Insulin Treatment Improves the Spontaneous Remission of Neonatal Streptozotocin Diabetes in the Rat

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SUMMARY

Neonatal rats injected with streptozotocin (STZ, 100 mg/kg) at birth exhibit an acute diabetes that is characterized by a spontaneous and incomplete remission. The short- and long-term effect of exogenous insulin on the course of this neonatal diabetes has been studied. Insulin treatment (20 mU/g body wt./day, for 4 days) diminished the percentage of glycosuric animals on day 5 after birth (10%) as compared with the percentage in the non-insulin-treated diabetics (STZ) (67%). On the 14th day, the body weight and the pancreatic insulin content of insulin-treated animals (STZ + I) were significantly higher than the corresponding values in the STZ animals. Glucose tolerance tests performed sequentially indicated that from 21 days to 7 mo, the plasma insulin response in the (STZ + I) females was clearly increased as compared with that observed in the STZ group. However, it did not reach the insulin response of the controls except in the 21-day-old females and, as a function of age, it declined progressively at variance with the normal age-related pattern. These findings indicate that insulin treatment (sufficient to reduce daily glycosuria) applied during the overtly diabetic period markedly improved the recovery of the insulin stores in the pancreas. Moreover, the long-term effect of the treatment was a long lasting if not permanent improvement of the in vivo insulin response to glucose.

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During the first months after the onset of juvenile-type diabetes in humans, a partial recovery of insulin release resulting in a decrease of daily insulin requirement is sometimes observed.[1–3] Careful control of blood glucose by continuous insulin infusion or multiple daily insulin injections for brief periods (generally 1 wk) has been reported to increase endogenous insulin release, as evaluated by the plasma C-peptide response.[4–6] In this case, it is postulated that pancreatic function may improve when the B-cells are relieved from the hyperglycemic stress. However, such remissions are usually not permanent.

The present study was designed to determine the effect of metabolic control by exogenous insulin on endogenous insulin release in a model of experimental diabetes in the rat. Neonatal rats treated with streptozotocin at birth were used because they, exhibit an insulin-deficient acute diabetes 4 days after birth which is characterized by a spontaneous remission,[7,8] in contrast with streptozotocin-induced diabetes in the adult, which is irreversible.[9] However, this remission is incomplete and leads to a stable and chronic pathologic pattern in the adult with slight glucose intolerance and low-insulin response to glucose but without glycosuria. There is a subnormal growth curve and a slight hyperphagia. This chronic state is maintained up to 12 mo.[8] In the present study, streptozotocin-injected newborn rats were treated with insulin after the streptozotocin injection and their pancreatic function was tested during a long-term longitudinal in vivo study.

MATERIALS AND METHODS

Albino rats (Sherman strain) were fed ad libitum with pelleted chow (UAR, Villemoisson s/Orge, France, carbohydrate 47%, protein 20%, fat 8%). Females were caged with a male for one night (5 p.m. to 9 a.m.) and pregnancy was detected by abdominal palpation 14 days later as previously described.[10] Natural birth occurred 22 days after mating. On the day of birth (day 1), the newborn rats received streptozotocin (Upjohn Chemical Co., Kalamazoo, Michigan) (100 μg/g) in 25 μl of citrate buffer (0.05 mol/L), pH = 4.5, through the saphenous vein directly accessible by transcutaneous puncture. They were left with their own mothers, with the number of animals per litter kept at 8. In separate litters, the animals received a daily injection (20 μl) of insulin mixte-lente (Novo, Copenhagen, Denmark): either 5 mU/g body wt. for 5 days (from day 2 to day 6), or 20 mU/g

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body wt. for 4 days (from day 2 to day 5). From day 2 after birth, all the neonates were tested each day for glycosuria with Clinistix (Ames Co., Division Miles Laboratories, Paris, France). Glycosuria was measured just before the daily insulin injection. In other litters, all the newborns received citrate buffer (25 μl) at birth and saline injections (20 μl) during 5 days, and they were used as controls. All the animals were weaned on day 21 after birth.

On day 7, 14, and 21 after birth, blood samples were collected from axillary vessels and immediately centrifuged at 4°C; plasma was stored at −20°C until assayed. After dissection, the pancreas was weighed and homogenized for 1 min by ultrasonic disintegration at 4°C (Sonifier B12, Heat Systems-Ultrasonics, Plainview, New Jersey) in acid-alcohol solution (75% ethanol, 1.5% v/v, 12 mol/L HCl, 23.5% distilled water). After one night at −20°C, the extracts were centrifuged and the supernatants kept at −20°C until assay. Glucose tolerance was determined in control females and in streptozotocin-injected females treated or not treated with insulin, at 21 days, 3 mo, and 7 mo. Each female was serially tested. Intravenous glucose tolerance tests (0.5 g glucose/kg body wt.) were performed under pentobarbital anesthesia (4 mg/100 g body wt. i.p.).

Blood was withdrawn from the tail vein. Blood samples (300 μl) were immediately centrifuged at 4°C; plasma was stored at −20° until assayed.

Plasma glucose was determined using a glucose analyzer (Beckman Instruments, Fullerton, California). Plasma immunoreactive insulin (IRI) was estimated using purified rat insulin as standard (R 171, Novo, Copenhagen, Denmark), antibody to mixed porcine + bovine insulin, and porcine monoiodinated ^{125}I-insulin.[11] The method allows the determination of 6 μU/ml (0.25 ng/ml) with a coefficient of variation within and between assays of 10%. Silicate was used to separate free from bound hormone.[12] Anti-insulin antibody levels were undetectable in the plasma of the insulin-treated animals after the 4-day treatment. The glucose tolerance and the insulin response to glucose in vivo have been calculated as the ΔG and the ΔIRI, respectively.[13] ΔG is the incremental integrated plasma glucose values integrated over the period (90 min) following the injection of glucose and ΔIRI is the incremental plasma insulin values integrated over the same period.

Results were given as mean ± SEM. Statistical analysis was performed using Student's unpaired *t* test.

RESULTS

NEONATAL DIABETES AND INSULIN TREATMENT

As compared with the non-insulin-treated group, the mortality rate of the STZ-injected newborns was not changed by the 5 mU insulin/g/day (50% in the two groups), whereas it was reduced in the group treated with 20 mU insulin/g/day (25%).

Efficacy of insulin treatment on blood glucose levels was evaluated by the percentage of animals with glycosuria (3 + value with the Clinistix test) each day after the induction of streptozotocin diabetes (Figure 1). Glycosuria was measured just before the daily insulin injection (and as a consequence, 24 h after the preceding injection). With 5 mU insulin/g/day, the percentage of glycosuric animals on day 5 after birth was only 24% and with the 20 mU/g/day protocol, it was only 10% as compared with 67% in the non-

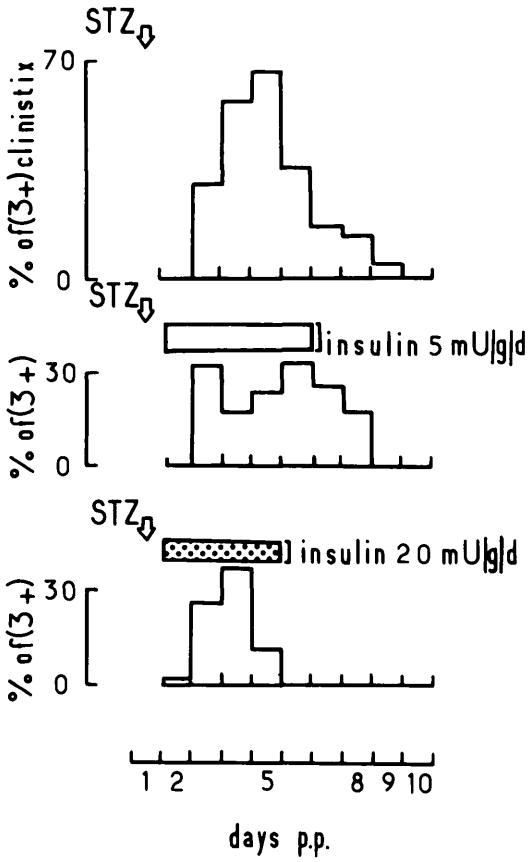


FIGURE 1. Effect of insulin treatment on daily glycosuria tested with Clinistix, in rats injected with streptozotocin (STZ, 100 mg/kg) at birth. Two groups of STZ-treated animals received 5 mU/g body wt. of insulin mixte lente per day for 5 days (▭) or 20 mU/g body wt. of insulin/day for 4 days (▨). Number of rats in each group ranged from 44 to 31.

insulin-treated diabetics. Moreover, in the animals treated with the high insulin dose, glycosuria disappeared in all rats on day 6 after birth whereas some untreated animals were glycosuric until day 9.

Animals from the two insulin-treated groups were killed on day 7 or day 14 and were compared with untreated diabetics and with nondiabetic animals (Table 1 and Figure 2).

On day 7, the body weight and the pancreatic insulin content (1.70 ± 0.13 mU/mg pancreas) of diabetic rats treated with the low insulin dose were not significantly different from the corresponding values in the non-insulin-treated diabetics (1.73 ± 0.16 mU insulin/mg pancreas). On day 14, animals taken from the same experimental group exhibited a significantly ($P < 0.05$) increased pancreatic insulin content (3.55 ± 0.43 mU/mg) as compared with the corresponding value in the non-insulin-treated diabetics (2.52 ± 0.15 mU/mg). Nevertheless, the pancreatic insulin content was still about half of that in the nondiabetic animals (6.85 ± 0.47 mU/mg). On day 7, in diabetic animals treated with the high insulin doses, the body weight (12.8 ± 0.3 g) and the pancreatic insulin content (4.04 ± 0.47 mU/mg) were significantly ($P < 0.02$) higher than the corresponding values in the non-insulin-treated diabetics (11.3 ± 0.5 g and 1.73 ± 0.16 mU/mg, respectively). On day 14, their body weight value was 90% of the value in nondiabetic animals as compared with 68% in the untreated diabetic rats (Table 1). The pancreatic insulin content of the diabetics

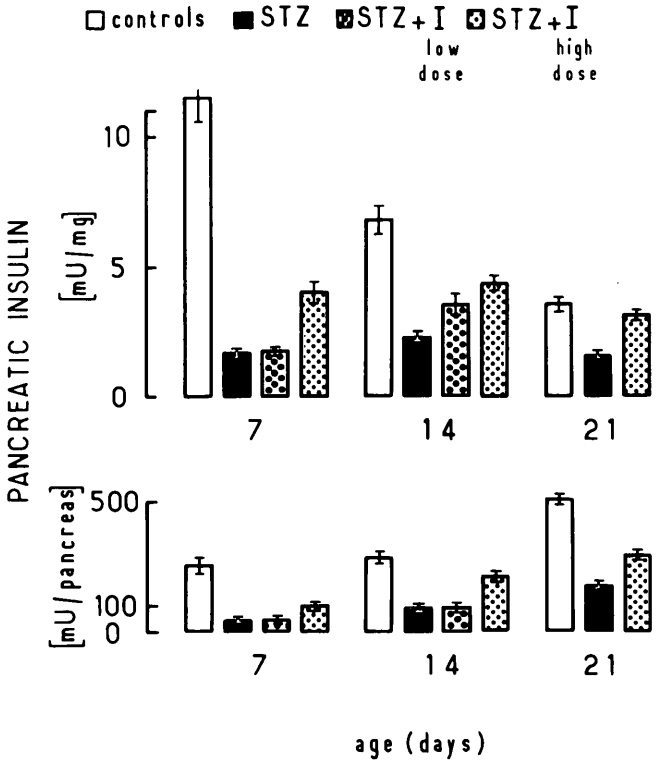
TABLE 1
Effect of insulin treatment on basal characteristics of rats injected with streptozotocin (STZ, 100 mg/kg) at birth. Two groups of STZ-treated animals received 5 mU insulin/day from day 2 to day 6 (STZ + I low dose) or 20 mU insulin/day from day 2 to day 5 (STZ + I high dose)

	Body weight (g)		Plasma glucose (mg/100 ml)		Plasma insulin (μU/ml)	
	7 days	14 days	7 days	14 days	7 days	14 days
Controls	12.3 ± 0.5 (7)	27.1 ± 0.4 (7)	106 ± 2 (7)	115 ± 5 (7)	31 ± 3 (7)	45 ± 4 (7)
STZ	11.3 ± 0.5 (7)	18.3 ± 0.7 (17)	130 ± 7 (7)	121 ± 6 (15)	25 ± 7 (7)	40 ± 1 (7)
STZ + I (low dose)	11.5 ± 0.3 (15)	19.7 ± 1.3 (10)	140 ± 7 (9)	142 ± 8 (10)	29 ± 6 (9)	45 ± 13 (8)
STZ + I (high dose)	12.8 ± 0.3* (12)	24.4 ± 0.5† (6)	125 ± 6 (12)	140 ± 3 (6)	40 ± 8 (12)	31 ± 3 (6)

The animals were killed on day 7 or day 14 after birth.
Values are the mean ± SEM. Number of rats is shown in parentheses.
* $P < 0.02$ when compared with related STZ group.
† $P < 0.001$ when compared with related STZ group.

treated with the high insulin doses was significantly ($P < 0.001$) increased (4.40 ± 0.28 mU/mg) as compared with the value in the non-insulin-treated diabetics (2.28 ± 0.20 mU/mg) and represented 65% of the value in nondiabetic animals (6.85 ± 0.47 mU/mg) (Figure 2). On day 21, the pancreatic insulin content of the rats treated with the high insulin dose (3.13 ± 0.18 mU/mg) was no longer significantly different from that in nondiabetic animals (3.62 ± 0.27 mU/mg) whereas it was only 1.59 ± 0.12 mU/mg in the non-insulin-treated diabetics (Figure 2).

FIGURE 2. Effect of insulin treatment on pancreatic insulin content in rats injected with streptozotocin (STZ, 100 mg/kg) at birth. Two groups of STZ-treatment animals received 5 mU/g of insulin mixte lente per day from day 2 to day 6 (STZ + I low dose) or 20 mU/g of insulin/day from day 2 to day 5 (STZ + I high dose). The animals were killed on day 7, 14, or 21 after birth. Each bar is the mean ± SEM of 6–12 observations in each group.



LONG-TERM STUDY OF RATS WITH NEONATAL DIABETES TREATED WITH INSULIN

Females taken from the group treated with the high insulin doses (20 mU/g/day) were bred and sequentially tested up to 7 mo. To ascertain recovery, they were tested for glycosuria two days a week up to adult age. Glycosuria disappeared after day 6 in all animals.

Baseline characteristics. In the streptozotocin and insulin-treated (STZ + I) females, as well as in the females treated with streptozotocin only (STZ), body weight was not significantly different from that of age-related normal females from 21 days to 3 mo (Table 2). However, at 7 mo, it was significantly decreased as compared with normal females in both groups receiving streptozotocin, treated or not treated with insulin. In the STZ + I rats the baseline plasma glucose in the fed state was not different from the controls, from 21 days to 7 mo (Table 2). In the STZ rats it was significantly increased at 7 mo. Baseline plasma insulin levels in both STZ and STZ + I groups were similar to those measured in the age-related normal group (Table 2).

Glucose tolerance. In the STZ group from 3 to 7 mo, 5, 15, and 90 min after glucose injection, the plasma glucose levels were slightly but significantly higher ($P < 0.05$) than levels found in age-matched control females (Figure 3). Glucose disposal was thus slightly decreased in these adult animals in accordance with our previous observations. With the calculation of the integrated plasma glucose area, it may be observed that the slight deterioration of glucose tolerance as a function of age in the STZ group was similar to that observed in the control group (Figure 4). In the STZ + I group, the plasma glucose area values were not significantly increased as compared with those obtained in the control group at 21 days, 3 mo, and 7 mo, (Figure 4).

Insulin response. In normal females, it can be seen that the plasma insulin response increases with age (Figures 3 and 4). In the STZ group, the 5- and 15-min plasma insulin values were considerably decreased at all ages. Calculation of the plasma insulin area indicated that the low insulin response to glucose did not spontaneously deteriorate or

TABLE 2
Effect of age on basal characteristics of control female rats and females injected with STZ (100 mg/kg) at birth and treated by 20 mU insulin/day during 4 days (STZ + I) or untreated (STZ). Animals were in the fed state

	Body weight (g) (mo)			Plasma glucose (mg/dl) (mo)			Plasma insulin (μU/ml) (mo)		
Age	0.75	3	7	0.75	3	7	0.75	3	7
Controls	45 ± 2 (8)	148 ± 5 (18)	250 ± 9 (10)	117 ± 2 (8)	135 ± 3 (18)	137 ± 3 (10)	12 ± 2 (8)	51 ± 5 (18)	49 ± 7 (10)
STZ	41 ± 2 (8)	139 ± 7 (18)	201 ± 7‡ (12)	123 ± 3 (8)	146 ± 9 (18)	180 ± 18* (12)	12 ± 1 (8)	43 ± 5 (18)	42 ± 6 (12)
STZ + I	39 ± 3 (9)	149 ± 4 (10)	205 ± 7† (9)	125 ± 3 (9)	144 ± 5 (10)	143 ± 4 (8)	28 ± 3 (8)	59 ± 6 (10)	56 ± 3 (8)

Values are the mean ± SEM. Number of rats is shown in parentheses.
* $P < 0.05$ when compared with related controls.
† $P < 0.01$ when compared with related controls.
‡ $P < 0.001$ when compared with related controls.

ameliorate with age (Figure 4). In the STZ + I group from 21 days to 7 mo, the plasma insulin response to glucose is clearly increased as compared with that measured in the STZ group. But it did not reach normal insulin response, except in the 21-day-old animals (Figure 4). Moreover, as a function of age, the insulin response to glucose in this STZ + I group declined progressively in contrast with the age-related pattern observed in the normal females (Figure 4).

DISCUSSION

We have previously reported[7] that the unique characteristic of STZ-diabetes in rat neonates was the transitory nature of the overt B-cell deficiency as compared with STZ-diabetes in the adult rat. Yet, the spontaneous recovery occurring in streptozotocin-treated neonates was incomplete.[8] The present study was designed to determine if improved control of plasma glucose in the diabetic neonate would allow the diabetic endocrine pancreas to improve its insulin response to glucose. The data presented here clearly indicate that insulin treatment (20 mU/g body wt./day) markedly improved the recovery of the insulin stores in the pancreas (Figure 2). Both the timing and completeness of reaccumulation of insulin in the tissue were affected. Moreover, the long-term effect of this treatment was a long-lasting (up to 7 mo) improvement of the in vivo insulin response to glucose.

In the untreated diabetic rat neonate, we have previously shown that the recovery of the pancreatic insulin content was related to an increase of the total B-cell mass[14] and that this regeneration was characterized by both formation of new islets budding from the ducts and some degree of reduplication of surviving B-cells in preexisting islets.[15] This is in accordance with the fact that even in normal neonates there is an active proliferation of the B-cell.[16]

In the insulin-treated diabetic rat neonate, it may be hypothesized that all these processes are favored but histologic data are presently not available.

FIGURE 3. Long-term study of rats with neonatal diabetes treated with insulin. Females injected with streptozotocin (STZ, 100 mg/kg) at birth received 20 mU/g of insulin mixte lente/day from 2 to day 5 (▲—▲). They were compared with females treated with STZ only (△—△) and with control females (dotted shadow). Glucose tolerance and plasma insulin response to glucose (0.5 g/kg i.v.) were determined at 21 days, 3 mo, and 7 mo in the same animals sequentially studied. Each point is the mean ± SEM of 6–10 observations in each group.

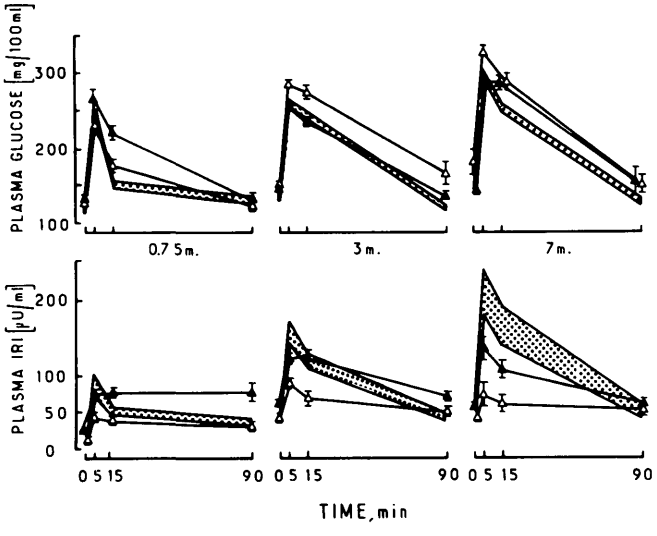
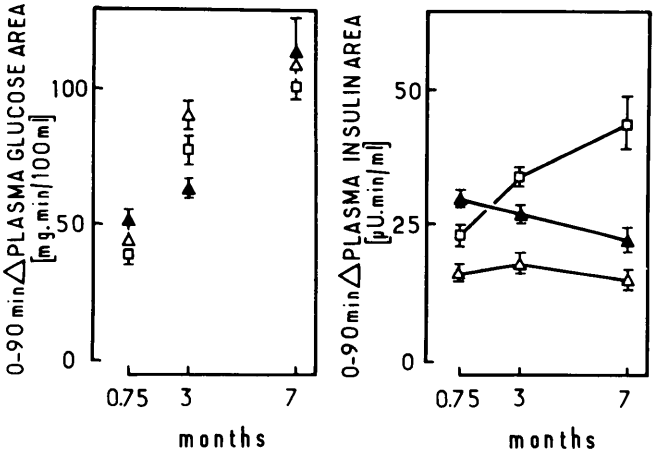


FIGURE 4. Long-term study of rats with neonatal diabetes treated with insulin. Mean incremental plasma glucose area and mean incremental plasma insulin area were determined in control females (□—□), STZ females (△—△), and STZ females treated with insulin (▲—▲). Parameters presented here have been calculated from values obtained during the 90-min intravenous glucose tolerance test (see Figure 3). In the three groups, the test was performed at 21 days, 3 mo, and 7 mo. Each point is the mean ± SEM of 6–10 observations in each group.



The mechanism by which insulin treatment enhances the growth and function of the neonatal diabetic pancreas is not clear. There is abundant evidence that supraphysiologic glucose concentrations stimulate B-cell proliferation in neonatal pancreatic cultures and result in an increased B-cell mass.[16] Nevertheless, hyperglycemia alone is probably not the only factor for this effect since the B-cell mass was the same in fetal pancreases transplanted either into normal rats or untreated diabetic rats.[17] Thus, diabetes neither stimulated nor inhibited B-cell proliferation. But the administration of insulin to the diabetic rats after transplantation resulted in a clear-cut increase in B-cell mass and insulin content of the transplanted tissue.[18,19] The question that arises is whether insulin directly stimulates B-cell proliferation or permits the proliferative stimulus of glucose to occur. A previous report indicated that mannoheptulose, an agent previously believed to inhibit insulin release by acting as a competitive inhibitor of glucose phosphorylation, also inhibits B-cell replication, thus suggesting that glucose must be metabolized by the B-cell to stimulate replication.[20]

Moreover, it has been recently reported that fetal rat pancreas explanted in vitro in the presence of added insulin had a greater B-cell volume and a greater insulin content than those grown without added insulin.[21] On the other hand, in insulin-treated chick embryos the histogenesis of the islets was not affected.[22] There is presently no clear-cut evidence for a direct action of insulin to stimulate B-cell proliferation.

The present data are consistent with the observations in humans that early insulin treatment of juvenile-onset diabetics with glucose-regulating devices may improve endogenous insulin release.[4–6] Moreover, the duration of the improvement after the period of strict control seems to be inversely related to the duration of the disease before the onset of the strict blood glucose control.[5,6] Recent studies in the genetically nonobese diabetic Chinese hamster indicate that improved control of blood glucose by continuous infusion of insulin can lead to improved endogenous insulin release at the end of the treatment.[23] But the duration of the improvement was not reported. In our experiments, insulin release was still significantly improved after 7 mo. Nevertheless, in rats with neonatal diabetes treated with insulin, insulin response to glucose and glucose tolerance decreased with age (Figure 4). This contrasts with the pattern noted in normal animals that exhibited increased insulin response as a function of age (Figure 4). In normals, increased insulin needs as a function of age are probably accounted for by decreased insulin sensitivity of the target tissues.[24,25] One may question, therefore, the possibility that the adult pancreas of the rat with neonatal diabetics treated with insulin is not able to match the increased insulin demand as a function of age. In any event, this deterioration of glucose handling after remission in these animals bears a resemblance to what usually occurs in insulin-dependent type I diabetics after temporary remission of their illness. We suggest that this experimental model in the rat provides opportunities for investigating the pathophysiology of remission, and especially of the mechanism leading to termination of these remission periods.

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