EFFECTS OF NICOTINAMIDE AND INSULIN ON GLYCOSYLATED HEMOGLOBIN AND BLOOD GLUCOSE IN THYROIDECTOMIZED STREPTOZOTOCIN-DIABETIC RATS

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Abstract—The present study deals with the effects of nicotinamide and insulin on glycosylated hemoglobin (HbA1) and blood glucose in thyroidectomized streptozotocin (STZ)-diabetic male rats. Nicotinamide (500 mg/kg body wt., i.p.) significantly decreased the levels of HbA1 and blood glucose in both STZ-treated (65 mg/kg body wt., i.v.) intact and STZ-treated thyroidectomized animals. Administration of NPH-insulin recovered hyperglycemia, and the increased HbA1 in thyroidectomized STZ-diabetic rats to control levels.

In our previous paper, we reported that glycosylated hemoglobin (HbA1) in thyroidectomized streptozotocin (STZ)-treated diabetic rats (two weeks after STZ i.v. injection) was higher than that in those which were thyroidectomized alone and that thyroidectomy abolished the reducing effect of both the norepinephrine-induced pressor response and the ACh-induced depressor response observed in STZ-diabetic rats. These results suggest that hypothyroid states may partially improve glucose metabolism and cardiovascular responses in STZ diabetic rats (1).

Nicotinamide given i.p. either prior to STZ or simultaneously with STZ protected against the diabetogenic effect of STZ in both rats and mice (2). Other prospective protecting agents such as nicotinic acid and N1-methylnicotinamide were not effective against STZ (3). The action of STZ was modified by nicotinamide adenine dinucleotide (NAD) (3).

In the present study, we examined the effects of nicotinamide and insulin on the levels of blood glucose and HbA1 in thyroidectomized STZ-diabetic rats.

MATERIALS AND METHODS

Adult male Wistar-Imamichi rats, weighing 200–250 g, were obtained from the Animal Bleeding Laboratory (Ohmiya, Japan). All animals were housed under the following conditions: temperature (23±2°C), humidity (55±5%), and lighting (14 hr-light and 10 hr-dark). The rats were maintained on a commercial laboratory chow (Labo MR Breeder, Nippon Nosan Kogyo Co., Yokohama, Japan) and tap water ad libitum. Bilateral thyroidectomy was performed surgically under light ether anesthesia seven days prior to experiment. STZ (Sigma Chemical Co., Saint Louis, Missouri, U.S.A.), freshly dissolved in 0.1 M citrate buffer (pH 4.6), was injected into a saphenous vein of the hind-limb (65 mg/kg body wt.). Control animals received citrate buffer (0.5 ml/100 g body wt.). Criteria for diabetes were decreased growth rate, glucosuria (determined by DIASTIX, Miles-Sankyo Co., Tokyo, Japan),
and hyperglycemia. Nicotinamide (Tokyo Kasei Kogyo Co., Tokyo, Japan) was dissolved in 0.9% saline and injected i.p. (500 mg/kg body wt.) 15 min before the administration of STZ. One group of diabetic rats was injected for 3 days with a daily dose of 6 units/animal of insulin (ISZILIN®-Shimizu, 40 units/ml, Shimizu Pharmaceutical Co., Shimizu, Japan) at twelve days after STZ. The other group of diabetic rats was injected for the same period with a daily dose of 4–10 units/animal of NPH-insulin (NPH-ISZILIN®-Shimizu, long-acting, 40 units/ml) the next day after STZ administration. The subcutaneous injections of insulin were performed twice a day, once in the morning and the other in the evening at the different sites. Three or 4 hr after the last injection, rats were decapitated. Body weights were determined initially, on days 4, 7, 11, and before sacrifice. Blood samples were obtained from the neck of the animals at decapitation. HbA₁ was determined by the Quik-Sep method (Seikagaku Kogyo Co., Tokyo, Japan) at 22-24°C. Blood glucose was measured by the conventional method using the o-toluidine-boric acid reagent. Data were analyzed by the Student's t-test; P<0.05 was considered to be significant.

RESULTS

1) Effects of nicotinamide on body weight in intact and thyroidectomized STZ-diabetic rats: As shown in Fig. 1, the increase in body weight was much reduced after STZ injection in both intact and thyroidectomized rats. On the other hand, the body weights of the intact and thyroidectomized rats in the nicotinamide-STZ group were decreased by approximately 5% of the age-paired control value at the next day after STZ injection. Thereafter, a progressive increase in body weight was observed in both groups in parallel with the age-paired control throughout the experimental period.

![Fig. 1. Effects of nicotinamide on body weight in intact and thyroidectomized streptozotocin-diabetic rats. Tx: thyroidectomized, STZ: streptozotocin, NicA: nicotinamide.](image)

2) Effects of nicotinamide and insulin on HbA₁ and serum glucose in intact and thyroidectomized STZ-diabetic rats: As shown in Fig. 2a, the value of HbA₁ was significantly increased after STZ in intact and thyroidectomized rats, and previous injection of nicotinamide protected rats from any increase of HbA₁ in both groups. Also, STZ induced hyperglycemia was completely prevented by the pretreatment with nicotinamide (Fig. 2b).

As shown in Fig. 3a, insulin abolished the increase in the levels of HbA₁ in thyroidectomized (P<0.01) or thyroidectomized STZ-diabetic rats (P<0.01), although no effect was observed in intact STZ-diabetic animals. Insulin partially prevented STZ-induced hyperglycemia in intact rats (P<0.05), but
Fig. 2. Effects of nicotinamide on glycosylated hemoglobin (HbA,) (a) and serum glucose (mean±S.E.) (b) in intact and thyroidectomized streptozotocin-diabetic rats. Numbers per group are indicated at the base of each column. *P<0.05, **P<0.01. Tx: thyroidectomized, STZ: streptozotocin, NicA: nicotinamide.

completely prevented it in thyroidectomized rats (P<0.01) (Fig. 3b).

3) Effects of NPH-insulin on body weight in intact and thyroidectomized STZ-diabetic rats: NPH-insulin clearly recovered the rate of body weight increase in parallel with the age-paired control throughout the experimental period in intact and thyroidectomized STZ-diabetic rats, although the growth was much suppressed in both STZ-treated groups (Fig. 4).

4) Effects of NPH-insulin on HbA1 and serum glucose in intact and thyroidectomized STZ-diabetic rats: As shown in Fig. 5, NPH-insulin prevented the increase of HbA1 in STZ-diabetic rats (Fig. 5a). Decrease in serum glucose was observed after NPH-insulin in intact and thyroidectomized rats. STZ-induced hyperglycemia was completely abolished by NPH-insulin in both groups (Fig. 5b).

DISCUSSION

The intraperitoneal injection of nicotinamide 15 min before STZ almost completely protected against STZ-caused hyperglycemia and increase of HbA1 in intact and thyroidectomized rats. It has been known that administration of nicotinamide results in a high NAD concentration. Kaplan et al. has reported that the level of NAD was as much as 8–10 times the control level after a single
Fig. 3. Effects of insulin on glycosylated hemoglobin (HbA1) (a) and serum glucose (mean ±S.E.) (b) in intact and thyroidectomized streptozotocin-diabetic rats. Numbers per group are indicated at the base of each column. *P<0.05, **P<0.01. Tx: thyroidectomized, STZ: streptozotocin.

Fig. 4. Effects of NPH-insulin on body weight in intact and thyroidectomized streptozotocin-diabetic rats. Tx: thyroidectomized, STZ: streptozotocin.

Injection in the mouse (4). Some explanations for this phenomenon have been proposed, and the most widely accepted is that it is caused by the stimulation of NAD synthesis (4). An alternative possible mechanism was proposed by Zatman et al. (5) who had found that nicotinamide non-competitively inhibited NADase so that newly synthesized NAD would accumulate. STZ reduced the NAD content of liver and certain tumors, and nicotinamide abolished this effect (3). It could be not exclusive that STZ causes the depletion of NAD content in the beta-cells of islets (6). However, the precise mechanism by which nicotinamide prevents the diabetogenic effect of STZ is not known at present.

The value of HbA1 was strongly correlated with the fasting blood sugar (7). It is well known now that HbA1 increases in patients with diabetes and that its value is considered to be one of the useful clinical parameters to indicate the state of long-term blood glucose control. However, only few studies have been reported on the changes in the values of HbA1 in rats and mice. An approximately two-fold elevation of HbA1 was observed in the genetically (C57BL/6J db/db and C57BL/KsJ ob/ob) and chemically induced (alloxan and STZ) diabetic mice. The degree of increase in HbA1 levels was not correlated with the severity of hyperglycemia, duration of diabetes, age of the mouse, or body weight. It is not known what factor(s) contributes to the steady state concentration of HbA1 (8).

The present authors found that the values of HbA1 were 1.413±0.071% (mean±S.E.)
Fig. 5. Effects of NPH-insulin on glycosylated hemoglobin (HbA1) (a) and serum glucose (mean±S.E.) (b) in intact and thyroidectomized streptozotocin-diabetic rats. Numbers per group are indicated at the base of each column.

**P<0.01. Tx: thyroidectomized, STZ: streptozotocin.

and 1.946±0.137% in intact and thyroidectomized rats, respectively. The HbA1 value increased in parallel with the serum glucose level after STZ in both intact and thyroidectomized rats. The treatment with nicotinamide before STZ-injection abolished the increase of HbA1 in both groups.

Trivelli et al. (9) reported that no appreciable differences in the values of HbA1 were observed between adult diabetic patients treated with and without insulin. In our animal experiments, the administration of insulin or NPH-insulin decreased the HbA1 and serum glucose levels in thyroidectomized STZ-diabetic rats. However, we cannot make any direct quantitative comparison of the activities of these different types of insulins because NPH-insulin and insulin were administered at different periods after STZ as described in Materials and Methods.

In conclusion, these results indicate that nicotinamide and insulin recovered hyperglycemia and the increased HbA1 in thyroidectomized STZ-diabetic rats to control levels.

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