Determination of Percent Incorporation of Radio Tracer (14c - Acetate and 14c-Cholesterol) in Wet Tissues of Streptozotocin Inducced Diabetic Rat Vis-Control Rats.

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Abstract

The effect of streptozotocin induced diabetes on the metabolism of 14c- cholesterol was investigated by Ip injection of uniformly labelled 14c- cholesterol and compared with sham control rats, siginficant metabolic changes were observed. Uptake of acetate (14c-U-acetate) label in all SZID tissues reduced (range by 32% to 80%; P<0.01) except pancreas (where uptake elevated by 393%, P<0.001) by streptozotocin induced diabetes mellitus. Thus, the tissue capacity for uptake of 14c- activity was reduced tremendously (73% -97%) P<0.001) by all tissues (liver, heart, kidney, muscle, pancreas, adipose, brain and serum) due to diabetes.

Uptake of cholesterol [U-14c- cholesterol] was reduced only in muscle and brain (by 13% and 26%) but elevated in all other tissues in diabetic rats as opposite to metabolic pattern of 14c-acetate (P<o.oo1), radiolabelling of cholesterol was reduced in muscle, pancreas and brain (P<0.001) while in all other tissues elevation of labelling occurred by 22%-433%(P<o.001) due to diabetes.

Introduction

Acetyl CoA, active acetate, arises partly from oxidative decarboxylation of pyruvate and partly from beta oxidation of long chain fatty acids and several amino acids. It is no exaggeration to call active acetate a metabolite of cardinal importance.

The energy content of acetyl CoA, i. e.-its free energy of hydrolysis is a little higher than that of ATP and yet this reactive, ester cannot be considered to be the universal reservoir of energy. Rather acetyl CoA is the reactive intermediate that provides the C_2 fragment for so many conversion and synthesis. By being converted to acetyl CoA the various intermediatary metabolites all enter the common terminal pathway of

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break-down, known as the citrate cycle. Active acetate, therefore, is essential for the proper functioning of the citrate cycle. Synthesis with active acetate- acetyl CoA is an important starting material for biosynthetic reactions. First of all, fatty acids can be formed from it. This is the major pathway followed during the conversion of carbohydrate to fat. A second important anabolic pathway of acetyl CoA produces the isoprenoid lipids, especially the steroids. From acetyl CoA, amino acids, protein and conbolydrate also are formed.

In insulin deficiency or in fasting, utilization of acetyl CoA by the tricarboxylic acid cycle is depressed, and also the synthesis of fatty acids. As a result of the tendency of the insulin-deficient animal is to convert all its metabolically available resources into blood glucose, Under these conditions acetyl CoA tends to be shunted into the formation of HMG CoA, leading to increased formation of ketone bodies and cholesterol. However, the amount of acetyl CoA that can be shunted into cholesterol formation is very limited, so the great bulk of the HMG CoA is converted into the Ketone bodies, acetoacetic and 13-hydroxy butyric acids, whose concentration in the blood and urine may become very high in diabetes mellitus or depancreatized animals. Thus, insulin deficiency causes profound alterations in a complex network of metabolic interrelationships(1)

Cholesterol which can be derived from the diet or manufactured De novo in virtually all the cells, plays a number of important roles. In diabetes the plasma cholesterol level is usually elevated and this may play a role in the accelerated development of the artheriosclerotic vascular disease which is a major long-term complication of diabetes in man. In severe diabetes cholesterol synthesis is decreased, part of the rise in plasma, cholesterol level is due to an increase in the cholesterol containing very low density lipoprotein and low density betaliporprotein secondary to the great increase in circulating triglycerides. Another factor may be a decline in hepatic degradation of cholesterol when it exceeds the decline in cholesterol synthesis contributes to the rise (2). Because cholesterol and acetyl CoA are very important metabolic precursor which play a central role in our biological system so that we were interested to investigate the metabolic pattern in diabetic condition of these two precursor.

In this paper we have seen the effect of steptozotocin induced on the metabolism of 14c-acetate and 14c-cholesterol. It was investigated by IP injection of uniformly labelled 14c-acetate and 14c-cholesterol, pair of rat was used separately in both cases and compared with Sham control rats. This investigations include the areas involved in the studies of the up take of radioactivity from the radio tracer compound by wet tissues, from which we

can get some information about the metabolic pattern of the respected compound.(3)

Materials and Methods

(a) **Animal Management :**

(i) **Animal** : Long-evens rats of black and white strain were used for this study. Adult animals between the ages of 75 to 120 days and weighing between 200 to 250 gms were selected. The experimental animals were obtained from the animal resources branch, diabetic association, Shahabag, Dhaka, Bangladesh.

(ii) **Maintenance :** Each of the selected rat was housed in a screen bottomed cage (Geo, H, Walsman Manufacturing Co. Baltimore; M.D) and minatained in a constant temperature enviroment with 12 hrs. of artificial light per 24 hours. The temperature of the room in which the rats were kept was 26°C+ 5°C. The rats were fed on a good quality basal diet and water ad libitum. The diet supplied for 7 days to each rat was approximately isocaloric, (i.e. Approximately, 20 gm of diet per rat per day(4).

(b) **Induction of diabetes :** Diabetes were induced in the experimental rats by injection of streptozotocin. The drug (1 ml/200 gm body wt)(1 ml contains: steptozotocin 100 mg and citric acid 22mg) was administered intraperitoneally after 24 hrs of fasting. The Sham control rats were given equal amount of normal saline, pair of rat was used for both cases. The degree of occurrence of diabetes was monitored by estimating blood glucose level using glucose strip in a reflectometer (Relemat,labora Mannheim; GMbH für labor-technik) and by urine analysis for detecting increased concentration of glucose by benedict reagent. One drop of blood was collected from a stab incision at the ventral position of the base of the tail for the examination of blood glucose level. Blood glucose was estimated by modified somgyi-Nelson Method (5).

(c) **Radiotracer Specification** : The compound 14c-acetate (U-14c). Specific activity of 57.9 μci/mmol and 14c-cholesterol 55.7 μci/mmol (Code CF A229, Batch 64) was supplied by ICN Isotope and Nuclear Division, 2727 Campus Dive, Irving, U.S.A. Its purity was 99%, as checked by paper Chromatography.

(d) **Administration of Radiotracers :** A tracer does of 4 μci/0.2 ml cholesterol (U-14c) were administered IP following an overnight fasting rats. Pair of rat was used for every cases separately. The Sham control rats were also given equal amount of radioactive acetate and cholesterol. Pair of rat was used in this case also.

(e) **Collection of Blood and Tissue samples from rat :** For measurement of radioactivity after 24 hrs of the IP injection of 0.2 ml of the radiotracer. The leg muscle, liver, kidney, heart, brain, pancreas and adipose tissues were excised from rats exsanguinated under ether anesthesia. The rat were anesthetized by an overdose of ether in a desicator and were surgically treated with fine pair of scissors to dissect the tissues. These were used for the study of the percent incorporation of the radiolabelled substrate in wet tissues. Blood was collected by puncturing the heart and was used for the same purpose.

(f) **Processing and storage :** Animal tissues excised for radioactivity measurement were washed several times with ice-cold normal saline 0.9% NaCl) and freed extraneous material. The organs of both sham control and experimental rats were blotted dry and weighed accurately in an electrical balance (Mechaniki Zaklades Precyzjnej, Gdnask, Poland). The weighed tissues were wrapped in aluminium foil and stored in a deep freeze (ultra low revco. MC. West Columbia) at -20°C until used.

(g) **Sample preparation :** About 5 to 50 mg wet tissue and 0.2 to 0. 6 ml serum, in 24 mm opening L. S. Counting vial was added 2.5 ml 10% KOH and digestion was allowed to proceed at 50°C-60°C in water bath . Acid hydrolysis was avoided because low PH produces very strong quenching. The chemiluminescence was miniminsed by alkali digestion. To get high counting efficiency, the digested coloured tissue was bleached by adding 0.04 ml 30% H_2 0_2 in each vial.

(h) **Measurement of Radioactivity :** Samples of wet tissue were prepared in 20 ml low K-glass. All samples were counted for thrice for one minute using a packard tricarb liquid scintillation spectrometer (Model No: 3255) with a Teletype electric type writer with automatic background substraction at BIRDEM. Result shown (count per min. CPM) are the mean of three counts, counting efficency (CE) was determined by using an internal standard (% counting efficency, between 86%- 88%) and standard curve for varying degree of quench was constructed using the formula

$$\% \text{ CE} = \frac{\text{CPM X100}}{\text{D.P.M}}$$

(i) **Radioactivity Calculations :**

Following formulations were used for radioactivity calculations : % incorporation of radiocativity per 100g wet tissue

$$= \frac{\text{CPM}}{\text{TWmg X x}\mu\text{ci}} \text{ X} \frac{100}{\% \text{ CE X } 2.22 \text{ X } 10^6}$$

Where, a) Twmg =tissue weight in milligrmme.

b) xμci= microcurie injected

c) $\text{dpm} = \frac{\text{CPM X 100}}{\% \text{ CE}}$

d) $2.22 \text{ X } 10^6$ is multiplied with x μci for converting μci into dpm.

(iii) 14C- activity interms of dpm per 100g tissue

$$= \frac{\text{CPM per100g wet tissue}}{\%\text{CE}} \text{ X } 100$$

Result

Tables 1 and 2 present data of the distribution of radioactivity, as incorporaration per 100gm wet tissue, 24 hrs after pulsing in Streptozotocin induced diabetic and Sham control rats in serum, Liver, muscle, kidney, heart, adipose tissue, pancreas and brain. Tissues uptake was examined in terms of 14c-activity (10^6 dpm 100g wet tissue).Standard deviation on which we found in our result, can be neglected because its range is very small.

Data of the percent incorporation of acetate (U-14c) in streptozotocin induced diabetic and Sham control rats has been reported in table 1. In all diabetic tissues percent incorporation was depresssed except in pancreas where it was accelerated by 393% compared to control. SZID caused a decrease in percent incoporation of radioactivity in liver, heart, kidney, muscle, adipose tissue, brain and serum by 31%(P<0.), 79%(P<0.1), 67% (P0.2.), 72%(P<0.2), 79%(p<0.100), 80%(P<0.100) and 63%(P<0.1) respectively. Fig: 1 showing baragraph which indicates a clear picture of the % incorporation of radio tracer (14c-acetate) in tissues of SZID and SC rats. Table ll reports the data of the distribution (U-14c) in SZID and SC rats. As

opposite to acetate (U-14c), the percent incorporation of cholesterol (U-14c) in all diabetic tissues was increased except in muscle and brain. SZID showed a decrease in muscle by 13% and brain by 26% compared to control. In SZID the precent incorporation was increased by 427% in liver, 6.89% in muscle, 220% in kidney, 193% in pancreas 59% in adipose and 451% in serum. Fig. 2 showing a baregraph which indicates a clear picture of the % incorporation of radio tracer (14c-cholesterol) in tissues of SZID and SC rats.

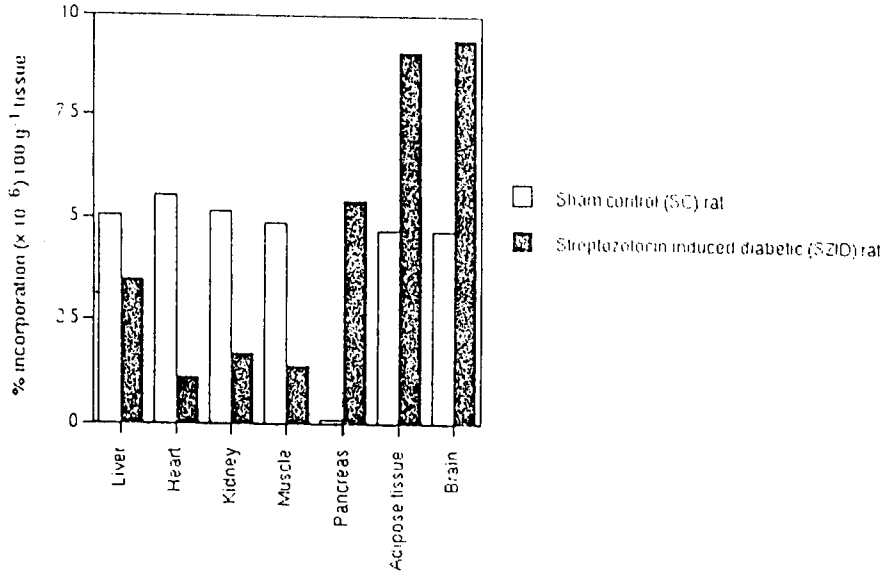

Fig. 1. % Incorporation of radioactivity by wet tissue after pulsing with uniformly labeled 14C-acetate.

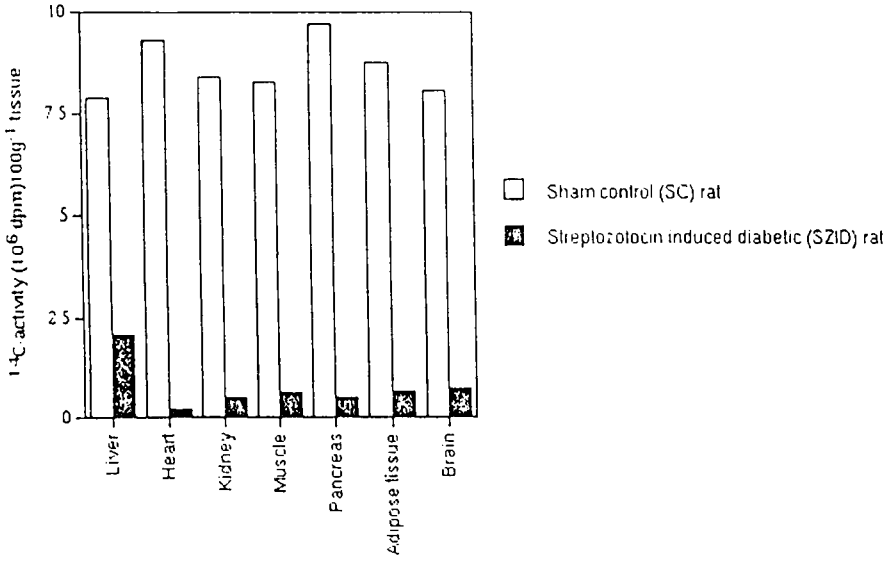

Fig. 2. Tissue distribution of radioactivity after pulsing with unformly labeled ^{14}C-acetate.

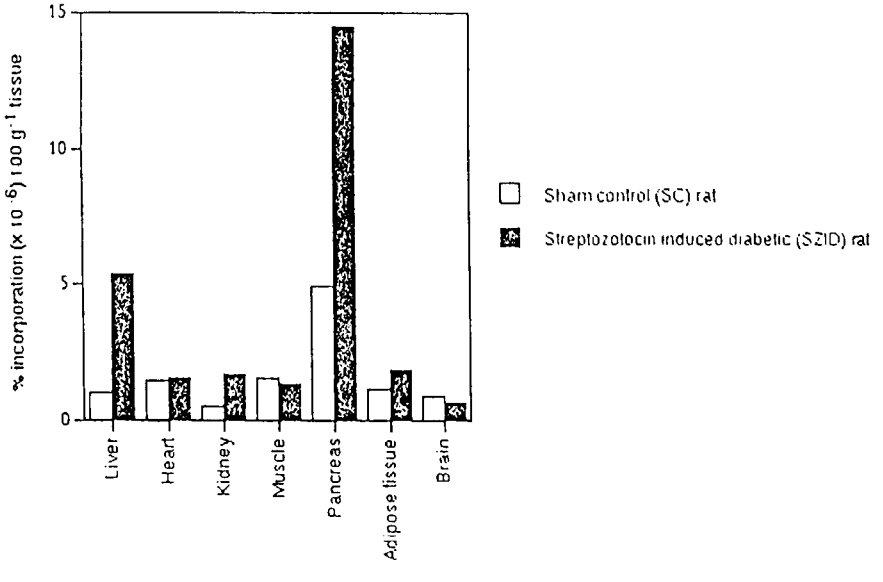

Fig. 3. % Incorporation of radioactivity by wet tissue after pulsing with uniformly labeled 14C-cholesterol.

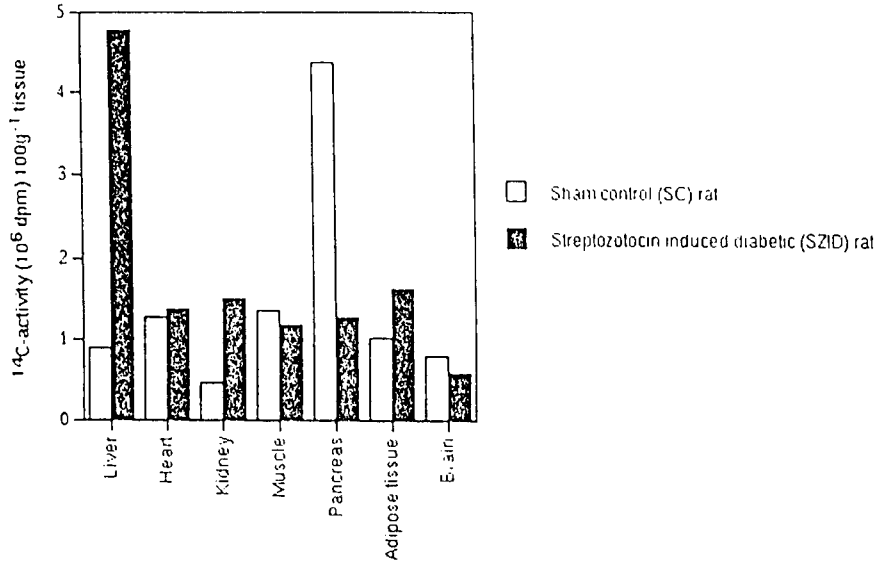

Fig. 4. Tissue distribution of radioactivity after pulsing with uniformly labeled 14C-cholesterol.

Discussion

The shifting of metabolic patterns as regulated by cellular receptors sensitivity in metabolic disorder was studied in streptozotocin induced diabetic (SZID) rats with uniformly labelled precursors viz. 14c-acetate and 14c-cholesterol and compared with Sham Control (SC) rats. In case of 14c-acetat and 14c-cholesterol metabolic study,uptake of acetate (U-14C) was increased by 39% (table-1). Interestingly, we noticed an increased uptake of cholesterol (U-14c) in almost all SZID tissue except in muscle and brain, where the uptake of cholesterol(U-14c) was decreased by 13% ($P<0.001$), and 26%($p<0.001$) respectively, (table ll).

The above findings are in accord with the observations of Goldstein (6) who reported that insulin stimulates the transport of sugar and amino acids by influencing membrane structure and function. The reports of Morgan et al.

(7) and SZID heart, muscle, adipose tissues, and kindey. An increased uptake of acetate, (U-14c) was observed in SZID pancreas only.

The increased incorporation of cholesterol in almost all SZID tissues except in muscle and brain indicates a change in metabolic pattern in diabetic condition. Their preferential incorporation by SZID rat tissues indicates that perhaps their transport across the cell membrane is more insulin independent except brain and muscle compared to acetate metabolites. However, it is established by our finding that diabetes mellitus defintely shift the metabolic pattern and distribution of radioactivity of different tissues invariably.

Refernce

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