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ALLOXAN DIABETES IN SWISS MICE: ACTIVITY OF NA^+-K^+-ATPASE AND SUCCINIC DEHYDROGENASE

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Abstract: The activities of two enzymes viz: Na^+-K^+-ATPase and succinic dehydrogenase (SDH) in brain and liver of alloxan diabetic Swiss albino mice are reported. Alloxan diabetes caused significant decrease in the activity of Na^+-K^+-ATPase reflecting reduced glucose transport across the cell membrane. On the contrary, the observed enhanced activity of the enzyme SDH is attributed to increased supply of TCA cycle substrates from accelarated oxidation of fatty acids.

Key words: alloxan diabetes, Swiss mice, Na^+-K^+-ATPase, succinic dehydrogenase

INTRODUCTION

Disorders in glucose homeostasis during diabetic syndrome are chiefly due to poor transport of glucose across the cell membrane. Glucose enters the cell by a specific transport system that is regulated by the hormone insulin (1). This possibly leads to lower intracellular glucose concentration making the cell deficient of this primary energy currency for further degradation and energy output. As might be expected, the activity of glycolytic enzymes decreased (2). Likewise, the enzyme pyruvate dehydrogenase responsible for irreversible funneling of glycolytic product i.e. pyruvate into TCA cycle showed lower activity (3) thus limiting free entry of substrates into TCA cycle. In view of the above facts, in the present study the activity of two enzymes, viz. Na^+-K^+-ATPase responsible for glucose symport and succinic dehydrogenase (SDH) responsible in part for the mitochondrial oxidation of fuel molecules was studied in liver and brain of alloxan diabetic Swiss mice.

METHODS

Swiss albino mice (Mus musculus) body wt. range 15-33 g (approximate age 2-3 months) of both sexes were procured from a commercial firm at Calcutta and were maintained at room temperature (30 $\pm$ 2°C) on a freshly prepared diet (500 g semolina, 50 g milk powder, 20 yeast tablets, NaCl salt 5 g, boiled to make paste to serve 20 animals) and water was provided *ad libitum*. A minimum acclimation period of 7 days was always allowed before the beginning of the experiments.

Alloxan treatment: After laboratory acclimation, 20 animals were starved for 48 hours and divided into control and experimental groups (ten animals each). Diabetes was induced in experimental groups of mice through intraperitoneal injection of alloxan (Fischameno, Loba Chemie, Wien, Austria) dissolved in distilled water at a dose of 100 mg/kg body wt (4), while the control group received an equal volume of distilled water. Diabetic state was maintained by administration of repeated doses of alloxan on every alternate day for 7 days.

Blood glucose level: On the 8th day of treatment, blood drawn from the subclavian vein of mice of both control and experimental groups under mild ether anaesthesia and collected in graduated centrifuge tubes containing 2 ml of 2% sodium citrate (E Merck)

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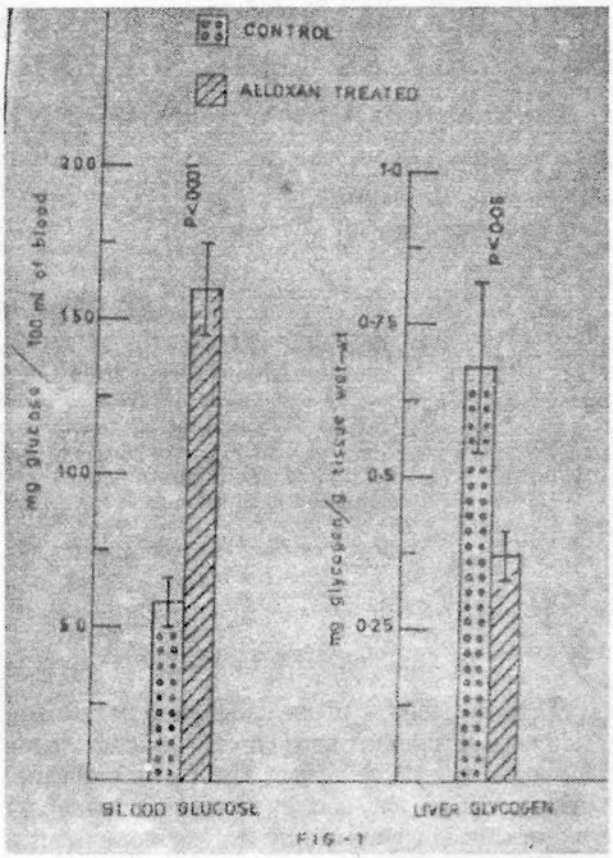
solution. Glucose contents of the sample was determined colorimetrically (5).

Tissue processing: The brain and the liver were quickly dissected out in precooled mammalian Ringer (Kreb's Ringer phosphate) and the adherent tissues were cleaned. A part of the liver was used for the estimation of glycogen content following standard procedures (6, 7). The entire brain and rest of the liver were blotted off in Whatman filter paper No.1 and weighed. A 5% homogenate of each tissue was prepared in 0.25M sucrose solution using a REMI homogenizer (Bombay) at a medium speed for 1 min. This homogenate was used for the assay of Na^+-K^+-ATPase and succinic dehydrogenase activity as described in our earlier publication (8). Significance of the result was analyzed using Students 't' test (9).

RESULTS

Blood glucose and liver glycogen level: Alloxan treatment for 7 days to mice increased the blood glucose content significantly as compared to the values of controls. On the otherhand, the liver glycogen levels declined significantly following alloxanization (Fig. 1). Such observations confirmed the induction of alloxan diabetes in mice as reported earlier (4).

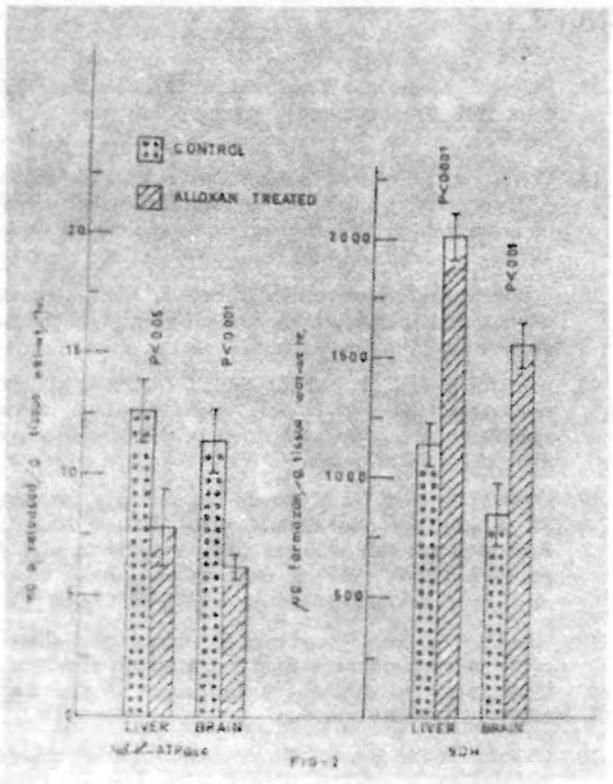


Na^+-K^+-ATPase activity: The enzyme (ATPase) activity in brain, and liver of mice decreased significantly following 7 days of alloxan treatment (Fig. 2).

SDH activity: Succinic dehydrogenase activity in the brain and the liver homogenates of Swiss mice increased significantly following 7 days of alloxan treatment (Fig. 2).

DISCUSSION

Alloxan, a B-cytotoxin, induces a "chemical diabetes" (alloxan diabetes) in a wide variety of animal species through damage of the insulin secreting cells (10). Induction of diabetes in these animals was confirmed by a significant rise in blood-glucose and fall in the liver glycogen level (11), as shown in other animal species. The lower activity of the glycolytic enzymes such as glucokinase, phosphofructokinase, glyceraldehyde-3- phosphate dehydrogenase (2) and of various isoenzymes of LDH (12) within a single metabolic pathway possibly points to decreased supply of glucose to the cells during induced diabetes. The entry of glucose into cell is mediated by specific transport ATPase such as Na^+-K^+-ATPase in conjuction with the movement of Na^+ and K^+ ions across the cell membrane (13) and the activity of this enzyme is largely influenced by the concentration of the hormone insulin in the blood (1). The results indicate a decreased activity of this enzyme in the brain and the liver of alloxan diabetic Swiss mice. Such an observation conforms to the earlier reports that the transport of ions and glucose across the cell membrane is reduced during diabetes (14). Most transport ATPases, so also the Na^+-K^+-ATPase require complete integration of cell membrane for their activity (15). A certain degree of fluidity seems essential for



Na^+-K^+-ATPase and the fluidity of the phospholipids bilayer of the membranes, to a large extent, is determined by the fatty acids (16). Decreased enzyme activity was reported with the decrease in phospholipid molecules (17). It is possible that the reductions in the activity of this enzyme in tissues of alloxan diabetic Swiss mice is chiefly due to decreases in both fatty acid and phospholipid levels since reductions in the contents of these liquid fractions has already been observed in erythrocyte membrane (18) and cardiac tissue (19) during diabetes mellitus. On the other hand, the possibility of nonenzymatic glycosylation of the above enzyme, as it occurs in human diabetes (20) leading to its malfunction (21) cannot be ignored.

Consequent upon the decreased activity of glycolytic enzymes, the concentration of the end product of glycolysis, i.e. pyruvate entering the oxidative pathway is low as evident by decreased rate of oxidative decarboxylation of pyruvate to Acetyl CoA through a reduction in the activity of pyruvate dehydrogenase complex (3). As such one might expect a low yield of TCA cycle enzymes and low energy output unless adequate acetyl CoA is derived from oxidation of lipids. Therefore the present study also deals with the activity of TCA cycle enzyme succinic dehydrogenase (SDH) in tissues of alloxan diabetic Swiss mice. This enzyme directly involved in the aerobic oxidation of food stuff and is probably the best candidate for such studies because it is tightly bound to the inner mitochondrial membrane and the oxidation of succinate to fumarate in animal tissues is linked to O_2 via cytochrome and cytochrome oxidase (22).

The results indicate that the activity of the enzyme SDH in brain and liver homogenates of alloxan diabetic Swiss mice increased significantly over the respective control values (Fig. 2) in conformity with the earlier observation (23). Such observations make the availability of acetyl CoA from non-carbohydrate precursors more plausible and free entry of this metabolite into TCA cycle in sufficient amounts so as to reflect an increase in the rate of aerobic oxidation. It is known that such a metabolite can be derived from oxidation of non-carbohydrate precursors such as lipids. Moreover, diabetes is invariably associated with increased fatty acid oxidation (18). Therefore, one might suggest that the increased activity of SDH in tissues of diabetic Swiss mice on the face of decreased rate of glycolysis, is mediated through increased supply of substrates from accelerated oxidation of fatty acids, as has also been pointed out earlier (23).

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