Exp. Clin. Endocrinol. Vol. 93, No. 2/3, 1989, pp. 225–230 VEB J. A. Barth, Leipzig

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Spontaneous Recovery of Streptozotocin Diabetes in Mice

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With 3 Figures

Summary. With the aim of developing a model of experimental diabetes by which spontaneous recovery processes can be investigated, we used a lower (1) and a higher (2) dose of streptozotocin (SZ) to find out the lowest possible dose definitely inducing diabetes in mice through beta cell loss but preventing excessive damage to the endocrine pancreas which would exclude restoration processes.

After application of SZ (1) to neonatal mice only male animals showed an overt diabetes in adult life. 70 percent of these mice had recovered 15 weeks after appearance of diabetes. Recovery was indicated by normalization of blood glucose, serum insulin, insulin secretion and biosynthesis of isolated pancreatic islets and a reenhancement of the pancreatic insulin content from lower than 10 to 30 percent of control values. After SZ (2) both sexes became hyperglycaemic, and the recovery rate was lower, but was increased by pregnancy in female mice. By means of this model it will be possible to investigate mechanisms and promoting factors of such restoration processes in more detail.

Key words: Recovery — Streptozotocin — Diabetes — Mice

Introduction

Only some cases of a spontaneous and long lasting recovery from severe insulin dependent diabetes mellitus in humans and from drug induced diabetes in animals are known. So Sodoyez-Goffaur and Sodoyez (1977) and Gentz et al. (1969) observed a transient diabetes mellitus in few neonates and recently was shown that rats with Cyclosporin-induced hyperglycaemia were able to recover spontaneously from a diabetes-like syndrome (Hahn et al., 1987). An amelioration of a streptozotocin (SZ)-diabetes of rats was found by the groups of Portha (Giroix et al., 1983) and Weir (Giddings et al., 1985) but after a recovery phase in the neonatal stage these rats developed non-insulin-dependent diabetes in adult life.

The regeneration processes involved can be studied in more detail using an animal model with reproducible drug induced diabetes. But it is difficult to destroy the pancreatic beta cells by drugs to such a degree that diabetes will be produced with certainty but that damage to the endocrine pancreas will not be as severe as to exclude restoration processes. We used two rather low doses of SZ to find out the lowest possible dose definitely inducing diabetes in mice.

Materials and Methods

Neonatal BALB/c mice, 5–7 days old, were given intraperitoneal SZ (Serva, FRG) injections of either SZ (1) = 50 or SZ (2) = 100 mg/kg b. w. The control groups received citrate buffer (10 mM; pH 4.2).

Blood samples were obtained after decapitation or from the retroorbital plexus of fed animals and blood glucose (FERMOGNOST Blutzucker Test, GDR) and serum insulin by a micro modification (Besch et al., 1987) of RIA (rat insulin standard R 171, NOVO, Denmark) were estimated.

The whole pancreas or only the ventral part of the tissue was prepared, immediately frozen, weighed and stored at −20°C until extraction for estimation of the pancreatic insulin content. The extraction was carried out with acidic ethanol (Ziegler et al., 1985a) using glass potter homogenisators with two different luminaries. Insulin content of the extract was analysed by RIA (Besch et al., 1984).

Pancreatic islets were isolated from the whole pancreas or the dorsal part of the tissue. The source of the used pancreas (whole, ventral or dorsal part) was always the same for the treated and for the control groups of mice, which should be compared regarding the pancreas insulin, the insulin secretion or biosynthesis of isolated islets.

The digestion of the pancreas was carried out according to Lernmark et al. (1981) with collagenase (1 mg/ml Hanks solution). Insulin release and insulin biosynthesis of pancreatic islets were estimated by incubating batches of 5 islets of similar size at 37°C for 2 h with 370 kBq ^{3}H-Leucin (Rossendorf, GDR) in 200 µl of a modified KRB, supplemented with 16 mM HEPES, 1 mg/ml bovine serum albumin and either 6 or 15 mM glucose. Insulin release and insulin content of the sonificated islets were determined by RIA (Besch et al., 1984). Insulin biosynthesis of the islets was estimated by incorporation of ^{3}H-Leucin into (pro)insulin using a double antibody method (Jahr et al., 1980) and LSC-measuring.

The results are given as mean values ±SD of n samples investigated. Statistical analysis was performed using Student's t-test.

Results

From Table 1 we can see that already 1 day after SZ (1) application the pancreatic insulin content is strongly diminished and the serum insulin concentration is increased, though the latter difference is not significant because of the great SD-values.

Six weeks after SZ (1) application almost only male mice developed hyperglycaemia with glucose values averaging 20 mmol/l. The pancreatic insulin content of these male mice is lower than 10% of control values and the serum insulin is decreased. The hyperglycaemic male mice progressively recovered and 70% of these mice reached approximate normoglycaemia 21 weeks after SZ application (Fig. 1). Eight weeks later the serum insulin values of these male mice were normal again and the pancreatic insulin content increased up to 30% of control values. At this time the pancreatic insulin content of female mice amounted to 70% of control values (Table 1). The insulin content of pancreatic islets of male and female mice was still diminished, but insulin biosynthesis (data not presented) and insulin secretion of islets were also back to normal (Fig. 2). Using the SZ (2) dose, both sexes became diabetic 4 weeks after application. The blood glucose values, averaging 26 mmol/l, were higher than after the SZ (1) dose and the recovery rate was much lower; only 30% of the female and 17% of the male diabetic mice reached approximately normal blood glucose values 11 weeks after SZ (2) application. Pancreatic insulin content of the non mated female SZ-group was still below 10% of the controls. Female diabetic mice which were mated about 4 weeks after SZ application and delivered living pups reached a pancreatic insulin content of 60% of normal values, whereas the pancreas insulin of mice which delivered dead pups remained on the low level (Fig. 3.)

Table 1 Blood glucose, pancreatic and serum insulin of mice after application of 50 mg streptozotocin per kg b. w. up to 29 weeks

Time after SZ	Sex	Group	Blood glucose mmol/l	Pancreatic insulin pmol/mg	Serum insulin pmol/l	n
1 d	—	C	5.2 ± 0.6	69.5 ± 15.3	106.2 ± 21.0	13
1 d	—	SZ	5.7 ± 0.9	36.0 ± 14.4***	169.0 ± 82.8	(9–14)
6 W	♂	C	7.1 ± 0.3	26.4 ± 0.8	205.9 ± 150.3	10
6 W	♂	SZ	20.2 ± 8.0**	2.22 ± 0.02***	102.0 ± 43.0*	(5–17)
29 W	♂	C	5.7 ± 1.6	27.4 ± 7.6	153.5 ± 72.5	7
29 W	♂	SZ	5.2 ± 1.9	8.7 ± 3.1***	140.0 ± 71.4	(4–11)
29 W	♀	C	6.3 ± 1.4	24.2 ± 12.7	200.6 ± 92.1	11
29 W	♀	SZ	5.3 ± 1.2	16.7 ± 8.2	200.0 ± 91.0	(8–14)

C = Controls; SZ = Streptozotocin; * $p < 0.025$, ** $p < 0.005$, *** $p < 0.001$.

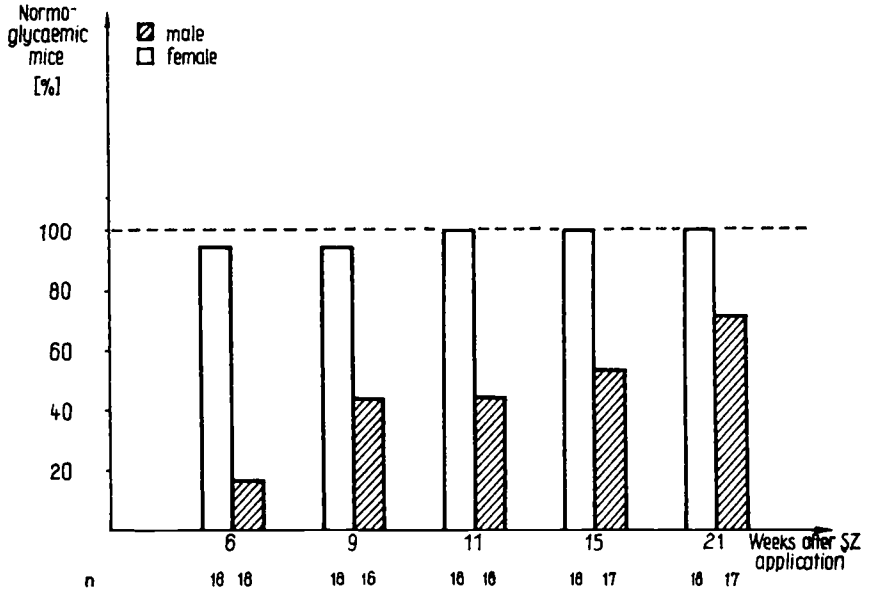

Fig. 1 Percentage of male and female mice with approximately normal blood glucose values (within 3 D range of age matched controls) up to 21 weeks after application of 50 mg streptozotocin (SZ)/kg b. w.

Discussion

The lowest SZ dose applied by us caused a marked decrease of the pancreatic insulin content already 1 day after application (Table 1). After a short intermediate recovery phase in the neonatal stage (Hartmann et al., 1986) diabetes developed in adult life, but almost only in male mice. The male sex seems to be more sensitive to the toxic effect of the drug (Kromann et al., 1981). Six weeks after SZ application the pancreatic insulin content is lower than 10% of control values and the decreased serum insulin

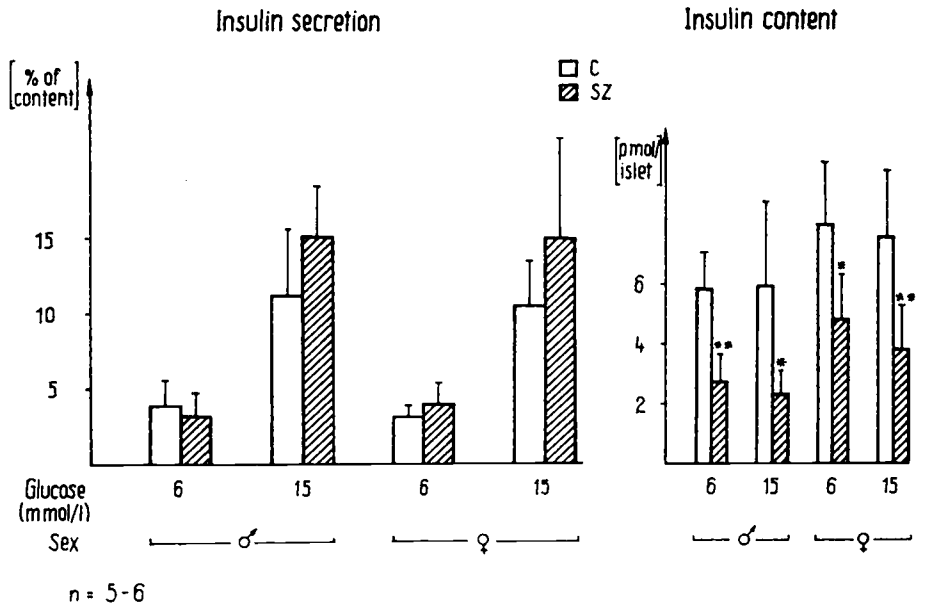

Fig. 2 Insulin secretion and insulin content of pancreatic islets isolated from male (♂) and female (♀) mice in the 29 th week after application of 50 mg streptozotocin (SZ)/kg b.w. C = Controls, significances: * $p < 0.025$ ** $p < 0.01$

(Table 1) is a sign of an insufficient insulin secretion of the surviving beta cells. Then progressive restoration processes started so that blood glucose and insulin secretion and biosynthesis of islets came back to normal as well as the pancreatic insulin content has reenhanced up to 30% of control values (Table 1). An interesting fact is that the pancreatic insulin content of female mice, even after the higher SZ (2) dose, reached 60% of control values after pregnancy when they had delivered living pups. As is well known beta cell growth (Aerts and Van Assche, 1975) and many islet functions are stimulated in pregnancy (Ziegler et al., 1985b).

It is remarkable that the mice were able to recover in spite of their hyperglycaemia, considering the fact that high blood glucose levels per se may have toxic effects on pancreatic beta cells (Curry, 1986). In SZ hyperglycaemia of rats neither the pancreatic insulin content nor the beta cell volume showed a tendency toward reenhancement, not even after longterm observations (Hahn et al., 1985). But there are also reports which show that glucose promotes beta cell proliferation increasing the pool of beta cells which are able to proliferate (Swenne, 1982); however, the beta cell genome is probably programmed only for a small number of mitotic cycles (Zühlke, 1982).

Up to now it has been widely acknowledged that a SZ-induced severe diabetes in rats (Hahn et al., 1985) and particularly in mice (Brosky and Logothetopoulos, 1969; Rerup, 1970), is permanent, because the regenerative capacity of the surviving beta cells is only limited (Brosky and Logothetopoulos, 1969). We were able to show a spontaneous recovery of a SZ induced diabetes in BALB/c mice and on the basis of this model it will be possible to investigate the mechanisms and promoting factors of such restauration processes in more detail.

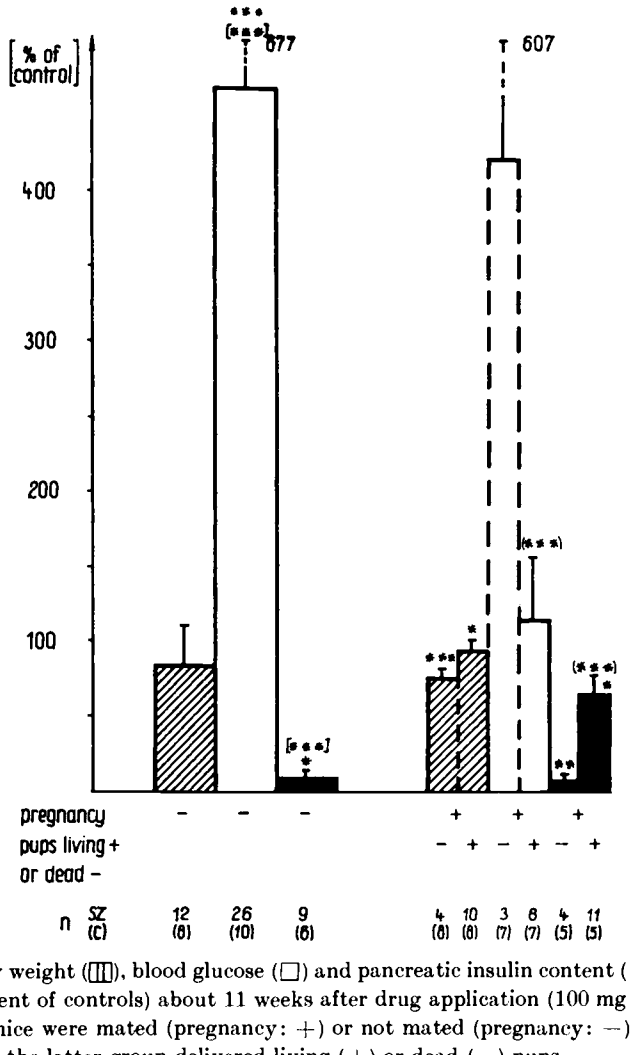

Fig. 3 Body weight (▥), blood glucose (□) and pancreatic insulin content (■) of SZ-injected female mice (in percent of controls) about 11 weeks after drug application (100 mg streptozotocin/kg b.w.). The female mice were mated (pregnancy: +) or not mated (pregnancy: −) about 4 weeks after SZ injection and the latter group delivered living (+) or dead (−) pups.
Pancreatic insulin content of female controls (C): 19.0 ± 7.0 pmol/mg (n = 5)
significances: SZ: C (absolute values not presented) = *$p < 0.05$; **$p < 0.02$; ***$p < 0.001$
SZ (pregnancy: −): SZ (pregnancy: +, pups living: +) = [***] $p < 0.001$
SZ (pups living: +): SZ (pups dead: −) = (***) $p < 0.001$

Acknowledgements. The authors are grateful to Mrs. C. Scheibner and Mrs. B. Parlow for skilful technical assistance.

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(Accepted 19 September 1988)

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