Mild streptozotocin diabetes in the Göttingen minipig. A novel model of moderate insulin deficiency and diabetes

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Larsen, Marianne O., Michael Wilken, Carsten F. Gottfredsen, Richard D. Carr, Ove Svendsen, and Bidda Rolin. Mild streptozotocin diabetes in the Göttingen minipig. A novel model of moderate insulin deficiency and diabetes. Am J Physiol Endocrinol Metab 282: E1342–E1351, 2002.—Nonrodent models of diabetes are needed for practical and physiological reasons. Induction of mild insulin-deficient diabetes was investigated in male Göttingen minipigs by use of streptozotocin (STZ) alone (75, 100, and 125 mg/kg) or 125 mg/kg combined with pretreatment with nicotinamide (NIA; 0, 20, 67, 150, and 230 mg/kg). Use of NIA resulted in a less steep slope of the regression line between fasting plasma glucose and changing doses compared with STZ (−7.0 ± 1.4 vs. 29.7 ± 7.0 mM·mg−1·kg−1, P < 0.0001). Intermediate NIA doses induced moderate changes of glucose tolerance [glucose area under the curve increased from 940 ± 175 to 1,598 ± 462 mM·min−1, P < 0.001 (100 mg/kg) and from 890 ± 109 to 1,669 ± 691 mM·min−1, P = 0.003 (67 mg/kg)] with reduced insulin secretion [1,248 ± 602 pM·min−1 after 16 days and 1,566 ± 190 pM·min−1 after 60 days vs. 3,251 ± 804 pM·min in normal animals (P < 0.001)] and β-cell mass [5.5 ± 1.4 mg/kg after 27 days and 7.9 ± 4.1 mg/kg after 60 days vs. 17.7 ± 4.7 mg/kg in normal animals (P = 0.009)]. The combination of NIA and STZ provided a model characterized by fasting and especially postprandial hyperglycemia and reduced, but maintained, insulin secretion and β-cell mass. This model holds promise as an important tool for studying the pathophysiology of diabetes and development of new pharmacological agents for treatment of the disease.

The pig is useful as a model for human physiology and pathophysiology, because many organ systems resemble those of the human. Of special interest for the study of diabetes is the similarities to humans found in the clinical chemistry (7, 10, 12, 14, 24, 26, 55), nutrition and gastrointestinal tract (4, 8, 11, 20, 35, 40, 51), pancreas development and morphology (21, 36, 37, 44, 49, 54), and metabolism (3, 35). These characteristics make swine an interesting species for studies of metabolic abnormalities in diabetes. The Göttingen minipig is especially suitable for long-term studies because of its small size and ease of handling, even at full maturity (6).

Pancreatectomy has been investigated as a method of inducing diabetes in pigs (33, 34, 50, 55). However, high rates of mortality have been observed postoperatively (50, 55), meaning that this technique should be used with great caution, and alternatives should be considered because of welfare considerations. Chemical induction of diabetes offers the advantage of preservation of both exocrine and endocrine cell populations other than β-cells, thus resembling the situation in human diabetes (55). Several stable models have been established for overt type 1 diabetes in the pig by the use of pharmacological induction of β-cell damage with streptozotocin (STZ), either as single or repeated injections (2, 15, 16, 27–29, 46, 55). Substantially increased fasting plasma glucose (FPG) levels and decreased insulin secretion in response to glucose stimuli have been obtained as well as increases in plasma triglycerides and total cholesterol (27, 29). Late complications typical of diabetes, such as capillary basement membrane thickening and cataracts, have also been shown in diabetic minipigs (28, 41).

In other studies, alloxan has been used for induction of diabetes in pigs (11, 25, 41). This compound, which has β-cell-toxic properties similar to those of STZ, in a dose of 200 mg/kg in Yucatan minipigs induced severe diabetes with high mortality due to hypoglycemia following acute hyperinsulinemia as a consequence of massive β-cell damage (41). Doses of 80 mg/kg have
been reported to induce mild diabetes with moderate hyperglycemia and partial loss of β-cell mass with impaired insulin secretion rates but normal fasting insulin levels in Göttingen minipigs (25). Due to its greater selectivity toward β-cells, its wider range between doses causing mild and severe changes in glucose tolerance compared with alloxan (22, 23), and the more extensive background literature on the effect of STZ in pigs, this compound was chosen for reduction of β-cell mass in the present study.

Despite the widespread use of STZ, its use results in a wide variability in the extent of diabetes depending on species, strain, age, and laboratory, thus limiting the predictability of its effects. Furthermore, the efficacy of STZ varies even in an apparently uniform group of animals receiving the same dose of the compound (13, 47).

In the present study, it was therefore investigated whether the use of nicotinamide (NIA) would have protective effects against the diabetogenic action of STZ in the Göttingen minipig, as previously reported in rats (31). The protective effect of NIA against the effect of STZ has been shown in vivo to be both dose and pretreatment time dependent, but even the most effective protective dose of NIA did not completely prevent the diabetogenic effects of STZ (23, 30). Thus a combination of these two compounds might be useful in the establishment of a nonrodent model of mild insulin-deficient diabetes, and in the present study, the dose-response relations on glucose metabolism using two different approaches of pharmacological induction of abnormalities in glucose tolerance are investigated in adult male Göttingen minipigs. The first approach was administration of a dose range of STZ (75, 100, and 125 mg/kg) alone, the second being administration of different doses of NIA (0–230 mg/kg) as a pretreatment in combination with STZ at a fixed high dose (125 mg/kg). The aim of the study was to obtain a reliable method of induction of impaired glucose tolerance and/or mild insulin-deficient diabetes in the adult Göttingen minipig, characterized by reduced β-cell mass and disturbed residual insulin secretion leading to a decreased ability to dispose of glucose and a following, modest hyperglycemia during an oral glucose tolerance test (OGTT).

MATERIALS AND METHODS

Animals

Adult male Göttingen minipigs 11–14 mo of age were obtained from the barrier unit at Ellegaard Göttingen Minipigs ApS, Dalmore, Denmark. Animals were housed in single pens under controlled conditions (temperature was kept between 18 and 22°C, relative air humidity was 30–70% with 4 air changes/h) with a 12:12-h light-dark cycle and fed twice daily: 140 g of SDS minipig diet (SDS, Essex, UK) and 240 g of a commercial swine fodder (“Svinefoder 22,” Slaegerup, Denmark) and allowed free access to water. The pigs were studied at least 2 wk after surgery and were trained carefully for all experimental procedures before the start of experiments.

For the dosing studies with STZ alone, 14 animals weighing 18 ± 3 kg (range 14–23 kg) were used. For the dosing studies using NIA and STZ in combination, 38 animals weighing 22.5 ± 3.25 kg (range 16.9 to 28.9 kg) were used. These animals served as their own control and were studied both before and after induction of diabetes.

Principles of laboratory animal care were followed, and the type of study was approved by the Animal Experiments Inspectorate, Ministry of Justice, Denmark.

Surgical Implantation of Central Venous Catheters

Two central venous catheters (Certo 455, B. Braun Melsungen, Melsungen, Germany) were surgically inserted under general anesthesia induced with a combination of 0.83 mg/kg zolazepam, 0.83 mg/kg ketamine (Ketaminol vet. [100 mg/ml], Bayer, Lyngby, Denmark), 0.83 mg/kg ketamine (Ketaminol vet. [100 mg/ml], Rosco, Taastrup, Denmark), and 0.20 mg/kg methadone (Metadon “DAK” [10 mg/ml], Nycomed, Roskilde, Denmark) and maintained with isoflurane (1–3%) (Forene, Abbot, Gentofte, Denmark) in 100% oxygen. Postsurgical analgesia was maintained by intramuscular injection of 0.03 mg/kg buprenorfine [Anorfin (0.3 mg/ml), GEA, Frederiksborg, Denmark] and maintained with isoflurane (1–3%) (Forene, Abbot, Gentofte, Denmark) in 100% oxygen. Postsurgical analgesia was performed by injection of dihydrostreptomycin sulfate (25 mg/kg) and benzylpenicillinprocain (20,000 IU/kg) [Streptocillin. vet. (250 mg + 200,000 IU benzylpenicillinprocain/ml), Boehringer Ingelheim] immediately after surgery and once daily for the 2 days after surgery by intramuscular injection of 4 mg/kg carprofen once daily. Postsurgical infection was prevented by injection of 4 mg/kg carprofen once daily. Postsurgical infection was prevented by injection of dihydrostreptomycin sulfate (25 mg/kg) and benzylpenicillinprocain (20,000 IU/kg) [Streptocillin. vet. (250 mg + 200,000 IU benzylpenicillinprocain/ml), Boehringer Ingelheim] immediately after surgery and once daily for the following 2 days. All animals were allowed 2–3 wk of recovery after the surgical procedure and had normal behavior and eating patterns at the start of the study period.

Protocol 1: Mixed-Meal OGTT

The mixed-meal OGTT was performed in all animals in the NIA and STZ combination study 1 wk before and 1 wk after exposure to NIA and STZ. The test was performed in nonrestrained, freely moving animals in their usual pens to reduce the amount of stress experienced by the animals during testing.

After an 18-h overnight fast, animals were offered a mixed-meal OGTT of 25 g of SDS minipig fodder and 2 g/kg glucose (500 g/l, SAD, Copenhagen, Denmark). The meal was eaten from a bowl, rapidly and without stress, under supervision.

Blood samples were obtained from the jugular vein catheters at t = −15, −5, 0, 15, 30, 45, 60, 90, 120, 150, and 180 min relative to the fodder and glucose load.

Protocol 2: Intravenous Glucose and Arginine Challenge

This test was performed 2 wk (16 ± 2 days; n = 6) or 2 mo (60 ± 0 days; n = 2) after dosing with NIA plus STZ in the 67 mg/kg NIA group and a control group of normal, age-matched, animals (n = 14) to evaluate insulin-secretory capacity. The test was performed in nonrestrained, freely moving animals to reduce the amount of stress experienced by the animals during testing.

After an 18-h overnight fast, animals were dosed with an intravenous bolus of glucose [500 g/l (0.3 mg/kg), SAD] at t = 0, and blood samples were obtained at t = −15, −10, −5, 1, 3, 5, 7, 10, 45, 50, 55, 61, 63, 65, 67, 70, 80, 85, 91, 93, 95, 97, and 100 min relative to the glucose load. At t = 60 min, another bolus of glucose [500 g/l (0.6 mg/kg), SAD] was dosed

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intravenously, and from \( t = 61 \) to \( t = 100 \) min, glucose was infused intravenously at \( 2 \) g·kg\(^{-1}\)·h\(^{-1} \) (200 g/l, SAD). At \( t = 90 \) min, arginine (L-arginine, Merck, art. 15:42) (67 mg/kg) dissolved in sterile saline (0.9%, SAD) was given intravenously. The insulin response to glucose and arginine was calculated as the area under the curve (AUC; baseline subtracted) during 10 min immediately after dosing.

**Protocol 3: Examination of STZ-Dosed Animals**

The examination of animals dosed with STZ alone was limited to measurement of fasting plasma values of glucose, insulin, and glucagon.

**Handling and Analysis of Blood Samples**

Blood samples (2 ml of whole blood) were immediately transferred to vials containing EDTA (1.6 mg/ml final concentration) and aprotinin [500 kallikrein inhibitor units (KIU)/ml (Trasylol, 10,000 KIU/ml, Bayer)] and kept on ice until centrifugation. Samples were centrifuged (4°C, 10 min, 3,500 rpm), and plasma was separated and stored at \(-20°C\) until analysis. Plasma glucose was analyzed using the immobilized glucose oxidase method: 10 μl of plasma in 0.5 ml of buffer (EBIO plus autoanalyzer and solution, Eppendorf, Hamburg, Germany). Plasma insulin was analyzed in a two-site immunometric assay with monoclonal antibodies as catching and detecting antibodies (catching antibody HUI-018 raised against the A-chain of human insulin; detecting antibody OXI-005 raised against the B-chain of bovine insulin (I)) and using purified porcine insulin for calibration of the assay. The minimal detectable concentration was 3.2 pM, the upper limit was 1,200 pM (no sample dilution), and the inter- and intra-assay variations at three concentration levels were 9.8, 9.9, and 7.6% (at 235 pM), 14.6 and 4.4% (at 87 pM). Recovery at concentration levels were 15.3 and 3.2% (at 342 pM), 9.9 and 7.6% (at 235 pM), and the inter- and intra-assay variations at three concentration levels were 97.1, 97.9, and 101%, respectively. A commercial kit from Linco was used to measure glucagon concentrations (glucagon RIA kit, cat. no. GL-32K). Performance data for the glucagon assay given by the supplier were: coefficient of variation (CV) of 10.3% (39 pg/ml, ED\(_{50}\)), 7.7% (104 pg/ml, ED\(_{50}\)) and 7.8% (271 pg/ml, ED\(_{20}\)).

**Induction of Diabetes**

Diabetes was induced by intravenous administration of STZ (Sigma S-0130) through the indwelling catheters over 2 min, either at variable doses alone [75 (\( n = 4 \)), 100 (\( n = 3 \)), or 125 (\( n = 7 \)) mg/kg] or at a fixed dose of 125 mg/kg in combination with NIA to accomplish a partial protection of the \( \beta \)-cells. NIA (Sigma N-3376) was administered intravenously 15 min before STZ. The doses of NIA used were \( 0 \) (\( n = 4 \)), 20 (\( n = 2 \), 67 (\( n = 11 \)), 100 (\( n = 11 \)), 150 (\( n = 6 \)), and 230 (\( n = 4 \)) mg/kg. Administration of NIA and STZ was performed after an 18-h overnight fast in conscious animals. NIA was weighed out in individual portions, protected from light, and dissolved immediately before injection in sterile saline (0.9%) to a concentration of 300 mg/ml. STZ was weighed out in individual portions and dissolved in sodium citrate buffer (no. 929546, pH 4.7, Bie & Berntsen, Roedovre, Denmark) to a concentration of 62.5 mg/ml immediately before injection.

Vomiting was seen in all animals during the first hours after administration of STZ, and this seemed to be unaffected by NIA pretreatment. Most animals were eating and behaving normally 24–48 h after dosing. Animals were offered SDS fodder 2 h after administration of NIA and STZ and were observed frequently during the first 2 days after administration of NIA and STZ. Blood glucose was monitored regularly to avoid episodes of hypoglycemia due to sudden hyperinsulinemia caused by necrosis of \( \beta \)-cells.

After 2 days, insulin therapy (Insulatard, 100 IU/ml, Novo Nordisk, Bagsvaerd, Denmark) was initiated if necessary, on the basis of individual clinical examination, with the aim of keeping fasting plasma glucose (FPG) below 10 mM.

**Histological Examination of Pancreas**

**Fixation and physical fractionation.** Histological examination was performed 1 mo (27 ± 8 days; \( n = 7 \)) or 2 mo (60 ± 0 days; \( n = 4 \)) after dosing with NIA (67 mg/kg) and STZ and compared with data from normal animals (\( n = 5 \)). Furthermore, an animal that received STZ alone was included for comparison. At the end of the study period, after euthanasia with pentobarbitone (20 ml/animal (200 mg/ml); Pharmacy of the Royal Veterinary and Agricultural University, Copenhagen, Denmark), the pancreas was immediately isolated in toto and fixed in paraformaldehyde (Bie & Berntsen) for 24 h. The following day, the pancreas was embedded in 3% agar solution (Meco-Benzon, cat. no. 303289, Copenhagen, Denmark) at 45°C. After cooling, the pancreas was cut into 3-mm slices (17), and every fifth tissue slice, starting at slice 1, 2, 3, 4, or 5, determined from a table of random numbers, was retained for sectioning into ~80 cubes of roughly equal size. Those cubes were arranged according to size, as practiced in the smooth fractionator method, with the largest cubes in the middle and the smallest cubes on the ends (5, 32). Every eighth cube, starting at cube 1, 2, 3 . . . 8, determined from a table of random numbers, was retained and placed in cassettes for dehydration and paraffin infiltration in a tissue processor (Leica TP 1050, Copenhagen, Denmark). The 10–12 pancreas cubes were embedded in paraffin, and sections 3 μm thick were cut on a Leica RM2165 microtome.

**Immunohistochemistry.** Sections were deparaffinized in xylene, brought to 99% ethanol, treated with 0.5% H\(_2\)O\(_2\) for 20 min to block endogenous peroxidase activity, and rinsed with Tris-buffered saline (TBS). Sections were then immersed in 0.01 M citrate buffer, pH 6, preheated to 90°C, and submitted to antigen retrieval by microwave oven treatment for 3 × 5 min of heating at 80% (Polar Patent, Umeå, Sweden). The slides were subsequently cooled, still in the citrate buffer, by immersion of the jar in running tap water and rinsed in TBS + 0.01% Triton X-100 (TBS-T; Sigma, St. Louis, MO), and the tissue sections were “ringed” with a DAKO-pen (DAKO, Copenhagen, Denmark).

**INSULIN STAINING.** The following staining steps were carried out in an Autostainer (DAKO). All dilutions were carried out with TBS-T. Sections were blocked with 5% normal rabbit serum (X0902; DAKO) and then incubated for 60 min with primary antibody guinea pig anti-insulin (651041, ICN, Costa Mesa, CA) diluted 1:3,500 in 7% normal rabbit serum, normal swine serum (X901; DAKO) in TBS-T. Sections were rinsed in TBS-T and incubated for 30 min in secondary antibody peroxidase-labeled rabbit anti-guinea pig IgG (P141; DAKO) diluted 1:300 in 7% rabbit + 3% swine serum in TBS-T. The sections were rinsed with TBS-T and then developed with 0.075% diaminobenzidine (DAB; DAKO) and 0.008% H\(_2\)O\(_2\) in TBS-T for 3 min. After a rinse in TBS-T, the slides were washed for 5 min in running tap water, counterstained with Mayer’s hematoxylin for 0.5 min, washed again in running tap water for 10 min, dehydrated, and mounted in Pertex (Histolab, Stockholm, Sweden).

**NON-\(\beta\)-CELL STAINING.** These staining steps were also carried out in an Autostainer. All dilutions were carried out with...
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Table 1. FPG and FPI in normal male Göttingen minipigs and after administration of STZ in various doses

<table>
<thead>
<tr>
<th>STZ Dose, mg/kg</th>
<th>0</th>
<th>75</th>
<th>100</th>
<th>125</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>38</td>
<td>4</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>FPG, mM</td>
<td>3.6 ± 0.7</td>
<td>4.0 ± 0.7</td>
<td>10.4 ± 1.9</td>
<td>10.8 ± 3.0</td>
</tr>
<tr>
<td>P value</td>
<td>0.034</td>
<td>0.025</td>
<td>&lt;0.001</td>
<td>0.006</td>
</tr>
<tr>
<td>FPI, pM</td>
<td>35.6 ± 26.0</td>
<td>23.2 ± 10.2</td>
<td>2.5 ± 1.4</td>
<td>3.0 ± 3.8</td>
</tr>
<tr>
<td>P value</td>
<td>0.094</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD of individual plasma concentrations. FPG, fasting plasma glucose; FPI, fasting plasma insulin. P values for comparison of streptozotocin (STZ)-dosed animals vs. normal animals, Student’s t-test.

TBS-T. Sections were incubated for 10 min with avidin-blocking solution (DAKO), washed with TBS-T, incubated for 10 min with biotin-blocking solution (DAKO) to block endogenous biotin-binding sites, and rinsed in TBS-T. Sections were then incubated with 5% normal donkey serum (D-9663; Sigma). The sections were then incubated for 60 min with a mixture of antibodies to glucagon (GLU), somatostatin (SOM), and pancreatic polypeptide (PP): monoclonal mouse anti-GLU (clone Glu-001, 1:2,500; Novo Nordisk) + rabbit anti-SOM (A566, 1:1,600; DAKO) + rabbit anti-PP (A619, 1:2,000; DAKO) diluted in TBS-T with 4% normal goat serum (X0907; DAKO) + 4% donkey serum + 3% normal swine serum. Sections were rinsed in TBS-T and incubated for 30 min with a mixture of biotinylated secondary antibodies; biotinylated donkey anti-mouse IgG (715–065–1501, 1:3,000; Jackson, West Grove, PA) + biotinylated goat anti-rabbit IgG (111–065–1441, 1:3,000; Jackson) diluted in the same serum-containing buffer as the primary antibodies. 4% goat + 4% donkey + 3% swine serum in TBS-T. Sections were rinsed in TBS-T, incubated 15 min with peroxidase-labeled streptavidin (P397, 1:300; DAKO) and rinsed in TBS-T. Peroxidase activity was developed for 5 min with 0.05% DAB + 0.006% H2O2 + 1.3% NiSO4 in TBS-T + imidazole. After a rinse in TBS-T, the slides were washed for 5 min in running tap water, counterstained with Mayer’s hematoxylin for 0.5 min, washed again in running tap water for 10 min, dehydrated, and mounted in Pertex (Histolab).

Stereological estimation of β- and non-β-cell mass. Non-β- and β-endocrine cell mass was evaluated stereologically in two to three sections 250 μm apart in an Olympus BX-50 microscope (Olympus, Copenhagen, Denmark) with video camera and monitor at a total on-screen magnification of ×960. The sections were analyzed by point counting of frames after systematic uniform random sampling using a PC-controlled motorized stage and the CAST-GRID software (Olympus).

Initially, the tissue sections were circumscribed using a ×1.25 objective, and the counting of endocrine and exocrine structures took place within this area. The volume fractions of β- or non-β-cells were estimated by point-counting stereological techniques (18) at a total on-screen magnification of ×960 obtained with a ×20 objective, a grid of 4 × 64 points, and step lengths of maximum 900 × 600 μm controlled by the CAST-GRID software. The sections were examined with the origin of the sections blinded to the observer. Mean values of estimated volume fractions were calculated with correction for the differences in total areas counted.

Statistics

Calculations of fasting values and AUC (baseline = 0) for glucose and insulin during OGTT (protocol 1), baseline subtracted for glucagon during OGTT (protocol 1) and for insulin during glucose and arginine stimulation test (protocol 2), and statistical evaluation of results was performed using paired two-tailed Student’s t-test, the Kruskal-Wallis test, and linear regression using Excel (2000) and GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, CA). Comparison of slopes of regression lines was performed using the method described by Zar (56). P values of ≤0.05 were considered significant.

RESULTS

Normal Plasma Profiles During OGTT (Protocol 1)

On the basis of the OGTT performed in all 38 animals before administration of NIA and STZ, curves showing the normal plasma concentrations of glucose, insulin, and glucagon were obtained. Normal FPG and fasting plasma insulin (FPI) are summarized in Table 1. The glucose area under the curve (AUCglucose) was 980 ± 200 mM·min, and the 2-h plasma glucose (2-hPG) was 4.7 ± 1.2 mM. Similarly, normal AUCinsulin was 42,087 ± 21,637 pM·min. Normal fasting plasma glucagon (FPGa) was 86 ± 22 ng/l, and AUCglucagon was −1,451 ± 2,886 ng·l·min⁻¹.

Fasting Plasma Values After Administration of STZ (Protocol 3)

Changes in FPG, FPI, and FPGa were evaluated by comparison with the normal values obtained in the 38 animals (Table 1). FPG was significantly increased in the 100 and 125 mg/kg STZ groups but not in the 75

Fig. 1. Relationship between fasting plasma glucose and dose of streptozotocin (STZ) (A; r² = 0.5924, P = 0.001, slope = 29.7 ± 7.0), or nicotinamide (NIA) combined with a fixed dose of STZ (125 mg/kg) (B; r² = 0.4339, P < 0.001, slope = −7.0 ± 1.4) in male Göttingen minipigs.

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mg/kg STZ group, and there was a significant correlation between log STZ dose and FPG ($r^2 = 0.5924$, $P = 0.001$) (Fig. 1). FPI was significantly decreased in the 100 and 125 mg/kg STZ groups but not in the 75 mg/kg STZ group, there being a significant correlation between log dose STZ and FPI ($r^2 = 0.6157$, $P = 0.002$). No significant change was detected in FPGs (data not shown).

**Plasma Profiles During OGTT (Protocol 1) After Administration Of NIA and STZ**

A significant increase in FPG was found in the 100, 67, 20, and 0 mg/kg NIA groups but not in the 230 and 150 mg/kg NIA groups (Table 2), the correlation being significant between log dose of NIA and FPG ($r^2 = 0.4339$, $P < 0.001$) (Fig. 1). A significant decrease in FPI was found in the 67 mg/kg NIA group, but this most probably is due to high values of FPI before NIA + STZ in this group, since changes in all other groups were nonsignificant. There was no significant correlation between log dose of NIA and FPI ($r^2 = 0.0052$, $P = 0.687$).

No significant changes in FPGs were detected in any of the dosing groups.

A significant increase in AUC$_{glucose}$ was found in all dosing groups except the 230 mg/kg NIA group (Table 3). Changes in glucose levels are illustrated in Fig. 2, and there was a significant correlation between the log NIA dose and AUC$_{glucose}$ ($r^2 = 0.2970$, $P < 0.001$) (data not shown). Furthermore, there was a significant correlation between AUC$_{glucose}$ and FPG ($r^2 = 0.880$, $P < 0.0001$) (Fig. 3). A significant decrease in AUC$_{insulin}$ was found in the 150, 67, and 0 mg/kg NIA groups. In the other groups, a nonsignificant trend toward lowering of AUC$_{insulin}$ was seen. When AUC$_{insulin}$ after 20 mg/kg NIA + STZ were compared with the AUC$_{insulin}$ (in pM-min) from the 38 normal OGTT profiles, there was a significant decrease (from 42.087 ± 21.637 to 7.970 ± 2.702, $P = 0.03$). There was no significant correlation between log NIA dose and AUC$_{insulin}$ ($r^2 = 0.00875$, $P = 0.599$). No significant changes in AUC$_{glucagon}$ were found in any of the groups.

**Duration Of Reduction Of β-Cell Function and Mass**

Over the period in which the intravenous glucose and arginine challenge (protocol 2) was performed, FPG (in mM) was consistently elevated in the NIA + STZ-dosed animals [4.0 ± 1.0 after 2 wk and 4.9 ± 1.2 after 2 mo vs. 3.2 ± 0.4 in normal animals ($P = 0.029$)], whereas FPI (in pM) did not differ between the groups [20 ± 11 after 2 wk and 27 ± 6 after 2 mo vs. 38 ± 30 in normal animals, nonsignificant (NS)]. No change in individual FPG was seen from 1 wk after dosing of NIA + STZ to the time of intravenous glucose and arginine challenge [4.6 ± 1.5 vs. 4.4 ± 1.0 mM (NS)]. Reduction in insulin response (pM-min) was seen both after intravenous glucose, 0.3 g/kg [1,248 ± 602 after 2 wk and 1,566 ± 190 after 2 mo vs. 3,251 ± 804 in normal animals ($P < 0.001$)], 0.6 g/kg [1,464 ± 693

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**Table 2. FPG and FPI in normal male Göttingen minipigs and after administration of NIA in various doses and STZ (125 mg/kg)**

<table>
<thead>
<tr>
<th>NIA Dose, mg/kg</th>
<th>230</th>
<th>150</th>
<th>100</th>
<th>67</th>
<th>20</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>4</td>
<td>6</td>
<td>11</td>
<td>11</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>FPG pre, mM</td>
<td>4.4 ± 0.4</td>
<td>4.4 ± 0.2</td>
<td>3.1 ± 0.8</td>
<td>3.6 ± 0.6</td>
<td>3.1 ± 3.2</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>FPG post, mM</td>
<td>3.8 ± 0.3</td>
<td>5.1 ± 1.0</td>
<td>4.5 ± 0.9</td>
<td>5.0 ± 2.1</td>
<td>14.1 and 13.8</td>
<td>13.4 ± 5.3</td>
</tr>
<tr>
<td>$P$ values</td>
<td>0.078</td>
<td>0.168</td>
<td>&lt;0.001</td>
<td>0.041</td>
<td>0.016</td>
<td>0.034</td>
</tr>
<tr>
<td>FPI pre, pM</td>
<td>21.6 ± 7.8</td>
<td>37.8 ± 30.1</td>
<td>22.7 ± 11.7</td>
<td>54.3 ± 35.4</td>
<td>27 and 46</td>
<td>29.3 ± 9.3</td>
</tr>
<tr>
<td>FPI post, pM</td>
<td>12.2 ± 5.1</td>
<td>36.9 ± 22.5</td>
<td>26.9 ± 17.0</td>
<td>25.5 ± 15.7</td>
<td>15 and 12</td>
<td>10.9 ± 11.1</td>
</tr>
<tr>
<td>$P$ values</td>
<td>0.073</td>
<td>0.958</td>
<td>0.547</td>
<td>0.006</td>
<td>0.291</td>
<td>0.194</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD of individual plasma values. $P$ values for comparison before and after dosing of nicotinamide (NIA) and STZ, paired Student’s $t$-test.

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**Table 3. AUC$_g$ and AUC$_t$ during OGTT in normal male Göttingen minipigs and after administration of NIA in various doses and STZ (125 mg/kg)**

<table>
<thead>
<tr>
<th>NIA Dose, mg/kg</th>
<th>230</th>
<th>150</th>
<th>100</th>
<th>67</th>
<th>20</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>4</td>
<td>6</td>
<td>11</td>
<td>11</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>AUC$_g$ pre, mM-min</td>
<td>1,245 ± 81</td>
<td>1,224 ± 155</td>
<td>940 ± 175</td>
<td>890 ± 109</td>
<td>787 ± 147</td>
<td>827 ± 69</td>
</tr>
<tr>
<td>AUC$_g$ post, mM-min</td>
<td>1,159 ± 185</td>
<td>2,094 ± 710</td>
<td>1,598 ± 462</td>
<td>1,669 ± 691</td>
<td>4,288 ± 135</td>
<td>3,801 ± 1,147</td>
</tr>
<tr>
<td>$P$ values</td>
<td>0.359</td>
<td>0.022</td>
<td>&lt;0.001</td>
<td>0.003</td>
<td>0.036</td>
<td>0.016</td>
</tr>
<tr>
<td>AUC$_t$ pre, pM-min</td>
<td>51,403 ± 37,630</td>
<td>66,432 ± 28,622</td>
<td>36,346 ± 14,653</td>
<td>35,434 ± 12,287</td>
<td>37,030 ± 12,657</td>
<td>32,108 ± 11,799</td>
</tr>
<tr>
<td>AUC$_t$ post, pM-min</td>
<td>19,052 ± 12,558</td>
<td>17,033 ± 11,852</td>
<td>29,435 ± 21,510</td>
<td>21,092 ± 10,354</td>
<td>7,971 ± 2,702</td>
<td>2,714 ± 2,225</td>
</tr>
<tr>
<td>$P$ values</td>
<td>0.098</td>
<td>0.002</td>
<td>0.408</td>
<td>0.006</td>
<td>0.227</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD of individual values AUC$_g$, glucose area under curve; AUC$_t$, insulin AUC; OGTT, oral glucose tolerance test. $P$ values for comparison before and after dosing of NIA and STZ, paired Student’s $t$-test.
after 2 wk and 1,173 ± 256 after 2 mo vs. 3,593 ± 1,253 in normal animals \((P < 0.001)\), and arginine \([1,424 ± 440 after 2 wk and 1,779 ± 1,027 after 2 mo vs. 3,283 ± 1,854 in normal animals \((P = 0.015)\)]\) (Fig. 4). Furthermore, β-cell mass \([mg/kg; 5.5 ± 1.4 after 1 mo and 7.9 ± 4.1 after 2 mo vs. 17.7 ± 4.7 in normal animals \((P = 0.009)\)]\) was reduced after NIA + STZ (Fig. 5).

During the histological examination of pancreata from animals dosed with NIA + STZ, no signs of development of tumors from surviving β-cells were found.

**DISCUSSION**

Attempts to genetically select a strain of diabetic pigs have been made (38, 39, 40) but have, so far, not been confirmed to be successful (19). The use of pancreatectomized animals as a model of diabetes (15, 33, 34, 50) has the disadvantage of also removing the exocrine function of the pancreas and the non-β-cell endocrine cells of the islets of Langerhans, and although performance of partial pancreatectomy might be useful for induction of mild diabetes, this method clearly is more invasive compared with the administration of STZ.

Pigs are more resistant to the diabetogenic effects of STZ than rats (2, 13, 15, 16, 23, 27–29, 31, 46, 55) and nonhuman primates (42), and a wide individual variability in the response to STZ was seen in the present study, as has also been shown in nonhuman primates (42). In the present study, only male animals were included to reduce variability in glucose tolerance due to estrous cycling, and probably the diversity in genetic background is the best explanation for the wide vari-
Fig. 4. Glucose concentrations (A) and insulin response (B) after intravenous glucose (0.3 g/kg at $t = 0$ and 0.6 g/kg at $t = 60$ followed by an infusion of $2$ g·kg$^{-1}$·h$^{-1}$ from 60 to 100 min) or arginine (67 mg/kg at $t = 90$ min) (dosing indicated by arrows) in normal animals (○) and animals 2 wk (△) or 2 mo (■) after dosing with NIA (67 mg/kg) and STZ (125 mg/kg). Data are means ± SE.

Fig. 5. Paraffin sections of pancreas from normal minipigs (A and D; Vvol/Vvol$^{non\%}$ 1.44/0.28% and 1.04/0.19%, where Vvol is volume fraction), mildly diabetic minipigs (B, C, and E; Vvol/Vvol$^{non\%}$ 0.41/0.17%, 0.27/0.34%, and 0.28/0.22%) and from an overtly diabetic minipig (F; Vvol/Vvol$^{non\%}$ 0.10/0.47%). The sections were doubly immunostained for insulin (brown cytoplasm) and the combination of glucagon + somatostatin + pancreatic polypeptide (black cytoplasm) before light hematoxylin counterstaining. Bar = 50 μm.
ability seen, because diet and nutritional status were standardized in the present experiment.

Previous observations showing that 35–40 mg/kg STZ did not influence glucose metabolism in pigs (15, 29) are consistent with data from the present study. In the present study, all of the animals in the 100 mg/kg STZ group and five of seven animals in the 125 mg/kg STZ group were classified as severely diabetic, whereas the remaining two animals from the 125 mg/kg STZ group had impaired fasting glucose (IFG). Thus these data demonstrate that adult male Göttingen minipigs can be made severely diabetic by using a dose of STZ of 100–125 mg/kg or above. This is in accord with previous observations in minipigs and domestic pigs, with 100–150 mg/kg STZ inducing overt diabetes (9, 15, 16, 55). The response to 100 or 125 mg/kg STZ showed some individual variation, indicating individual grades of sensitivity to STZ alone, in accord with previous observations (13, 47). The effects of STZ alone, expressed as increased FPG and decreased FPI and FPC, are significantly correlated with log dose of STZ and show a clear dose-response relationship, and despite the individual variation, this dosing regimen seems to be a reliable method for pharmacological induction of overt diabetes. However, on the basis of the present results, the dosing window in which induction of mild, insulin-deficient diabetes can be accomplished seems very narrow due to the steepness of the dose-response curve to STZ alone.

The present results show that NIA is indeed capable of partially protecting β-cells from the damaging effects of STZ in the Göttingen minipig, as has also been shown previously in rats (23, 30, 31).

The use of NIA seems feasible for induction of mild type 1 insulin-deficient diabetes, since the changes in FPG in response to changing doses of NIA are significantly smaller compared with changing doses of STZ. This can be seen when the regression lines for log dose NIA or STZ and FPG (Fig. 2) are compared, with the regression line based on changing STZ dose having a much steeper slope (30 ± 7) compared with that for the changing NIA dose (7 ± 2) (P < 0.001). NIA in a dose of 67 mg/kg in combination with STZ seems to be a useful regimen for induction of mild insulin-deficient diabetes.

However, the protective effect of NIA is not complete, since even the highest dose of NIA could not fully prevent the effect on the β-cells, as has also been shown in rats (23, 30).

The ratio of insulin to glucose at 30 min during the OGTT was decreased even in the animals that had normal glucose tolerance (NGT) post-NIA+STZ dosing compared with pre-NIA+STZ values (25.8 ± 11.5 vs. 61.2 ± 28.0, P < 0.001). Furthermore, there was a gradual decrease in the ratio post-NIA+STZ dosing from the NGT animals, through the glucose-intolerant (17.7 ± 11.1) and mildly diabetic animals (9.2 ± 8.8), to the overtly diabetic animals (2.6 ± 3.2, P < 0.001; Fig. 6), which is similar to what has been observed in humans (52). The fact that this ratio is also decreased in the animals with NGT post-NIA+STZ dosing compared with pre-NIA+STZ values indicates some deterioration of insulin-secretory response to glucose even in these animals.

The protection by NIA seems to be negligible when doses of 20 mg/kg or below are used. In the dosing range 150–67 mg/kg NIA, mild diabetes could be induced, even though some variability existed within the dosing groups, with the diabetic animals having moderately increased FPG and higher residual insulin-secretory capacity compared with diabetic animals from the 20 and 0 mg/kg NIA groups.

Administration of NIA and STZ in combination has been known to induce insulin-producing tumors in the pancreas of rats (45). In the present study, no tumors were found during histological examination of pancreatic tissues from animals for up to 2 mo after dosing with NIA+STZ. Diabetes induced by STZ has previously been shown to be stable in some experiments (9, 55), whereas other studies have shown a gradual improvement in glucose tolerance (27). In the present study, the elevated FPG after NIA+STZ was stable in the seven tested animals for a period of from 2 wk to 2 mo after dosing. Furthermore, there was a very significant reduction in insulin-secretory response to both glucose and arginine after the same period of time. Finally, the in vivo measures of reduced insulin-secretory capacity were confirmed histologically by reduced β-cell mass in the model. The stability of the IGT/diabetes in this model beyond 2 mo after dosing has yet to be investigated in detail.

In conclusion, these studies have shown that varying degrees of glucose intolerance and diabetes can be induced in male Göttingen minipigs with STZ and that NIA has protective effects in β-cells in these animals, as has also been shown in rats. There seems to be a rational reason for including NIA together with STZ for induction of diabetes in the minipig, because NIA produces a less steep dose-response curve with respect to effects of changing doses on FPG compared with STZ. Even though the protection of 67 mg/kg NIA was too high to induce frank diabetes in some of the animals, this dose still results in a significant change in
glucose tolerance, making it a suitable model for studies of new pharmacological agents for the treatment of diabetes. The model has proven to be stable for up to 2 mo, both functionally and histologically. The characteristics of the model include a reduced β-cell mass and disturbed residual insulin-secretory capacity, leading to a decreased ability to dispose of glucose, seen as both fasting, and especially postprandial hyperglycemia. The pathogenesis of latent autoimmune diabetes in adults (LADA) is closely related to a primary reduction in β-cell function, whereas obesity and the metabolic syndrome are not thought to be of primary importance (43, 48, 53). Because the primary defect in the present syndrome are not thought to be of primary importance in adults (LADA) is closely related to a primary reduction fasting, and especially postprandial hyperglycemia.

Furthermore, the good condition of the animals after induction of diabetes with NIA+STZ makes this a valuable alternative to pancreatectomy for induction of diabetes with respect to animal welfare measures. Thus a model of IGT/mild insulin-deficient diabetes has been developed that can be very useful in short- and long-term studies of pathophysiology and treatment of human diabetes/IGT due to reduced insulin secretion capacity.

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