
Liver glycogen, %	4.57± 0.22	<0.001	0.43± 0.03	0.79± 0.15	NS	0.63±0.06
p′				<0.001		<0.05

[1] Rats received a single intraperitoneal injection of streptozotocin (65 mg/kg) and were killed 3 days later. Pieces of liver were frozen within 10 sec in liquid N_2. The results are given as means ± SEM of 7–8 animals/group. P corresponds to the differences between fed and 48-hour starved groups and p′ to the difference of each streptozotocin group with the controls under the same dietary status. NS, not significant, i.e. p or p′ >0.05.

of starvation made the liver glycogen in the controls fall while in the streptozotocin-treated animals it did not change, being higher than in the controls (table I).

The levels of finer metabolites in the liver are summarized in table II. Acetyl-CoA in the liver of the animals treated with streptozotocin, when they were fed, was higher than in the controls. Fasting produced an increase in the liver steady state of this metabolite in the controls as on other occasions [1, 10, 11], while it did not change in the rats injected with streptozotocin; the differences between both groups disappearing. The citrate level was lower in the liver of the fed streptozotocin-treated animals than in their controls. 48 h of starvation made this parameter fall in the liver of the controls, as normally happens [1, 10, 11], while it did not change in the liver of the animals treated with streptozotocin. Thus, here again the differences between both groups disappeared with fasting.

These metabolic alterations in the liver were accompanied by changes in the circulating metabolites, as summarized in table III. Blood glucose was 3.1 times higher in the rats treated with streptozotocin than in their controls when fed and although it decreased in both groups with fasting, the differences between them was maintained (table III). Blood ketone bodies were also higher in the fed streptozotocin animals than in their controls, but with fasting they did not change in the first group while they increased in the second, and thus the difference between the groups disappears after 48 h starvation. The levels of plasma immunoreactive (IRA) insulin in the experimental groups are also shown in table III. As expected, the streptozotocin-treated rats had lower plasma IRA insulin than their controls, when both were fed. Fasting produced a decrease in the IRA insulin in the controls while it did not change in the streptozotocin animals, there being now no difference between both groups.

Discussion

We have observed here that, as described by others [4, 22, 26], the administration of streptozotocin to experimental animals is diabetogenic, as shown by the hyperglycemia, the hyperketonemia and the hypoinsulinemia found in the rats treated with the drug. We have also shown here that these alterations partially disappear with a 48-hour starvation. An attempt has been made to attain a better understanding of the factors contributing to these manifestations.

Table II. Effects of streptozotocin injection on the steady state of acetyl-CoA and citrate in the liver of the fed and the 48-hour starved rat[1]

	Controls			Streptozotocin		
	fed	p	48-hour starved	fed	p	48-hour starved
Acetyl-CoA, nmol/g	52.8 ±2.9	<0.05	65.2 ±4.1	63.9 ±3.7	NS	57.2 ±3.0
p′				<0.05		NS
Citric acid, μmol/g	0.340±0.043	<0.02	0.211±0.020	0.190±0.039	NS	0.229±0.025
p′				<0.05		NS

[1] Conditions of the animals, details of their sacrifice and statistical comparisons as in table I.

Table III. Effect of streptozotocin injection on circulating components in the fed and the 48-hour starved rat[1]

	Controls			Streptozotocin		
	fed	p	48-hour starved	fed	p	48-hour starved
Blood glucose, mg/100 ml	101.8±3.7	<0.001	66.0± 2.4	314.3±42.7	<0.01	178.5±39.7
p′				<0.001		<0.02
Blood ketone bodies, μmol/l	117.3±5.6	<0.001	574.6±34.6	335.4±71.7	<0.02	616.0±73.0
p′				<0.02		NS
Plasma IRA insulin, μU/ml	71.7±5.6	<0.001	31.0± 2.6	32.7± 2.9	NS	26.1± 4.8
p′				<0.001		NS

[1] Conditions of the animals, details of their sacrifice and statistical comparisons as in table I.

Rats fed *ad libitum* showed, after 72 h of treatment with streptozotocin, alterations in their livers similar to those found normally in the fasted animals [1, 10, 11], as shown by the decreased body and liver weights and liver glycogen, the maintenance of liver cellularity and the increased phospholipids and total fatty acid concentration in the liver. These changes were also accompanied by alterations in the steady state concentration of regulatory metabolites in their livers. Acetyl-CoA, the allosteric activator of pyruvate carboxylase [15], was increased in the rats made diabetic with streptozotocin, probably as consequence of an enhanced oxidation of fatty acids. The removal of acetyl-CoA via the formation of citrate seemed to be attenuated in the streptozotocin-treated rats, as suggested by their reduced concentration in the liver of this metabolite, while it was shifted into the formation of ketone bodies which were increased in the blood of these animals. This ketosis in animals having received streptozotocin seems in apparent contradiction with the difficulties of other authors in obtaining elevated levels of blood ketone bodies in these animals [13, 23], but in those studies they used fasted rats only while in the present work we have found ketosis in fed animals in a condition where insulin depletion is most marked and accelerated mobilization of stored fat must be maximal. The fall in liver citrate and increase in blood ketones could be induced by the concomitant effects of an increased β-oxidation of fatty acids and a fall in the oxaloacetate concentration of the liver probably due to the rapid conversion of this metabolite into phosphoenolpyruvate and subsequently to glucose. Actually, a decrease of oxaloacetate concentration in the liver has been found in the severe ketosis of the alloxan diabetic rat [27]. All these metabolic alterations in the fed animals treated with streptozotocin seemed to be primarily induced by a decreased function of the pancreatic β-cells [22, 26], which was the cause of the low circulating levels of insulin. Actually, these changes were similar to those found in the fasted control rats, as seen here and on other occasions [1, 10, 11] where insulin circulating levels are also low. The fact that several of these alterations in the fasted animals disappeared a few minutes after the administration of insulin could be in agreement with this interpretation [6, 10]. The possibility exists too, that the observed changes in the streptozotocin-treated rats were also facilitated by parallel variations in other endocrine sites such as an elevation of circulating glucagon levels, as reported by KATSILAMBROS *et al.* [14] after the treatment with streptozotocin *in vivo*, although this was not confirmed under *in vitro* conditions [16] and deserves further investigation. The concomitant effects of low insulin and high glucagon levels would also be inducing not only a hightened hepatic gluco-

neogenesis and decreased peripheral glucose utilization but also an enhanced glycogenolysis, all of which would be contributing to the hyperglycemia and the low hepatic glycogen concentration found here in the fed rats treated with streptozotocin. We believe that neither of these alterations found in the streptozotocin animals was induced by a reduced food intake, because at the time of the sacrifice, the intake in these animals was the same as that in the controls.

Fasting altered the whole metabolic picture in the controls in the expected direction, as shown and discussed previously [10, 11]. However, most of the parameters studied here were slightly or not changed with fasting in the diabetic group in such a way that differences with the respective controls were aminorated or even disappeared. We do not yet have enough experimental support to understand completely this lack of response to fasting in the streptozotocin-treated animals but the possibility exists that this is the consequence of the unchanged circulating IRA insulin with fasting in these animals. This was also observed by JUNOD *et al.* [13] in fasted animals having received intravenous streptozotocin doses of 65 mg/kg or less. Thus, circulating insulin concentrations are relatively fixed in the streptozotocin-treated animals at a level achieved by the controls when fasting, where gluconeogenesis must be activated to sustain glucose-requiring tissues during starvation. The turn-on failure in fed streptozotocin-treated rats due to the cytotoxic action of the drug on the pancreatic β-cell may yield the overproduction of glucose postprandially and contribute to the hyperglycemia. On the other hand, the fact that blood glucose and liver glycogen were higher in the fasted streptozotocin-treated animals than in their controls in the presence of equally circulating IRA insulin levels, would suggest a different biological activity of the hormone, a point which needs further investigation.

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