

Effects of tungstate in neonatally streptozotocin-induced diabetic rats: mechanism leading to normalization of glycaemia

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Summary The effects of oral administration of tungstate to an animal model of non-insulin-dependent diabetes mellitus (NIDDM), the neonatally streptozotocin-induced diabetic rat was studied. Islet insulin content and beta-cell mass were lowered in these animals. Furthermore, the islets lost their ability to release insulin in response to an increase in glucose concentration. However, the hepatic glucose metabolism in these diabetic animals before the treatment was not significantly altered with regard to glycogen content, or glucokinase or glycogen phosphorylase activities compared with healthy animals. On the other hand, the activation state of glycogen synthase was higher although the total activity was unchanged. Moreover, a 20% increase in the concentrations of liver glucose 6-phosphate compared to their healthy siblings was observed. Oral administration of tungstate for 15 days normalized glycaemia in these

diabetic animals (4.6 vs 7.8 mmol/l). Tungstate administration was also able to normalize beta-cell insulin secretion in response to 16.7 mmol/l glucose stimulus, reaching values similar to those observed in healthy animals. Concomitantly, a partial recovery in the insulin content and in preproinsulin mRNA levels was found in the islets of treated animals, which was associated with an increase in the number of beta-cells in the pancreas (1.73 vs 0.86%). The treatment did not change the liver parameters studied, except that it restored glucose 6-phosphate concentrations to healthy values. These data suggest that tungstate administration causes a normalization of glycaemia through the restoration of islet function. [Diabetologia (1997) 40: 143–149]

Keywords Tungstate, nSTZ-diabetic rats, islets, insulin, glycogen.

When adult rats injected with streptozotocin (STZ-diabetic rats) were treated orally with tungstate a reduction in blood glucose levels and a normalization of hepatic glucose metabolism was observed [1]. Tungstate administration decreased glycaemia to levels similar to those observed in healthy animals, while

no effect was found in healthy animals. Moreover, in the liver of STZ-diabetic rats, tungstate treatment increased glucokinase, 6-phosphofructo-2-kinase and pyruvate kinase activity and decreased phosphoenolpyruvate carboxykinase expression to almost the same levels as those measured in healthy animals. These changes led to a partial restoration of glycogen, glucose 6-phosphate and fructose-2,6-bisphosphate levels [1]. These combined changes result in a reduction of glucose production and also in an increase in glucose disposal, thus reducing hyperglycaemia. Therefore, tungstate is an efficient tool to reduce hyperglycaemia in insulin-deficient rats. We therefore examined whether tungstate could exert effects on other animal models of diabetes. We selected adult rats injected with streptozotocin during the neonatal period (nSTZ-diabetic rats), an animal model of

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Abbreviations: STZ, Streptozotocin; nSTZ-diabetic rat, neonatally streptozotocin-injected rats; STZ-diabetic rat, adult streptozotocin-injected rats; ALT, alanine transaminase; AST, aspartate transaminase; Glc 6-P, glucose 6-phosphate; SSC, saline sodium citrate.

non-insulin-dependent diabetes mellitus (NIDDM). In these animals mild hyperglycaemia appears between 1 and 2 months of life together with a partial deficiency in insulin. It is also known that the functional behaviour of the endocrine pancreas is characterized by a preferential impairment of the secretory response to D-glucose as distinct from other nutrient or non-nutrient secretagogues [2–4]. In this paper we describe the effects of tungstate administered orally in this animal model, and the mechanism by which tungstate was able to normalize glycaemia in these diabetic animals.

Materials and methods

Materials. Sodium tungstate was from Carlo Erba (Milano, Italy) and STZ was from Sigma (St. Louis, Mo., USA). Enzymes and biochemical reagents were either from Boehringer Mannheim (Mannheim, Germany) or Sigma. All other chemicals were of analytical grade.

Animals and experimental design. Wistar rats obtained from Charles River were allowed to breed. Diabetes was induced in new-born males by a single injection of STZ (100 µg/g body weight) in 0.9% NaCl with 100 mmol/l sodium citrate (pH 4.5) on their first day of life [5]. The animals were monitored for hyperglycaemia after the first month of life. The animals that became diabetic were used for the experiments when they were 9 weeks old. The STZ-injected rats that did not develop diabetes were discarded. At the beginning of the experiment, both the diabetic animals and their healthy siblings were divided into two different groups (untreated and tungstate treated). In the first group (untreated), rats received distilled water as drinking water. The other animals (treated) were given a solution of 2 mg/ml of sodium tungstate in distilled water. The animals were kept under a constant 12-h light-dark cycle (lights on at 08.00 hours) and were allowed to eat and drink ad libitum. The treatment was carried out for 15 days. During this period, the fluid and food intake was measured between 10.00 and 12.00 hours; and the body weight and blood glucose (Reflotron; Boehringer Mannheim) were measured every 2 days.

At the end of the treatment, rats were killed by decapitation. Immediately, blood was collected to measure serum parameters and additional blood samples were collected on EDTA. After centrifugation at 1000 *g* for 15 min at 4 °C, the plasma was removed and stored at –20 °C. Plasma insulin was measured in 100 µl of plasma by radioimmunoassay (CIS, Bioternational, Gif-Sur-Yvette, France). Livers were excised and sliced; fragments were either used immediately to measure enzyme activities or rapidly frozen in liquid N₂ for later processing. For islet isolation, the pancreas was inflated with Hank's Balanced Salt Solution (HBSS) solution by cannulation of the pancreatic duct and then dissected and incubated with collagenase. When the pancreas was used for morphometry, it was dissected and immediately frozen in liquid N₂ until the procedure.

Analytical procedures in serum samples. Serum alanine transaminase (ALT), aspartate transaminase (AST), urea and creatinine were measured spectrophotometrically by standard techniques adapted to a Dax 72 analyser (Bayer, Bayer Diagnostics, Leverkusen, Germany).

Enzyme assays. Glycogen synthase and phosphorylase activities were determined in fresh liver samples homogenized in 10 volumes of ice-cold 10 mmol/l Tris/HCl buffer (pH 7.4) containing 150 mmol/l KF, 15 mmol/l EDTA, 0.6 mol/l sucrose, 1 mmol/l phenylmethanesulfonyl fluoride, 1 mmol/l benzamidine, 25 µg/ml leupeptin and 50 mmol/l β-mercaptoethanol. The homogenates were centrifuged at 10000 *g* for 15 min at 4 °C and the supernatants were used for determinations. Glycogen synthase activity ratio (-Glc 6-P/ + Glc 6-P) and total activity were measured as previously described [6]. Glycogen phosphorylase *a* activity was determined as previously described [7]. Glucokinase activity was measured in fresh liver samples homogenized in 10 volumes of ice-cold 50 mmol/l Tris/HCl buffer (pH 7.4) with 1 mmol/l EDTA, 100 mmol/l KCl, 300 mmol/l sucrose and 10 mmol/l β-mercaptoethanol. The homogenates were centrifuged at 10000 *g* for 15 min at 4 °C. Glucokinase activity of the supernatants was determined as previously described [8].

Metabolite assays. Liver glycogen content was determined as previously described [9]. Frozen liver samples were homogenized with 10 volumes of ice-cold 30% (w/v) KOH and heated at 100 °C for 15 min; glycogen was determined after ethanol precipitation and enzymatic hydrolysis to glucose. Glc 6-P levels were determined in the neutralized perchloric extracts from frozen samples, as described previously [10].

Pancreatic islet isolation and analysis. The pancreatic islets were isolated by the collagenase method [11]. Islets were separated from the remaining exocrine tissue by handpicking under a stereomicroscope. Batches of eight islets were incubated in a shaking water bath for 90 min at 37 °C in 1.0 ml bicarbonate-buffered medium containing bovine serum albumin (5.0 mg/ml) and D-glucose at 2.8 or 16.7 mmol/l. For the first 10 min of incubation, the vials containing the incubation medium with the islets were gassed with O₂: CO₂ (95:5). At the end of the incubation period supernatants were stored at –20 °C until assayed for insulin by RIA. Insulin content was measured in these islets after they were disintegrated by ultrasonic disruption at 4 °C in 0.5 ml acid-alcohol solution (75% (v/v) ethanol, 23.5% bi-distilled water and 1.5% (v/v) 10 mol/l HCl), and centrifuged at 1500 *g* for 10 min at 4 °C, and the supernatants were kept at –20 °C until insulin was assayed by RIA.

Insulin mRNA. Total islet RNA was prepared from 250 islets by a modification of the Chomczynski method [12] (RNAzol B, TEL-TEST, Friendswood, Texas, USA) and analysed by Northern blotting. Aliquots of 20 µg RNA were separated by electrophoresis on a 1% agarose-5% formaldehyde gel and transferred to a nylon membrane (Hybond N, Amersham, UK). Membranes were prehybridized for 1 h and hybridized in Rapid Hyb buffer (RPN 1636, Amersham) for 8 h at 65 °C with an insulin cDNA probe (gift of G. Bell, Chicago, Ill., USA) labelled with ³²P by random priming. The blots were washed once in 2 × saline sodium citrate (SSC) 0.1% SDS at room temperature and three times in decreasing concentrations (1 × to 0.1 ×) of SSC 0.1% SDS at 65 °C. They were then exposed to a Kodak X-AR film and an intensifying screen at –70 °C. The resulting autoradiographs were quantified by scanning densitometry using Cue-2 (Olympus, Tokyo, Japan), and values for insulin mRNAs were normalized to the ethidium bromide-stained 28S ribosomal RNA.

Morphometric analysis of the islets. Serial sections were cut from each pancreas block and immunostained for insulin using the indirect peroxidase technique. Antisera to insulin (Dako, Glöstrup, Denmark), was used at a dilution of 1/10 and peroxidase conjugated anti-guinea pig (Sigma) was used as the

Table 1. Fluid and food intake, and tungstate intake in tungstate treated or untreated diabetic and healthy rats

| | Fluid intake (ml · kg ⁻¹ · day ⁻¹) | Food intake (g · kg ⁻¹ · day ⁻¹) | Weight gain (g/15 days) | Tungstate intake (mg · kg ⁻¹ · day ⁻¹) |
|----------------------|--|--|----------------------------|--|
| <i>Healthy rats</i> | | | | |
| Untreated (10) | 100 ± 5 | 75 ± 4 | 52 ± 7 | 0 |
| Treated (12) | 77 ± 5 ^a | 71 ± 4 | 41 ± 9 | 154 ± 9 |
| <i>Diabetic rats</i> | | | | |
| Untreated (12) | 145 ± 10 ^b | 80 ± 4 | 36 ± 8 | 0 |
| Treated (12) | 85 ± 7 ^c | 68 ± 3 ^d | 21 ± 4 ^a | 169 ± 13 |

Results are expressed as the means ± SEM for the number of animals indicated in parentheses

^a $p < 0.01$ compared to untreated healthy rats;

^b $p < 0.0001$ compared to untreated healthy rats;

^c $p < 0.0001$ compared to untreated diabetic rats;

^d $p < 0.05$ compared to untreated diabetic rats

second antibody. The islet surface was calculated after o-toluidine staining. Morphometry was performed using a manual optical picture image analyser (Model MOP-01, Olympus, Tokyo, Japan) on a projected image of the histological sections of the pancreas as described previously [13].

Statistical analysis

Results are given as the mean ± SEM for the indicated number of rats. Comparisons among the different experimental groups were carried out by unpaired Student's *t*-test and ANOVA. Differences were considered statistically significant at $p < 0.05$.

Results

Effects of tungstate treatment on physical status and blood parameters Untreated nSTZ-diabetic rats showed a mild hyperglycaemia (7.8 ± 0.2 mmol/l) which was normalized by tungstate treatment. After 6 days of tungstate administration the glycaemia of these animals reached levels (4.6 ± 0.1 mmol/l) equal to those of their healthy siblings (4.6 ± 0.1 mmol/l), that were maintained throughout the treatment. Tungstate administration to healthy animals did not exert any significant effect on glycaemia (Fig. 1).

In nSTZ-diabetic rats, liquid consumption increased compared with untreated healthy rats (Table 1). Administration of tungstate to diabetic rats reduced liquid intake to levels similar to those of untreated healthy rats (Table 1). Oral administration of tungstate to healthy rats slightly reduced their liquid intake (Table 1).

To ascertain whether tungstate induced hepatic or renal damage, serum catalytic concentrations of ALT and AST, and creatinine and urea concentrations were measured. Tungstate treatment did not induce any significant modification in these parameters, either in healthy or in diabetic animals, indicating that tungstate did not exert any appreciable toxic effect on the liver or the kidney (Table 2).

Characterization of hepatic glucose metabolism in nSTZ rats. In a previous report [1] we showed that

Table 2. Effect of tungstate treatment on various blood parameters

| | ALT (U/l) | AST (U/l) | Creatinine (μmol/l) | Urea (mmol/l) |
|----------------------|--------------|--------------|------------------------|------------------|
| <i>Healthy rats</i> | | | | |
| Untreated (10) | 84 ± 6 | 151 ± 13 | 34 ± 6 | 36 ± 6 |
| Treated (12) | 68 ± 9 | 125 ± 11 | 39 ± 3 | 40 ± 4 |
| <i>Diabetic rats</i> | | | | |
| Untreated (12) | 87 ± 11 | 133 ± 18 | 42 ± 2 | 36 ± 4 |
| Treated (12) | 85 ± 8 | 110 ± 17 | 34 ± 6 | 36 ± 4 |

Results are expressed as the means ± SEM for the number of animals indicated in parentheses

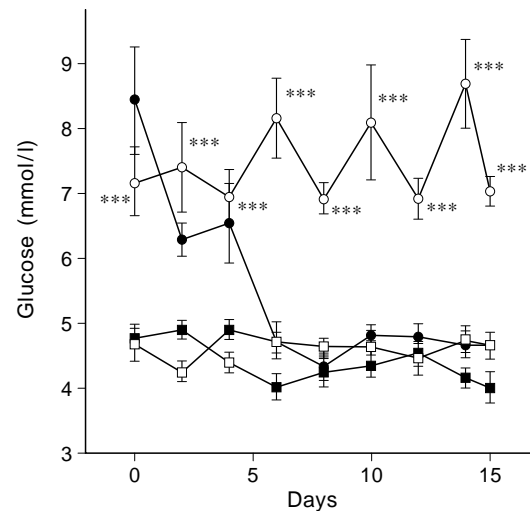


Fig. 1. Effects of tungstate treatment on blood glucose levels in vivo. Glycaemia was measured throughout the treatment from untreated healthy (HU, □), treated healthy (HT, ■), untreated diabetic (DU, ○) and treated diabetic (DT, ●) animals. Values are mean ± SEM ($n = 12$). *** $p < 0.0001$ compared with untreated healthy animals

in the STZ-diabetic rat hepatic glucose metabolism was markedly altered. However, hepatic glycogen levels were unchanged in untreated nSTZ diabetic rats (Fig. 2A). Glc 6-P concentration was in fact increased by about 20% in comparison to untreated healthy animals (Fig. 2A). We next measured the

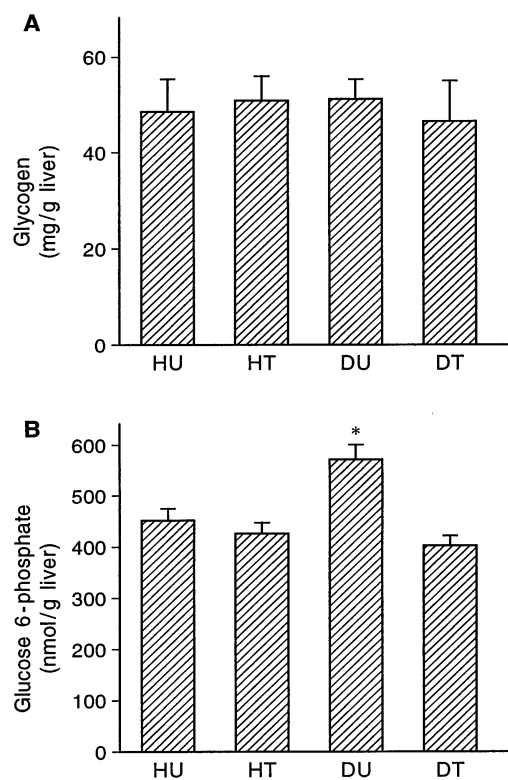


Fig 2A, B. Effects of tungstate treatment on hepatic glycogen and glucose 6-phosphate levels in vivo. **A** Glycogen level was measured in hepatic samples from untreated healthy (HU), treated healthy (HT), untreated diabetic (DU) and treated diabetic (DT) rats. Values are mean \pm SEM ($n = 10$). **B** Glu 6-P level was measured in hepatic samples from untreated healthy (HU), treated healthy (HT), untreated diabetic (DU) and treated diabetic (DT) rats. Values are mean \pm SEM ($n = 8$). * $p < 0.05$ compared to untreated healthy animals

activities of liver glycogen phosphorylase *a* and glucokinase. These two enzymes are key in the regulation of hepatic glucose metabolism, and variation in their activity may explain the increase observed in Glc 6-P. However, no significant differences were observed in glycogen phosphorylase *a* or glucokinase activity in nSTZ-diabetic rats when compared to healthy animals (Fig. 3). Furthermore, the total activity of glycogen synthase, the key enzyme in the control of glycogen biosynthesis, was not significantly modified in any group of animals (Table 3). In spite of that, diabetic rats showed an increase in the glycogen synthase (-Glc 6-P/+Glc 6-P) activity ratio (Table 3), indicating that the enzyme was in fact in a more active state than in their healthy counterparts.

Effect of tungstate treatment on hepatic metabolism. Tungstate did not modify hepatic glycogen concentration either in healthy or in nSTZ-diabetic rats (Fig. 2A), although in the latter it provoked a significant decrease in the hepatic concentration of Glc 6-P, to the same levels observed in healthy animals.

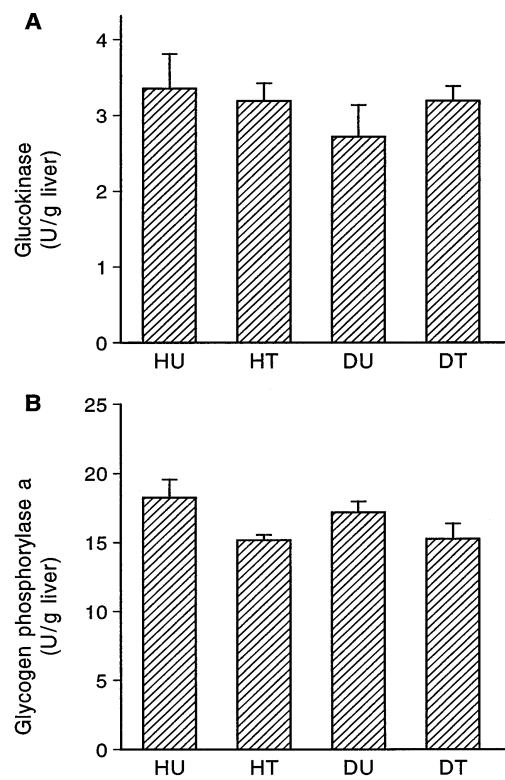


Fig 3A, B. Effects of in vivo tungstate treatment on hepatic glucokinase and glycogen phosphorylase activity. **A** Glucokinase activity was measured in hepatic samples from untreated healthy (HU), treated healthy (HT), untreated diabetic (DU) and treated diabetic (DT) rats. Values are mean \pm SEM ($n = 12$). **B** Glycogen phosphorylase *a* activity was measured in hepatic samples from untreated healthy (HU), treated healthy (HT), untreated diabetic (DU) and treated diabetic (DT) rats. Values are mean \pm SEM ($n = 12$)

Table 3. Effect of tungstate treatment on glycogen synthase activity

| | Activity ratio (-Glc6P/+Glc6P) | Total specific activity (U/g liver) |
|----------------------|--------------------------------|-------------------------------------|
| <i>Healthy rats</i> | | |
| Untreated (10) | 0.16 \pm 0.03 | 0.39 \pm 0.04 |
| Treated (12) | 0.17 \pm 0.02 | 0.37 \pm 0.03 |
| <i>Diabetic rats</i> | | |
| Untreated (12) | 0.22 \pm 0.03 ^a | 0.36 \pm 0.03 |
| Treated (12) | 0.25 \pm 0.03 ^a | 0.38 \pm 0.04 |

Results are expressed as the means \pm SEM for the number of animals indicated in parentheses

^a $p < 0.05$ compared to untreated healthy rats

Thus, the administration of tungstate normalized the concentration of hepatic Glc 6-P (Fig. 2B). However, tungstate did not significantly modify Glc 6-P concentration in healthy rats. Tungstate treatment did not alter the activity of the key enzymes of liver glycogen metabolism either in healthy or in diabetic rats (Fig. 3), nor did it induce any significant variation in glycogen synthase activity ratio (Table 3).

Table 4. Effects of tungstate on islet performance

| | Glycaemia (mmol/l) | Insulinaemia (pmol/l) | Insulin/glucose ratio | Insulin content (pmol/islet) ^a |
|----------------------|------------------------|-----------------------|--------------------------|---|
| <i>Healthy rats</i> | | | | |
| Untreated (12) | 4.6 ± 0.1 | 408 ± 42 | 88.7 ± 25.8 | 10.8 ± 0.7 (26) |
| Treated (12) | 4.4 ± 0.1 | nd | – | 10.8 ± 0.6 (39) |
| <i>Diabetic rats</i> | | | | |
| Untreated (12) | 7.8 ± 0.2 ^b | 330 ± 48 | 42.3 ± 4.2 ^b | 0.6 ± 0.1 ^b (35) |
| Treated (12) | 4.6 ± 0.1 ^c | 384 ± 132 | 83.5 ± 15.0 ^c | 3.7 ± 0.2 ^d (28) |

Results are expressed as the means ± SEM for the number of animals indicated in parentheses

^a The experiments were performed with the number of islet indicated in parenthesis pooled from four animals.

^b $p < 0.001$ compared to untreated healthy rats;

^c $p < 0.001$ compared to untreated diabetic rats;

^d $p < 0.001$ compared to untreated healthy and diabetic rats

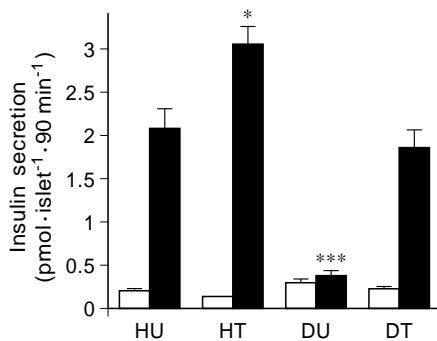


Fig. 4. Insulin secretion of the islets in tungstate treated or untreated healthy and diabetic animals. The secretion of insulin was measured in islets from untreated healthy (HU), treated healthy (HT), untreated diabetic (DU) and treated diabetic (DT) rats. The experiments were performed at 2.8 mmol/l glucose (□) and at 16.7 mmol/l glucose (■). The values are mean ± SEM (4 rats, 8 islets with 12 determinations). * $p < 0.01$ compared with untreated healthy animals; *** $p < 0.0001$ compared with untreated healthy animals

Effect of tungstate treatment on islet performance. Since the changes induced by tungstate in the metabolic state of the liver of the nSTZ-diabetic rats could hardly be responsible for the observed normalization of hyperglycaemia, we decided to evaluate whether tungstate treatment modified pancreatic beta-cell function, islet content or secretion. The insulin content was markedly decreased in the islets of diabetic animals (Table 4). Tungstate treatment partially restored islet insulin content in nSTZ rats to approximately 30% of that in healthy animals; however, it did not affect insulin content in healthy animals (Table 4). Moreover, preproinsulin mRNA levels also decreased in the islets of the nSTZ rats to approximately 45% of that in healthy rats. This value recovered up to about 75% in treated-diabetic animals.

The beta-cell response to glucose was also analyzed. As expected, in healthy animals there was a significant increase in insulin release when the glucose concentration was shifted from 2.8 to 16.7 mmol/l (Fig. 4). In contrast, the increase in insulin release evoked by 16.7 mmol/l glucose stimulus in pancreatic

islets was totally abolished in islets from nSTZ rats. When islets from treated diabetic rats were stimulated with a shift in glucose concentration, an increase in insulin secretion was observed from 0.36 ± 0.05 to 1.83 ± 0.21 pmol · islet⁻¹ · 90 min⁻¹. These values are similar to those observed in islets isolated from untreated healthy animals. A slight increase in insulin release was observed at 16.7 mmol/l glucose in islets from treated healthy animals (Fig. 4). Thus, tungstate administration restored the ability of beta cells to respond to an increase in glucose concentration.

Morphological data. We next determined whether the number of beta cells and/or islet surface area was changed after the treatment. No significant differences were observed in the mean islet surface area between the experimental groups. However, diabetic animals had a significant decrease (40%) in the beta-cell/exocrine tissue ratio compared with untreated healthy animals. The treatment of nSTZ-diabetic rats increased this ratio to values similar to those in healthy animals (Table 5). Tungstate administration to healthy rats did not modify the beta-cell/exocrine tissue ratio.

Discussion

The results presented in this paper show that tungstate, administered orally, can normalize glycaemia in an animal model of NIDDM, the nSTZ-diabetic rat. In a previous report [1], we showed that tungstate is effective in reducing hyperglycaemia in STZ-diabetic rats, a model of insulin-dependent diabetes mellitus (IDDM). Therefore, tungstate is useful as a hypoglycaemic agent in animal models of both types of diabetes. However, important differences have been observed regarding the mechanism by which tungstate exerts its action in each case.

In STZ-diabetic rats, the main action of tungstate appears to be the restoration of the hepatic glucose metabolism [1], by increasing the capacity of the liver to utilize glucose through glycolysis and glycogenesis.

Table 5. Morphometric analysis of pancreatic islets

| | Beta cell/exocrine tissue (%) | Islet surface (μm^2) ^a |
|----------------------|-------------------------------|--|
| <i>Healthy rats</i> | | |
| Untreated (4) | 1.45 \pm 0.05 | 10 897 \pm 1788 (21) |
| Treated (4) | 1.35 \pm 0.15 | 11 092 \pm 1033 (20) |
| <i>Diabetic rats</i> | | |
| Untreated (4) | 0.86 \pm 0.07 ^b | 12 219 \pm 1298 (41) |
| Treated (4) | 1.73 \pm 0.38 ^c | 10 479 \pm 936 (60) |

Results are expressed as the means \pm SEM for the number of animals indicated in parentheses.

^a The experiments were performed with the number of islet indicated in parentheses pooled from four animals.

^b $p < 0.001$ compared to untreated healthy rats;

^c $p < 0.05$ compared to untreated diabetic rats

Moreover, tungstate also decreases the hepatic potential for glucose output through reduction of the expression of the principal key enzyme in gluconeogenesis, phosphoenolpyruvate carboxykinase. However, this does not appear to be the case in nSTZ-diabetic rats. In these animals, liver glucose metabolism is only slightly altered, and glycogen levels are not decreased, as they are in the STZ-diabetic animals. Activities of glucokinase and glycogen phosphorylase, key enzymes of glucose metabolism, which are decreased in the STZ-diabetic rats, are not affected in the nSTZ-diabetic animals. Surprisingly, the only two differences observed in the livers of nSTZ-diabetic rats with respect to healthy animals are increases in Glc 6-P concentrations and in the activation state of glycogen synthase. These changes are the opposite to those observed in STZ-diabetic rats, in which these two parameters decrease. Nevertheless the modifications in hepatic glucose metabolism observed can hardly be responsible for the hyperglycaemia observed in nSTZ-diabetic rats. Therefore, although it has been recently demonstrated [14] that liver alterations are the main cause of hyperglycaemia in STZ-rats, this is probably not the case for nSTZ-diabetic rats.

Tungstate treatment of nSTZ-diabetic rats does not modify the hepatic parameters studied, although it restores Glc 6-P levels. The normoglycaemic effect of tungstate in these animals cannot be attributed to the small changes observed in this metabolite.

The main cause of hyperglycaemia in nSTZ-diabetic rats appears to be the lack of response of beta-cells to a glucose stimulus. These animals can maintain normal levels of basal insulinaemia [15], whereas the response to an increase in blood glucose levels (as in the postprandial state) is probably impaired. These data correlate with the observed decrease in beta-cell insulin content and beta cell mass in the pancreas of these diabetic animals. Tungstate treatment restores the ability of beta cells to respond to glucose, and causes an increase in insulin content, and beta-cell mass. Therefore, these results suggest that tungstate

may exert two different types of effect on the islets. On the one hand it may increase the sensitivity of beta cells to glucose. On the other, it may raise the insulin content, probably as a result of an increase in insulin gene expression as indicated by a rise in preproinsulin mRNA levels. This effect could be caused by an increase in insulin biosynthesis by the remaining beta cells or by an increase in the beta-cell mass in the pancreas. This latter effect was observed in morphometric analysis. This trophic effect of tungstate on the beta cells could be direct or indirect. In experiments performed in islet cell lines we observed that tungstate, when added to the culture medium, increased the incorporation of (³H)-thymidine to these cells (unpublished data) suggesting a direct effect. Taken together, these results indicate that tungstate may exert a repairing action in the beta cells of the nSTZ-diabetic rats. When healthy animals were treated with tungstate a slight increase in insulin secretion of their beta cells in response to an increase in glucose concentration was also observed. This indicates that in healthy animals tungstate can still increase sensitivity to glucose.

In summary, tungstate appears to exert its action by two different mechanisms. In STZ-diabetic rats, it acts by normalizing liver metabolism, while in nSTZ-diabetic rats its main effect is exerted at pancreatic level. In both cases, an important feature is that tungstate does not cause hypoglycaemia in healthy animals. Other insulin-like effects of tungstate have also been reported in studies "in vitro" [16, 17]. Thus, tungstate may offer the basis for a new oral treatment of both types of diabetes.

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