

LOW DOSE STREPTOZOTOCIN-INDUCED DIABETES IN WISTAR RATS IS ASSOCIATED WITH REDUCED INSULIN IMMUNOREACTIVE PANCREATIC ISLETS.

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ABSTRACT

Streptozotocin, which induces diabetes mellitus in laboratory animals, has been reported to be interred into β -cells by means of the glucose transporter 2 (GLUT2) and then reduced the cellular level of NAD⁺ leading to necrosis of the β -cells. In this experiment we investigated the effect of low dose streptozotocin (50 mg/kg) on insulin immunoreactivity in pancreatic islets. Two groups of rats were studied, the first group received streptozotocin (STZ) and the second was used as a control. Rats treated with STZ became gradually hyperglycemic on day 21 STZ: 318.8 ± 24.2 mg/dl vs CTRL: 65.1 ± 8.2 mg/dl; $P < 0.001$ with significant reduction of plasma insulin 6.3 ± 1.36 μ UI/ml vs CTRL 22.4 ± 2.8 μ UI/ml; $P < 0.01$). The immunohistochemical findings of insulin IR in the pancreatic tissue were in accordance with the results obtained for glucose and plasma insulin. In conclusion, we can say that a single low dose of STZ provoke destruction of pancreatic β -cells and the diabetogenic action of this drug is probably mediated by GLUT2.

Key words : Streptozotocin, Immunoreactivity, Insulin, GLUT2.

INTRODUCTION

Streptozotocin-induced diabetes in laboratory animals has been widely used to study diabetes research and its long term complications. Streptozotocin was originally isolated from cultures of streptomyces Achromogenes in 1960 (1). The glucose transporter (GUT2) is expressed in rodent β -cells of the pancreas, and in liver (2,4). GLUT2 is responsible for the glucose uptake into β -cells (2) and it has recently been suggested also to facilitate uptake of streptozotocin (STZ) (3). This may explain why β -cells especially are affected by the drug. Streptozotocin causes fragmentation of DNA in rat pancreatic β -cells through the formation of free alkylating radicals, leading to the reduction in the NAD⁺. The later causes a rapid necrosis of the β -cells (5,8). A recent study on cows showed that the injection of 100 mg/kg of streptozotocin had mild to marked decrease in islet immunoreactivity for insulin and pancreatic islets density (22). Therefore, the present study was undertaken to examine the insulin immunoreactive pancreatic islets and plasma insulin concentration during three weeks after induction of experimental diabetes with streptozotocin.

MATERIALS AND METHODS

Animals

Eight-week-old male wistar rats, weighing 200-250 g (Pasteur Institute, Algiers, Algeria), were randomly

allocated into two groups, streptozotocin-induced diabetic (STZ) and control rats (CTRL). All animals were allowed free access to water and laboratory food *ad libitum*. Temperature 21 ± 1 , humidity 70% and photoperiod 12 light/12 dark.

A single ip injection of 50 mg/kg streptozotocin (Sigma Chemical Co., St. Louis, MO) dissolved immediately before administration in 50 mM citrate buffer, (pH 4.0) was given to STZ rats on day 0. The injection was given at 16.00h. Control animals were received also an equivalent volume of citrate buffer.

The rats were anesthetized, and the blood was obtained from the tail vein for glucose and insulin determination. The whole blood glucose concentration was measured using the glucose oxydase method (Roche diagnostic kits) on day 0, 7, 14, and 21 and plasma insulin was also determined at the same time points by enzyme linked immunosorbent assay (ELISA) using kits supplied by Boehringer Mannheim Germany (enzyme-test insulin, ref. 199648).

Tissue samples for immunohistochemistry were obtained from the dorsal region of pancreas on day 0 (n=4, CTRL group), on day 7, 21 (n=4, STZ group). Tissue samples were immediately fixed by immersion into ice-cold, freshly prepared, buffered 4% paraformaldehyde. The fixed tissue samples of pancreas were embedded in paraffin and cut into 7 μ m section using a microtome. The unlabeled antibody peroxidase-antiperoxidase technique was applied, as described by Sternberg (9). Insulin antiserum (N°2004, Zymed Laboratories) were diluted 1:400 with 0.1 mol/liter phosphate buffer (pH 7.6) (Zymed

Laboratories) containing human serum albumin. The sections were examined by means of a Zeiss microscope (Carl Zeiss, Germany). Data were expressed as means $\pm$ SEM, and their statistical comparison was performed using ANOVA (one way), followed by multiple range test of Duncan.

RESULTS

Hyperglycemia was observed in STZ rats within 7 days after the administration of streptozotocin (CTRL: 67.4 ± 6.2 mg/dl vs STZ: 126.4 ± 11.3 mg/dl, $P < 0.01$) (Fig.1). Two weeks after streptozotocin injection the mean blood glucose concentration was above 200 mg/dl, and it remained at this level throughout the 3 weeks of the study (day 21 CTRL: 65.1 ± 8.2 vs STZ: 318.8 ± 24.2 , $P < 0.001$). Streptozotocin treatment inhibits efficiently insulin secretion and release. The plasma concentration of insulin on day 7 was significantly lower than of control rats (CTRL: 21.1 ± 1.7 μ UI/ml vs STZ 10.2 ± 1.2 μ UI/ml; $P < 0.01$). This reduction of plasma insulin persists throughout the experimentation (day 21 CTRL: 22.4 ± 2.8 μ UI/ml vs 6.3 ± 1.36 μ UI/ml; $P < 0.01$) (Fig.2).

In the control rats, the insulin immunoreactivity (IR) β -cells, were located centrally in the islets of Langerhans (Fig.3A). However, the STZ rats, the number of insulin-IR β -cells was markedly reduced (Fig.3B), in most islets insulin-IR cell were completely absent (Fig.3C).

DISCUSSION

The present study was undertaken to investigate the diabetogenic action of streptozotocin on insulin immunoreactivity of pancreatic B-cells. The exact mechanism of the β -cytotoxic action of streptozotocin is still conjectural, although it has been intensely investigated. The GLUT2 transporter has been known to facilitate the glucose uptake into rodent -cell (2) it also assumed to mediate the cellular uptake of streptozotocin (3).

In contrast to GLUT2, the GLUT1, which is an isoform of GLUT2, has very less affinity to streptozotocin (3), is predominately expressed on human β -cell (10). This may explain why human fetal pancreatic B-cells are resistant to the toxic effect of streptozotocin (11). It was found that patients had endocrine tumors and treated with streptozotocin they do not develop diabetes. In addition Okamoto (5,7) showed that the streptozotocin induced injuries in rodent β -cells provoke DNA repair mechanisms involving the action of the NAD⁺ degrading enzyme poly-(ADP-ribose) synthetase, and that the subsequent depletion of the cellular NAD⁺ pool depressed cell function, leading to degeneration β -cells (5,8,12). In support of this suggested action of streptozotocin, inhibitors of poly(ADP-ribose) synthetase nicotinamide and aminobenzamide, have

been shown to prevent streptozotocin diabetes through protection of the intracellular NAD⁺ pool (13,14).

In the present study, a low dose injection of streptozotocin increase plasma glucose concentration, on the other hand insulin plasma concentration was markedly reduced. The severity of experimental diabetes and its persistence in rats depend on the dose of streptozotocin used (15). Intraperitoneal injection of streptozotocin in a dose of 45 mg/kg is known to cause a transient state of diabetes (16), and spontaneous recovery from experimental diabetes within 36 weeks in rats injected with streptozotocin in doses up to 50 mg/kg (17,18). However, a high dose of streptozotocin (60 mg/kg) has been shown to induce a persistent diabetic state (16,19).

In the present study, we used a low dose of streptozotocin that it is revealed of a singular toxicity on β -cells. Immunohistochemical examination of pancreatic islets from CTRL rats, revealed a large number of insulin IR B-cells, in contrast to markedly reduction of insulin-IR in islets of STZ rats. In this context it has been reported that streptozotocin induced diabetes is associated with a reduction of insulin-IR concentration but glucagon and glucagon-like peptide (GLP1) immunoreactivity increased dramatically in the remaining islet cells (20, 21).

In conclusion, the present experiment suggests that the diabetic action of streptozotocin is most likely due to the combination of an increased activity of β -cells and increased number of GLUT2 transporters on B-cells, which have been implicated as important factors in the diabetic effect of streptozotocin.

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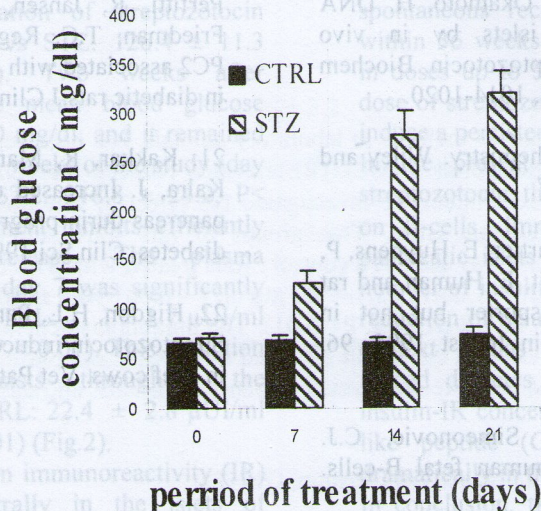


Figure 1 : Blood glucose concentration (mg/dl) measured

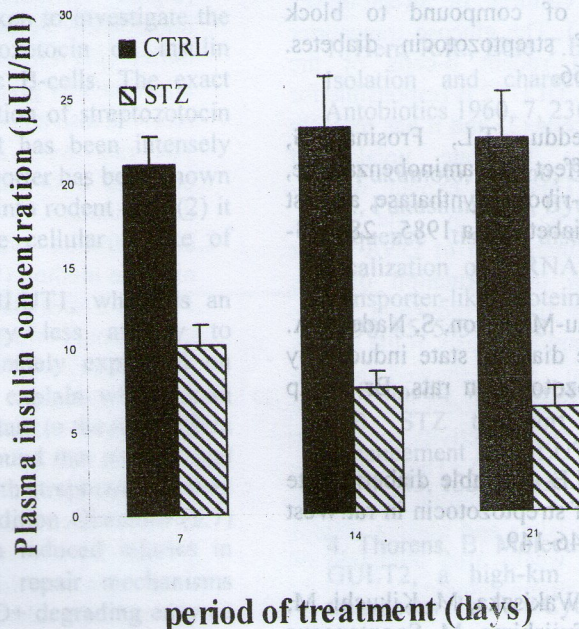


Figure 2: Plasma insulin concentration