The Effect of Streptozotocin on the Pancreatic A Cell Function

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Synopsis

The effect of a single i.v. injection of 30, 50, and 65 mg of streptozotocin (Sz) per kg of body weight on blood glucose, plasma insulin and glucagon levels was studied in rats. Responses to glucose of these three parameters were also examined in rats one week after the administration of various doses of Sz alone, Sz with nicotinamide or with picolinamide. Nicotinamide, 500 mg/kg, and picolinamide, 250 mg/kg, were given i.p. 15 min before the injection of 65 mg/kg of Sz.

The triphasic pattern was observed after the injection of 65 mg/kg of Sz not only in blood glucose and plasma insulin but also in plasma glucagon level fluctuations, the last of which showed a similar pattern to that of blood glucose responses. Further, the initial hyperglucagonemia had a delay of 2 hr in onset, when treated with 30 and 50 mg/kg of Sz, respectively.

Oral glucose loading resulted in a significant increase of plasma glucagon levels in rats injected with 30 and 50 mg/kg of Sz, respectively. The paradoxical rise of plasma glucagon and glucose intolerance was observed in rats given Sz with nicotinamide, and Sz with picolinamide, as well as in those given Sz alone, 65 mg/kg, while there was no significant difference in insulin responses between the pretreated groups of rats and controls.

These results suggest that streptozotocin, even in a nondiabetogenic dose, has effect(s) on the A cell function, and that nicotinamide and picolinamide are active in protecting B cells against the cytotoxic effect, but they do not modify the effect of Sz on the A cell function.

In various species of animals, streptozotocin (Sz) has a diabetogenic action (Rerup, 1970), which is mediated through the destruction of B cells of pancreatic islets. It has further been shown that nicotinamide protects B cells against the cytotoxic effect (Schein et al., 1967; Schein and Bates, 1968; Dulin and Wyse, 1969; Stauffacher et al., 1970). Picolinamide was found to prevent a diabetogenic effect of Sz (Doi, 1975).

Though there are lots of morphological and biochemical studies on B cells after Sz treatment, in vivo studies on the pancreatic A cell function, as far as we know, are sparse. The present study was undertaken to determine whether the agent had an effect on the A cell function of the pancreas in reference to acute responses of plasma glucagon to various doses of Sz. Glucagon responses to oral glucose loading were also examined in rats given Sz with nicotinamide, and Sz with picolinamide, as well as those given various doses of Sz alone.
Materials and Methods

Male Wistar rats, weighing 150 to 200 g, were used in this study. They were starved for 16 hr before a single injection of various doses of Sz into the tail vein under light ether anesthesia. Blood samples were taken thereafter at time intervals as indicated. Those animals continued to fast for 7 hr after treatment and subsequently were placed in metal cages with free water and laboratory chow pellets. Nicotinamide, 500 mg/kg body weight, and picolinamide, 250 mg/kg body weight, were given i.p. 15 min before the injection of 65 mg/kg body weight of Sz.

Streptozotocin (Upjohn, Michigan, U.S.A., Lot U9889. 11837 GGS-22B) was dissolved in 0.01 M citrate buffer adjusted to pH 4.5 immediately before the injection. Nicotinamide (Nakarai, Kyoto, Japan, Lot MG38325) and picolinamide (Nakarai, Lot H00423) were dissolved in distilled water, respectively.

Control animals were given an injection of a similar volume of citrate buffer.

Oral glucose tolerance tests were performed 1 week after treatment under pentobarbital anesthesia. Rats were starved for 16 hr and 3 g/kg body weight of glucose in a 50 percent solution was administered into the stomach via a polyethylene tube.

Blood specimens (1 ml each) were withdrawn from the jugular vein by syringes rinsed with a 10 percent of EDTA and collected in plastic microfuge tubes containing 0.1 ml of Trasylol (1,000 Kallikrein Inactivator Units/ml of blood). The plasma was then separated by centrifugation and stored at -20°C until assayed.

Blood glucose was measured by the method of Hoffman (1937) using a Technicon Auto-Analyzer. Plasma insulin was determined by radioimmunoassay using the double antibody system (Morgan and Lazarow, 1963) with rat insulin as a standard. Glucagon was assayed by radioimmunoassay using a dextran charcoal technique (Aguilar-Parada et al., 1969). The antiguacagon serum employed was 30K.

Statistical comparisons between groups were made using Student's "t" test.

Results

Acute Response to Sz of Blood Glucose, Plasma Insulin and Glucagon (Fig. 1)

The administration of 65 mg/kg of Sz resulted in the expected triphasic responses of blood glucose (Type P4) levels was observed at the 2nd and 4th hr with a significant decrease of plasma insulin levels. At the 7th hr, there was severe hypoglycemia associated with significantly increased plasma insulin levels. Twenty-four and 48 hr after treatment, all animals were severely diabetic, characterized by hyperglycemia and a fall in plasma insulin concentrations in the fed states. With inverse proportional changes between blood glucose and plasma insulin responses, unexpected triphasic responses of plasma glucagon concentrations were observed following treatment. Plasma glucagon levels rose markedly 2 and 4 hr after treatment and subsequently fell to the initial values at the 7th hr. Twenty-four and 48 hr after treatment (in fed states), the mean plasma glucagon levels were significantly elevated as compared to those of controls. The pattern was similar to that of blood glucose responses.

In comparison with 65 mg/kg of Sz, 30 mg/kg of Sz resulted in no significant variations of blood glucose levels within 7 hr after treatment, despite significant decreases of plasma insulin levels. It also resulted in a later appearance of the initial hyperglucagonemia, that is, at the 4th hr. Mild but definite hyperglycemia was observed 24 and 48 hr after treatment, associated with less pronounced decreases in plasma insulin concentrations. At that time, the elevation of plasma glucagon values was still definite but less pronounced than those of 65 mg/kg of Sz.

Responses of plasma glucagon to 50 mg/kg of Sz were nearly the same as those of 30 mg/kg of Sz, whereas the ensuing patterns of blood glucose levels were intermediate between those of 30 and 65 mg/kg of Sz.

Rats given an injection with buffer alone showed no significant deviations of blood glucose and plasma insulin levels, except for the significant rises of plasma insulin levels in the fed states (at 24 and 48 hr). Small but significant rises of plasma
glucagon concentrations were observed 2 and 4 hr after treatment.

**Oral Glucose Tolerance Tests 1 Week After the Administration of Sz (Fig. 2)**

Injection of 65 mg/kg of Sz resulted in gross intolerance to glucose with flat and depressed insulin responses. Furthermore, plasma glucagon responses to glucose were elevated much more greatly than those of controls, while no difference in fasting plasma glucagon levels was observed.

On the contrary, there were no significant differences in blood glucose and plasma insulin levels during the oral glucose tolerance tests between rats treated with 30 mg/kg of Sz and controls. However, the mean plasma glucagon level was significantly elevated 0.5 hr after glucose loading.

Responses to glucose of blood glucose and plasma insulin in rats treated with 50 mg/kg of Sz were intermediate between those of 30 and 65 mg/kg of Sz and the mean plasma glucagon level at the 2 hr was significantly elevated as compared with those of controls.

The diabetogenic action of Sz was prevented by pretreatment with nicotinamide. While plasma insulin responses to glucose did not show any significant deviations as compared with those of controls, blood glucose values at 1 and 2 hr were elevated. Concomitantly, plasma glucagon values were significantly elevated.

**Fig. 1.** The effect of a single intravenous injection of 30 (△–△), 50 (□–□), and 65 (●–●) mg of Sz per kg of body weight on blood glucose and plasma insulin and glucagon levels in rats. Control rats (○–○) were given an injection of a similar volume of citrate buffer. Rats were starved for 16 hr and continued to fast for 7 hr after treatment and, subsequently, were placed in metal cages with free water and laboratory chow pellets. The number in parentheses indicates the number in each group. Vertical bars represent SEM. Significance of differences vs control values: *p<0.05, **p<0.01, +p<0.005, ++p<0.001.
Picolinamide, the isomer of nicotinamide, also prevented the diabetogenic action of Sz, although pretreatment with the isomer induced severer glucose intolerance than nicotinamide-pretreatment. Glucagon responses to glucose in pretreated rats were nearly the same as those of 65 mg/kg of Sz.

Neither the pretreated rats nor those treated with 30 mg/kg of Sz showed any signs of overt diabetes, whereas, those given an injection with larger doses of Sz developed overt diabetes.

**Discussion**

As previously reported (Schein and Bates, 1968; Stauffacher et al., 1970) and confirmed by the present study, diabetes onset after i.v. injection of Sz, 65 mg/kg, is followed by a triphasic pattern with an early...
hyperglycemia, followed by hyperglycemia, and finally, by permanent hyperglycemia. The detailed mechanisms of Sz-induced blood glucose changes are not yet completely clarified. As shown in Fig. 1, the injection of a diabetogenic dose, 65 mg/kg, of Sz was followed by the triphasic pattern in not only blood glucose and plasma insulin but also plasma glucagon level fluctuations, and the pattern of plasma glucagon fluctuations was similar to that of blood glucose responses. These results suggest that the early hyperglycemia is, in part, responsible for the early hyperglucagonemia and hypoinsulinemia. The observation (Golden et al., 1971) that pancreatic islets 1 hr after the injection of Sz fail to secrete insulin in response to glucose in vitro supports the hypothesis that the hypoinsulinemia is responsible for a direct inhibitory action of Sz on B cells. The early hyperglucagonemia agrees with the enhanced baseline glucagon secretion following the acute treatment with a diabetogenic dose of Sz (Weir et al., 1976). The detailed mechanisms of the early hyperglucagonemia are also obscure. The present observations that a nondiabetogenic dose of the agent was also followed by a significant rise of plasma glucagon, which had a delay of 2 hr in onset and a similar severity to that observed after a diabetogenic dose, indicate that Sz, even in a nondiabetogenic dose, has a direct effect on the function of not only B cells but also A cells. This hypothesis is supported by the observation (Lazarus and Shapiro, 1972) that some necrotic A cells with pyknotic nuclei are observed after Sz.

Oral glucose tolerance tests revealed that a glucose loading was followed by a significant rise of plasma glucagon levels in rats 1 week after treatment with Sz, irrespective of the dose, while control rats showed no deviation of plasma glucagon levels. Besides, the paradoxical rise of plasma glucagon was seen not only in rats given a diabetogenic dose of Sz, but also in those given the same dose of Sz with nicotinamide, and Sz with picolinamide, while the insulin response to glucose was not impaired in the pretreated animals. Oral glucose loading caused a decrease in plasma glucagon in normal human subjects (Müller et al., 1970) but not in normal control rats used in this study. The reasons for this are unclear. Coexistence of the capacity of B cells to secrete insulin and the paradoxical glucagon rise found in pretreated rats provides further evidence that Sz has a direct effect on the A cell function and rules out the possibility that the paradoxical rise is a consequence of insulin deficiency.

References