A sigmoidal relationship between liver stearoyl CoA desaturase activity and serum hormone concentrations caused by streptozocin and its antagonists

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Summary. Stearoyl CoA desaturase activity in liver microsomes, and insulin, thyroxine, and triiodothyronine levels in serum were measured after administration of streptozocin (STZ) and its antagonists to rats. The effect of STZ, which caused hyperglycemia and inhibited the desaturase activity, was antagonized by 2-desoxyglucose and 3-O-methyl-glucose; 1-O-methyl-3-desoxyglucose and 1-O-methyl-3-O-methylglucose were without any effect. The enzyme activity plotted against insulin levels showed a broad sigmoidal curve, whereas the activities versus thyroid hormone levels showed steeper sigmoidal curves.
Key words. Stearoyl CoA desaturase; streptozocin; streptozocin antagonist; insulin effect on desaturase; thyroid hormone effect on desaturase.

The stearoyl CoA desaturase belongs to the class of Δ^9-desaturases. It converts stearic acid to oleic acid in the liver and other tissues. The biological significance and regulatory properties of this enzyme have been investigated extensively[1,2]. Regulatory modifiers of the enzyme include hormones[1-3], nutrients[1,4] and chemicals[5]. Among these modifiers, hormones regulate de novo synthesis of the enzyme protein[6]. Joshi and Aranda[7] have shown that insulin plays an obligatory role in inducing the desaturase activity, but triiodothyronine (T_3) or hydrocortisone act only as potentiators of the insulin effect. However, their experiments were performed with a chick liver explant culture system. Many related studies were carried out using subcellular fractions of animal tissues in which only the effect of a fixed dose of hormone was examined[1-6]. Thus, there is no reliable information about the correlation between desaturase activity in tissues and varying hormone levels in blood.

In the present study, we determined how significantly cellular enzyme activity relies on blood hormone concentrations. Since we had confirmed earlier that streptozocin (STZ) reduced both blood insulin and thyroid hormone levels[8], STZ and its antagonists were administered to rats, either singly or in combination, to modulate the hormone concentration in serum. At each hormone concentration, simultaneous changes in the desaturase activity in microsomes were measured.

Materials and methods. The STZ antagonists, 3-O-methyl-glucose (3-OMG) and 2-desoxyglucose (2-DG), were obtained from Nakarai Chemicals Ltd. (Kyoto, Japan). C_1-methylated analogs of 3-OMG and 2-DG, 1-O-methyl-3-O-methylglucose (1-OM-3OMG), and 1-O-methyl-2-desoxyglucose (1-OM-2DG) were synthesized in our laboratory[9]. [^{14}C]-Stearic acid (59.0 mCi/mmol) was purchased from New England Nuclear Co. (Boston, MA, USA). Other chemicals of special reagent grade were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and Nakarai Chemical Ltd. (Kyoto, Japan).

Male Wistar rats, weighing 180–200 g, were given an i.v. injection of a single dose of 0.226 mmoles (60 mg) STZ/kg b.wt to induce diabetes. The inhibitors of STZ were administered in combination with STZ as follows: 2-DG, 10 mmoles/kg; 1-OM-DG, 10 mmoles/kg; 3-OMG, 6.9 mmoles/kg and 1-OM-3OMG 5 mmoles/kg. After 2 months, animals were sacrificed under a light anesthesia with ether. Blood glucose level was measured by a method described previously[10]. Serum glutamate-oxalacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) activities were measured by using a commercially available kit (type RM101-k, Yatoron, Ltd., Tokyo, Japan). The serum concentration of STZ was spectrophotometrically measured by the method of Forist[11].

The microsomal preparation of livers was obtained basically as described by the method of Hogeboom[12]. Stearoyl CoA desaturase assay was carried out by the method of Mercuri et al.[4] with the slight modification of the addition of 4 μM rotenone to the medium[13]. The analysis of oleic acid was performed by the method of Jones et al.[14]. Protein determination was done by the method of Lowry et al.[15] with bovine serum albumin as the standard.

Insulin was assayed by Insulin EIKEN RIA KIT (Tokyo, Japan). T_3 and thyroxine (T_4) were measured by SPACK T_3 and SPACK T_4 RIA KITs (Mallincrodt, St. Louis, MO, USA), respectively.

Results. Figure 1 shows the combined effect of STZ and various glucose derivatives on the blood glucose level and the stearoyl CoA desaturase activity in microsomes. When hyperglycemia due to STZ became severe, the enzyme activity decreased. The administration of 2-DG or 3-OMG[16] with STZ returned the glucose level to normal and the enzyme activity was restored. The STZ-effect was not affected by C_1-substituted analogs of the antagonist, 1-OM-2DG or 1-OM-3OMG, and the enzyme activity remained low. GOT and GPT activities were measured to examine tissue damage

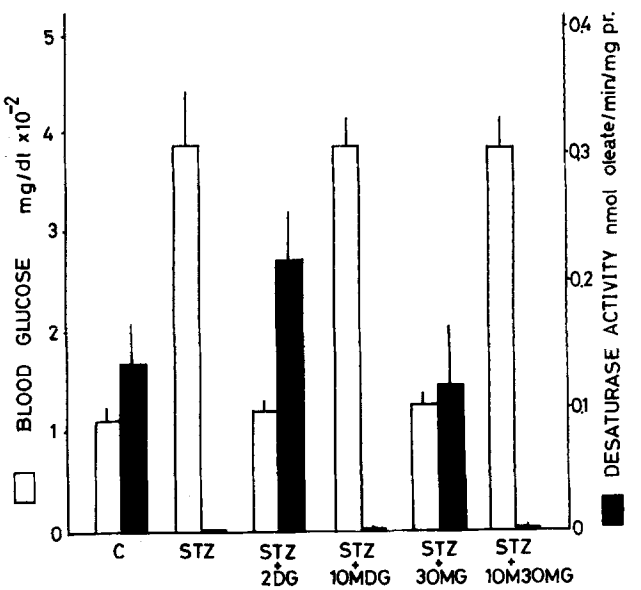

Figure 1. Effect of STZ with various glucose derivatives on blood glucose levels and microsomal stearoyl CoA desaturase activity. Each blood glucose level is expressed as the mean ± SD of six animals in each group. The enzyme activity represents the mean ± SD of five animals at each point. At least duplicate measurements were carried out for each specimen. Abbreviations; C: control, STZ: streptozocin 60 mg/kg intravenously injected. The following drugs were combined with STZ: 2 DG, 2-desoxyglucose; 1 OMDG, 1-O-methyl-2-desoxyglucose; 3 OMG, 3-O-methyl-glucose, and 1OM3OMG, 1-O-methyl-3-O-methyl-glucose.

by STZ. The values for GOT in the control and in the STZ-treated group were 125 ± 18 (n = 6) and 156 ± 20 (n = 6), respectively and for GPT, 51 ± 4 (n = 6) and 55 ± 8 (n = 5), respectively. These results indicated no significant tissue damage by STZ.

In figure 2, the activities of the stearoyl CoA desaturase after the combined administration of STZ and glucose analogs plotted against serum hormone levels are shown. Enzyme activity was not detected below 20 μU/ml of insulin, but became maximal when the hormone level reached approximately 80 μU/ml (fig. 2a). The relationship between the serum insulin level and the enzyme activity was not linear, but sigmoidal. When serum T_4 (fig. 2b) and serum T_3 (fig. 2c) were below 2.5 μg/dl and 70 ng/dl, respectively, the enzyme activity was not detectable. At higher levels of thyroid hormones, enzyme activities were readily observed, exhibiting steeper sigmoidal curves than that of insulin. The results also indicated that the optimal concentration of hormone for maximal activity of enzyme was within a physiologically normal range; namely 30–50 μU/ml for insulin, 4–5 μg/dl for T_4, and 80–100 ng/dl for T_3.

In order to examine directly whether STZ inhibits stearoyl CoA desaturase activity, various concentrations of STZ were added to the microsomal fraction containing stearoyl CoA and the enzyme activity was assayed. Stearoyl CoA desaturase activity was not affected by STZ over a concentration range of 2 μg/ml to 100 μg/ml which corresponded to the maximum serum STZ concentration detected 1 min after an i.v. administration of 60 mg STZ/kg of body weight to rats.

Discussion. Stearoyl CoA desaturase is impaired by STZ[4,16], and 3-OMG or 2-DG interferes with the diabetogenic effect of STZ[17], but the desaturase has not been examined in animals receiving both STZ and one of its inhibitors. The enzyme activity was protected by the addition of the antagonists of STZ, 2-DG and 3-OMG. However, 1-OM-2DG or 1-OM-3OMG neither reduced the effect of STZ nor restored the enzyme activity. Combining STZ with an analog, we could modify the blood hormone levels in the whole animal and examine the altered desaturase activity in liver. Since the half-life of the desaturase in situ is only 4 h[18], and the serum insulin or thyroid hormone level decreased severely with the drug-induced hyperglycemia, the enzyme activity in subcellular organelles was affected by the impaired de novo synthesis of enzyme protein and the deficiency of energy supplying materials.

When STZ was injected i.v., it disappeared rapidly from the blood within the first 30 min, and the maximal concentration of the drug was observed 1 min after the injection. Since the enzyme assay was carried out with liver microsomes from animals which had been diabetic for 2 months and the reported half-life of stearoyl CoA desaturase is 4 h[18], the possibility of a direct toxic effect of STZ on the enzyme would be small. However, to exclude the possibility, the in vitro effect of STZ on stearoyl CoA desaturase was tested at the maximally detectable concentration of the drug in serum, and the enzyme was found to be unaffected by STZ. Furthermore, the assessment of GOT and GPT indicated no appreciable damage of hepatic integrity by STZ. Thus, the direct damage to the liver would be minor, if any.

Evidence shown in figure 2 suggested a minimal threshold concentration of insulin or thyroid hormones in the blood for sustaining desaturase activity in the endoplasmic reticulum of rat liver. When the serum concentrations of insulin and thyroid hormones were modified from a low to a high level, the insulin-dependent desaturase activity (fig. 2a) was different from the thyroid hormone-dependent enzyme activities (fig. 2b and 2c). The slope of the former curve was less steep than the slopes of the latter, indicating that the blood insulin controls the tissue enzyme gradually in a broad range of its concentration, while the thyroid hormones mod-

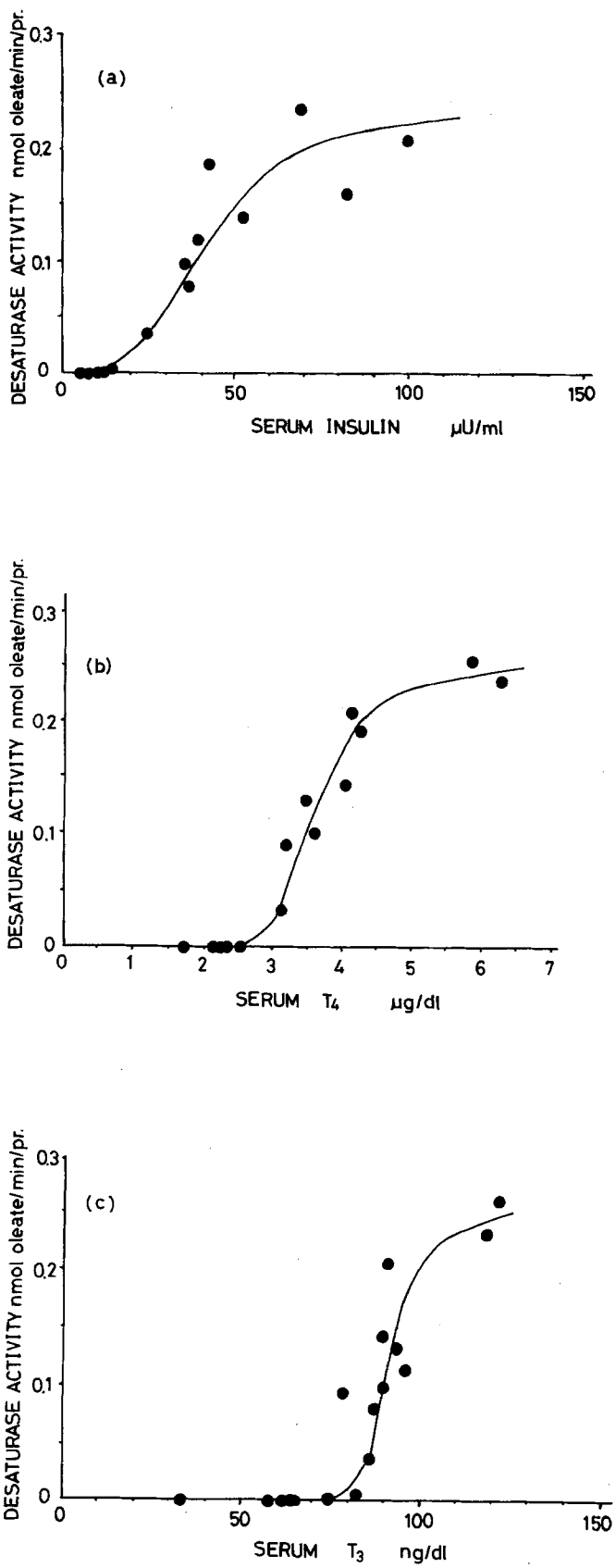

Figure 2. Relationship between serum hormone levels and desaturase activity in microsomes. Two or three animals in a group were given STZ singly or in combination with drugs as indicated in Materials and Methods. The enzyme activity of each individual animal was plotted against serum hormonal levels.

ulate the enzyme activity more drastically in a narrow range of their concentration. Both types of hormonal control may cooperate with each other to modulate an appropriate enzyme activity. This hypothesis is compatible with the results of Joshi and Aranda who showed that insulin is obligatory and thyroid hormone is secondary[7].

Our preliminary data also showed that starvation reduced serum insulin levels, thyroxine levels and thyroxine 5'-deiodinase activity in liver, but refeeding with glucose restored the hormonal levels to normal and simultanously elevated the enzyme activity (unpublished). The response in deiodinase activity to starvation and refeeding resembles that for stearoyl CoA desaturase[18]. Therefore, it seems likely that this 'multi-type' regulatory mechanism by hormones is of universal importance.

Since several biological factors[2], in addition to hormones, are involved in regulating the desaturase in vivo, it is difficult to determine the ranking of factors contributing to the enzyme activity. However, it is possible that each biological regulator functions to control the enzyme activity in its own characteristic way.

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Hepatic phosphatidylcholine synthesis in deficiency of lysine and threonine: effect of malathion

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Summary. Rats fed on a rice diet deficient in lysine and threonine showed increased activities of CDP-Choline pathway enzymes and incorporation of (methyl-^{3}H)-choline into hepatic microsomal phosphatidylcholine, compared to rats fed on the same diet supplemented with lysine and threonine. However, the amount of microsomal phosphatidylcholine was significantly decreased in rats fed a deficient rice diet. These results suggest an enhanced phosphatidylcholine catabolism in rats fed on a rice diet deficient in lysine and threonine. Malathion administration reduced the amount of phosphatidylcholine in all the groups.

Phospholipids are important structural and functional components of biological membranes. Reduced hepatic phospholipids after feeding low quality protein or protein-deficient diets have been reported by several investigators[2–4]. The active role of phosphatidylcholine (PC) in microsomal drug metabolism has been well established[5]; PC probably helps in electron transfer processes and also in accumulating nonpolar substrates. Conney et al.[6] have reported that malathion, chlorothion and parathion inhibit the in vitro microsomal metabolism of testosterone.

In the liver, PC is synthesized by more than one pathway; however, the major pathway is the CDP-choline pathway[7]. The rate-limiting steps of PC synthesis by this pathway are catalyzed by choline kinase and cholinephosphate cytidylyltransferase[8,9]. While studying the effect of malathion in various diets in rats, we found that the pesticide reduces metabolism of foreign compounds and inhibits hepatic PC synthesis. We reported earlier that malathion inhibits hepatic PC synthesis in rats by inhibiting CDP-choline pathway enzymes[10]. In the present study, we investigated whether the reduced microsomal PC in rats fed a diet deficient in lysine and threonine is due to reduced synthesis by the CDP-choline pathway and whether it is further reduced by malathion treatment.

Materials and methods. Adenosine triphosphate, choline chloride, cytidine triphosphate, activated charcoal, Dowex 1 × 8 (200–400 mesh), dithiothreitol, and 1-amino, 2,4 naphthol sulphonic acid were obtained from Sigma Chemical Co., St. Louis, MO. All organic solvents were purchased from British Drug House, Ltd., India. Malathion was a gift from M/S Excel Industries, India. (methyl-^{3}H)-choline chloride (sp. act. 6.4 mCi/m mole), phosphoryl-(methyl^{14}C)-choline (sp. act. 52 mCi/m mole) and CDP-(methyl-^{14}C)-choline (sp. act. 51 mCi/m mole) were obtained from Radiochemical Center, Amersham, England.

Male Wistar rats (40–50 g) obtained from the V.P. Chest Institute were fed a rice diet with and without lysine and threonine for fifteen days. The diets were prepared as described by Viviani et al.[11]. Malathion (100 mg/kg b.wt/day dissolved in ground nut oil) was administered orally each day. The controls received the vehicle only. After 15 days the rats were killed, livers were removed, microsomes and soluble fractions were separated. The yield and protein content of microsomes/g liver were not significantly different in all the groups. Choline kinase activity was determined by a modified method of Weinhold and Rethy[12] as described by Ishidate et al.[13]. Cholinephosphate cytidylyltransferase activity was assayed by the modified method of Ansell and