Sensory nerve inactivation by resiniferatoxin improves insulin sensitivity in male obese Zucker rats

Sophia G. Moesgaard,1,2 Christian L. Brand,1 Jeppe Sturis,1 Bo Ahérn,3 Michael Wilken,1 Jan Fleckner,1 Richard D. Carr,1 Ove Svendsen,2 Anker J. Hansen,1 and Dorte X. Gram1,2

1Discovery, Novo Nordisk A/S, Maaloev; 2Department of Pharmacology and Pathobiology, The Royal Veterinary and Agricultural University, Copenhagen, Denmark; and 3Department of Medicine, Lund University, Lund, Sweden

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Moesgaard, Sophia G., Christian L. Brand, Jeppe Sturis, Bo Ahérn, Michael Wilken, Jan Fleckner, Richard D. Carr, Ove Svendsen, Anker J. Hansen, and Dorte X. Gram. Sensory nerve inactivation by resiniferatoxin improves insulin sensitivity in male obese Zucker rats. Am J Physiol Endocrinol Metab 288: E1137–E1145, 2005; doi:10.1152/ajpendo.00356.2004.—Recent studies have suggested that sensory nerves may influence insulin secretion and action. The present study investigated the effects of resiniferatoxin (RTX) inactivation of sensory nerves (desensitization) on oral glucose tolerance, insulin secretion and whole body insulin sensitivity in the glucose intolerant, hyperinsulinemic, and insulin-resistant obese Zucker rat. After RTX treatment (0.05 mg/kg RTX sc given at ages 8, 10, and 12 wk), fasting plasma insulin was reduced (P < 0.0005), and oral glucose tolerance was improved (P < 0.005). Pancreas perfusion showed that baseline insulin secretion (7 mM glucose) was lower in RTX-treated rats (P = 0.01). Insulin secretory responsiveness to 20 mM glucose was enhanced in the perfused pancreas of RTX-treated rats (P < 0.005) but unaffected in stimulated, isolated pancreatic islets. At the peak of spontaneous insulin resistance in the obese Zucker rat, insulin sensitivity was substantially improved after RTX treatment, as evidenced by higher glucose infusion rates (GIR) required to maintain euglycemia during a hyperinsulinemic euglycemic clamp (GIRmax−120min= 5.97 ± 0.62 vs. 11.65 ± 0.83 μU·kg−1·min−1 in RTX-treated rats, P = 0.003). In conclusion, RTX treatment and, hence, sensory nerve desensitization of adult male obese Zucker rats improved oral glucose tolerance by enhancing insulin secretion, and, in particular, by improving insulin sensitivity.

capsaicin; hyperinsulinemic euglycemic clamp; obesity

Type 2 diabetes is characterized by a combination of diminished tissue sensitivity to insulin and pancreatic β-cell insufficiency resulting in chronic hyperglycemia (4). The pathogenesis of type 2 diabetes is complex and not completely understood; however, one factor that might contribute is the autonomic nervous system, which has an established role in glucose homeostasis (1, 37, 50). Parasympathetic and sympathetic nerves have been shown to affect both pancreatic islet function and insulin action (2, 3, 11, 13, 37, 40), but it is less established that sensory nerves influence the secretion and action of insulin. Sensory nerve inactivation (desensitization) through neonatal capsaicin treatment has been shown to improve glucose tolerance and insulin sensitivity in normal Sprague-Dawley rats (17, 26). Preliminary studies have investigated whether capsaicin desensitization also affects glucose homeostasis in type 2 diabetic subjects and have shown that desensitized adult Zucker diabetic fatty (ZDF) rats display improvement of glucose tolerance (14, 15).

The main focus of this study was to investigate whether sensory nerves influence insulin sensitivity in a nondiabetic but insulin-resistant animal model. The obese Zucker rat develops obesity, mild hyperglycemia, impaired glucose tolerance, extreme hyperinsulinemia, and insulin resistance at the age of 7–9 wk but, in contrast to the ZDF rat it retains the capacity to secrete insulin (36). In this study, the effect of sensory nerve desensitization on oral glucose tolerance, insulin secretion, and whole body insulin sensitivity was examined in the obese Zucker rat. To achieve sensory nerve desensitization, resiniferatoxin (RTX), a vanilloid that shares the same mechanism of action as capsaicin, was used (42). RTX acts selectively on primary sensory neurons (unmyelinated C fibers and thin myelinated Aδ fibers) in the rat to produce ultrastructural alterations and depletion of the sensory neurotransmitters (46, 49). Consequently, RTX desensitization may improve glucose homeostasis by inhibiting the release of neuropeptides from sensory afferent nerves. Furthermore, RTX has some advantages compared with capsaicin, e.g., a wider therapeutic index and a higher potency (46).

Materials and Methods

Experimental animals. Male obese Zucker rats, purchased from Charles River Laboratories (Wilmington, MA) at the age of 6 wk were housed at the Animal Unit (Novo Nordisk, Bagsvaerd, Denmark) under controlled ambient conditions with a constant temperature (20 ± 2°C) and a fixed 12:12-h light-dark cycle (light off at 6:00 PM). The rats had free access to standard rat chow (Altromin 1324; Brogaarden Aps., Gentofte, Denmark) and fresh tap water. After allocation to study groups, rats were housed two rats per cage separated by a fence. Food and water consumption was measured for each rat twice weekly. Rats were weighed once weekly. The experiments were approved by the Danish Animal Experiments Inspectorate and followed Principles of Laboratory Animal Care (EU Directive 86/609/EEF, 24.11.1986).

Resiniferatoxin desensitization. Resiniferatoxin (RTX, 1 mg; Sigma-Aldrich Chemie, Schnelldorf, Germany) was dissolved in 5 ml of ethanol and mixed with 45 ml of Tween in isotonic saline [5 ml of Tween 80 (Sigma-Aldrich Chemie) dissolved in 40 ml of isotonic saline]. A dose of 0.05 mg/kg RTX was given in a volume of 2.5 ml/kg to the scruff of the neck of the rats at the ages of 8, 10, and 12 wk. Because RTX acutely evokes pain and neurogenic inflammation (20), rats were anesthetized (halothane) before RTX dosing and administered 5 mg/kg carprofen subcutaneously (Pfizer Animal Health, St. Louis, MO). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Sensory nerve inactivation improves insulin sensitivity

Ballered, Denmark) and 0.3 mg/ml buprenorphine subcutaneously (GEA, Hvidovre, Denmark) for analgesic relief. Rats were gradually taken off anesthesia, placed in a heating chamber (25°C) 30 min after dosing, and monitored carefully for the next 2 days (activity level, food and water intake). The analgesic treatment was repeated the day after RTX administration. Control rats were anesthetized and treated with an equal amount of vehicle (VEH) and analgesics as the RTX-treated rats. The eye wipe response was examined before the experimental procedures to confirm desensitization (30, 43, 45). All RTX-treated rats and one control rat were tested with an ocular capsaicin application [1 mg capsaicin (Fluka Chemica, Buchs, Switzerland) in 100 µl ethanol, 100 µl of Tween 80, 9800 µl saline]. Desensitization should lead to a negative response (blinking without wiping). After the third RTX treatment (i.e., 12 wk), rats were randomly divided into groups for pancreatic perfusion (n = 8), isolation of pancreatic islets (n = 6), and hyperinsulinemic euglycemic clamp (n = 15).

Oral glucose tolerance test. Before RTX treatment, rats were allocated to groups (RTX or VEH) according to their body weight and blood glucose levels during an oral glucose tolerance test (OGTT before RTX). A second OGTT (OGTT after RTX) was performed after the second RTX treatment (11-wk-old rats) to follow a possible change in glucose tolerance in all the rats before any of the final, more specific tests.

Rats were fasted from 3:00 PM for 18 h before the test. Rats had been gavaged with saline 2 days before the OGTT. Before the OGTT, the rats had been trained to the handling and blood sampling procedures, and restraining was therefore not necessary. Glucose (2 g/kg) was given by gavage, and rats were bled from the tail tip capillary for measurement of blood glucose (BG) and plasma insulin (PI) at 0 (baseline), 30, 60, and 120 min after the glucose load. Blood samples for measurement of BG were collected in 10-µl heparinized glass capillary tubes, immediately suspended in 500 µl of EBIO analysis buffer, and analyzed on the test day. Blood samples for PI determination were collected in precooled 100-µl glass capillary tubes containing heparin, and plasma separated after centrifugation (8,000 × g for 6 min at 4°C) was kept at −20°C until assayed. During the test, no adverse signs of disturbed general condition were observed and therefore no adverse signs of stress noted.

Pancreas perfusion. Before the pancreas perfusion, four VEH- and four RTX-treated rats (fasted for 17–20 h) were weighted and anesthetized by 2 ml/kg of Hypnorm-Dormicum 1:1 [Hypnorm is 0.315 mg/ml fentanyl citrate and 10 mg/ml fluanisone (Janssen Pharmaceutica, Beerse, Belgium); Dormicum is 5 mg/ml midazolam sodium chloride, 5 mg/ml 25% acidic hydrochloride, and 4 mg/ml sodium hydroxide (Hoffmann-La Roche, Basel, Switzerland)], and the pancreas was isolated and perfused using an oxygenated Krebs-Ringer buffer with 4% dextran T-70 and 0.2% BSA, as previously described (41). A constant flow rate of 1.9 ml/min was used. After a 20-min equilibration period at 7 mM, glucose fractions of perfusate effluent from the portal vein were collected at 1-min intervals for a 50-min period. The fractions were collected in tubes placed in ice water and were stored at −20°C until assayed for determination of insulin concentrations. The perfusion protocol consisted of three consecutive periods under the following conditions: 7 mM glucose (10 min), 20 mM glucose (25 min), and 20 mM glucose plus 10 mM arginine (15 min).

Isolation and incubation of pancreatic islets. Before the isolation of pancreatic islets, three VEH- and three RTX-treated rats were sedated with chloral hydrate, and islets were isolated by the collagenase digestion technique, as previously described (28). All isolated islets from each group of rats were pooled, and the RTX islets and VEH islets were cultured overnight in 10 ml of RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 2.05 mM l-glutamine, 2.5 µg/ml amphotericin B (all Gibco-BRL, Paisley, Scotland, UK). The following morning, RTX- and VEH-treated islets were preincubated for 60 min in modified HEPES medium at 3.3 mM glucose in an atmosphere of 5% CO2 at 37°C (pH 7.4). Thereafter, the islets were incubated for 60 min in different glucose concentrations (3.3, 5.6, 8.3, 11.1, 16.7, and 22.2 mM glucose) in air equilibrated with 5% CO2 at 37°C (pH 7.4) in HEPES medium supplemented with 0.1% human serum albumin. Per glucose concentration, eight incubations of three islets were performed. The insulin response to glucose of three islets was assessed for each of the eight incubations by sampling 25 µl of the medium surrounding the islets. These samples were frozen at −18°C for subsequent analysis of insulin.

Surgical preparation and hyperinsulinemic euglycemic clamp studies. One to two weeks before the clamp studies, catheters (Tygon S-50-HL, ID 0.016 in., OD 0.031 in.; Norton Performance Plastics, Akron, OH) were inserted (under halothane anesthesia) in the left carotid artery (blood sampling) and jugular vein (infusions) and exteriorized on the back of the neck as described (32). Prophylactic antibiotic (0.6 ml/kg Tribribsin sc, Schering-Plough, Farum, Denmark) and analgesic treatments (5 mg/kg carprofen sc, Pfizer Animal Health) were employed for 3 days after surgery. Rats recovered for ≥7 days before the clamp study.

The hyperinsulinemic euglycemic clamp studies were performed over a 2-wk period, with the test of one RTX and one VEH rats per day. In all, seven VEH- and eight RTX-treated rats were tested. The rats were fasted overnight for 18 h, and in the morning the catheters were checked for patency and were then connected to the infusion system. The rats were placed in clamp cages and allowed to settle for 45–60 min. Blood was sampled for determination of fasting plasma glucose (PG) and fasting plasma insulin (PI). During the 120-min clamp study, insulin was given as a primed continuous (5 mU·kg−1·min−1) infusion, and a variable glucose infusion rate (GIR) was adjusted to maintain euglycemia. At the last blood sampling, plasma was saved for the determination of PI. The general behavior of the rats during the clamp was not affected, and no difference in general behavior between the RTX and control groups was noted.

Glucose and insulin assays. BG and PG concentrations were analyzed by the immobilized glucose oxidase method using either an EBIIO Plus autoanalyzer (Eppendorf, Hamburg, Germany) or, during clamp studies, a YSI 2300 STAT Plus (Yellow Springs Instrument, Yellow Springs, OH).

Pl and pancreas perfuse insulin concentrations were determined using an in house monoclonal/polyclonal mouse/guinea pig antibody ELISA technique (HUI-018 and GP4042D, Novo Nordisk).

In vitro insulin from stimulated pancreatic islets was analyzed radioimmunologically using a guinea pig anti-rat insulin antibody, mono-225I-labeled human insulin as tracer, and rat insulin as standard (Linco Research, St. Charles, MO). For the separation of free and bound radioactivity, the double-antibody technique was used.

Peroxisome proliferator-activated receptor transactivation assays. RTX was tested for capability to activate peroxisome proliferator-activated receptor (PPAR) receptors in transactivation assays. The PPAR transactivation assay has been described previously. Briefly, the ligand-binding domains of the three human PPAR receptor subtypes were fused to the DNA-binding domain (amino acids 1–147) of the yeast transcription factor Gal4. HEK293 cells were transiently transfected with an expression vector for the respective PPAR chimeras along with a reporter construct containing five copies of the Gal4 DNA binding site driving expression of a luciferase reporter gene. RTX and positive control compounds (rosiglitazone, Wy-14643, caycycelin) were dissolved in DMSO and diluted 1:1,000 upon addition to the cells. Compounds were tested in five concentrations ranging from 0.01 to 30 µM. Cells were treated with compound for 24 h, followed by luciferase assay.

Statistical analysis. Results are shown as means ± SE. Areas under the curve (AUC) were calculated by the trapezoidal rule. Statistical comparisons of effect of RTX treatment on body weight, food and water intake, BG, PI, insulin secretion during pancreas perfusion, and in vitro β-cell stimulation were performed using an unpaired Student’s t-test. In case group variances differed, Satterthwaite’s method was used. A repeated measurements analysis was performed with the
following explaining variables: RTX treatment (qualitative) and PG and body weight (quantitative/covariates). Each rat had random effect, and all other variables had fixed effects. An antedependence-1 covariance structure was used based on Akaike’s Information Criterion and Schwarz’s Bayesian Criterion. This test estimated the effect of RTX treatment on the GIR in the 60- to 120-min interval and furthermore determined whether nonexperimental features (covariates) such as body weight and PG values had also affected the GIR. $P < 0.05$ was considered significant.

RESULTS

Sensory nerve desensitization by RTX. All RTX-treated rats had a negative eye wipe response before experimental procedures, as they did not exhibit eye wipe response to topical application of a capsaicin solution (43, 45). For comparison, one control rat reacted immediately to the application and was wiping its eyes for 30–60 s.

Body weight and food and water intake. Figure 1A shows the growth curves of the two groups. The RTX-treated group members lost $\sim10\%$ of their weight during the week after the first RTX treatment (at the age of 8 wk). This slight weight drop was not completely regained; otherwise, the two growth curves were similar with regard to slope. The RTX group had a marked decrease in both food and water intake for the first 2 days after the first RTX treatment but quickly regained a more normal intake (Fig. 1B). Average water intake (8–14 wk), was not affected by RTX treatment (32 ± 1 g vs. 31 ± 1 g in RTX-treated rats, $P = 0.52$); however, average food intake (8–14 wk) was significantly decreased in RTX-treated rats (32 ± 1 g vs. 28 ± 1 g in RTX-treated rats, $P = 0.002$).

OGTT. Before RTX treatment, rats were allocated to groups with matching body weight and OGTT before RTX (Table 1 and Fig. 2). Three weeks later (after the second RTX treatment), the second OGTT (OGTT after RTX) was performed, and fasting BG tended to be lower, although not statistically significant, in the RTX-treated rats (Table 1). Oral glucose tolerance was significantly improved in the RTX-treated rats (AUCBG: 0–120 min: 980 ± 29 vs. 860 ± 27 mM·min/mg in RTX-treated rats, $P < 0.005$; Fig. 2). Fasting PI during OGTT after RTX increased in both RTX- and VEH-treated rats, yet the hyperinsulinemia was significantly lower in RTX-treated compared with VEH rats ($P < 0.0005$; Table 1). The PI response at 30 min after the oral glucose load was similar in the two groups. However, PI decreased faster and to a significantly lower level in the RTX-treated group at 60 and 120 min after the glucose load ($P < 0.01$; Fig. 2). In general, the PI level was significantly reduced in RTX-treated rats during OGTT after RTX (AUCPI: 0–120 min: 230 ± 112 vs. 187 ± 11 nM·min in RTX-treated rats, $P < 0.01$).

Pancreas perfusion. Four days before pancreas perfusion was performed, RTX rats had a lower, although not a statistically significant, fasting BG level compared with VEH-treated rats (4.53 ± 0.14 vs. 4.18 ± 0.10 mM in RTX-treated rats, $P = 0.09$). As shown in Fig. 3, RTX-treated rats had a significantly lower baseline (7 mM glucose in perfusate) insulin response compared with VEH rats (1.37 ± 0.17 vs. 0.54 ± 0.17 pmol/min in RTX-treated rats, $P = 0.01$; Fig. 3B). The increased glucose concentration (20 mM glucose in perfusate) caused a biphasic insulin release. There were no group differences in insulin secretion in absolute terms. However, both first- and second-phase insulin secretory responsiveness, defined as percent increase above baseline, were significantly enhanced in RTX-treated rats (first phase: 326 ± 111 vs. 98 ± 860 in RTX-treated rats, $P < 0.005$; second phase: 25 vs. 11 nM·min in RTX-treated rats, $P < 0.005$; Fig. 3C). Insulin secretion during arginine stimulation of the perfused pancreas did not differ between the two rat groups (total insulin secretion during 15-min arginine stimulation: 522 ± 187 vs. 111 ± 413 pm in RTX-treated rats, $P = 0.41$, data not shown).

Incubation of isolated pancreatic islets. There was no significant difference between the stimulated in vitro insulin response in RTX-treated and VEH rats (AUC: 187 ± 23 vs. 158 ± 25 mM·nM in RTX-treated rats, $P = 0.45$). The insulin response to the glucose gradient is shown in Fig. 4.

Hyperinsulinemic euglycemic clamp. On the day of the individual clamps, the selected RTX-treated rats had a significantly lower fasting PG (7.8 ± 0.2 vs. 6.9 ± 0.3 mM in RTX-treated rats, $P < 0.05$) as well as a lower body weight (434 ± 17 vs. 382 ± 13 g in RTX-treated rats, $P < 0.05$)

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**Fig. 1.** A: weight curves of resiniferatoxin (RTX)-treated (○) and vehicle (VEH) group (●) measured individually every week during the experimental period (8–16 wk). B: food intake (g) of the RTX-treated (○) and VEH group (●) measured individually twice a week after the 1st RTX treatment at the age of 8 wk. Values are expressed as means ± SE. Arrows indicate time when RTX or VEH was given.
compared with the VEH rats. These findings were adjusted for when the mean GIR was calculated. PI levels at the beginning of insulin infusion (PIclamp: 0 min) were significantly lower in the RTX-treated rats, whereas the PI levels at the end of the clamp (PIclamp: 120 min) as well as the rise in PI levels (ΔPIclamp) did not differ between the two groups (Table 2). Mean GIR, therefore, was not adjusted for this parameter.

During the clamp, the coefficient of variation in PG level was 10% at 60–120 min (MeanPG60–120: 6.71 ± 0.17 vs 6.98 ± 0.12 mM in RTX-treated rats). Whereas the mean GIR of the VEH rats seemed to reach steady state after 30 min, the GIR of the RTX-treated rats kept increasing until 60 min during the clamp (Fig. 5). In general, the mean GIR of the RTX-treated rats was markedly increased compared with VEH rats (Mean GIR60–120: 5.97 ± 0.62 vs. 11.65 ± 0.83 mg·kg⁻¹·min⁻¹ in RTX-treated rats, P = 0.003; Fig. 6).

**DISCUSSION**

The results of the present study demonstrate that RTX treatment and, hence, sensory nerve desensitization of adult male obese Zucker rats improves oral glucose tolerance by enhancing insulin secretion and, in particular, by improving insulin sensitivity.

**RTX sensory nerve desensitization.** The participation of sensory nerves in physiological processes has for several years been studied with the use of the drugs capsaicin and RTX (19, 42). Capsaicin and RTX act on small and medium-sized, peptidergic sensory neurons with primary afferent unmyelinated C fibers and thin myelinated Aδ fibers (24, 46, 49). RTX administration acutely leads to an activation of sensory nerves with release of sensory neurotransmitters such as substance P and calcitonin gene-related peptide (CGRP), rapidly followed by a prolonged yet reversible impairment of sensory effector function through neuropeptide depletion termed desensitization (20, 42, 44). Studies in normal Sprague-Dawley rats suggest a dose of 0.10 mg RTX/kg as the dose for sensory nerve desensitization, with a reversible effect (evaluated by the loss of pain sensation) lasting for at least 2 wk (12). To our knowledge, desensitization has not previously been performed in obese Zucker rats. We therefore performed pilot dose-range experiments in 8-wk-old obese Zucker rats, showing that 0.05 mg RTX/kg was both a well-tolerated and an effective dose for desensitization. To ensure an effective desensitization throughout the present study the tolerable dose of 0.05 mg RTX/kg was repeated every 2nd wk (at the ages of 8, 10, and 12 wk). The desensitization was confirmed by lacking eye wipe response to capsaicin.

**RTX activation of PPARs.** To test whether the mechanism of RTX-mediated insulin sensitization could be through PPAR activation, RTX was assayed along with compounds known to activate PPARs in transactivation assays. None of the PPAR subtypes, α, δ, or γ, showed any response to RTX, whereas rosiglitazone, Wy-14643, and carbacyclin activated their cognate receptor as expected (data not shown).

**Table 1. Characteristics of basal values including body weight, blood glucose, and plasma insulin in fasted rats used in OGTTbefore RTX and OGTTafter RTX**

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Test OGTTbefore RTX (8 wk)</th>
<th>Test OGTTafter RTX (11 wk)</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>Weight, g</td>
</tr>
<tr>
<td>VEH</td>
<td>36</td>
<td>238 ± 7</td>
</tr>
<tr>
<td>RTX</td>
<td>38</td>
<td>247 ± 6</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. OGTT, oral glucose tolerance test; RTX, resiniferatoxin; VEH, vehicle; BG, blood glucose; PI, plasma insulin. †P < 0.0005 vs. VEH rats.

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Fig. 2. Oral glucose tolerance tests (OGTT) of RTX-treated (●) and VEH group (○). Blood glucose and plasma insulin variations before RTX (OGTTbefore RTX) and after RTX (OGTTafter RTX). Glucose (2 g/kg) was given by gavage to fasted rats at 0 min. Samples for measurement of blood glucose and plasma insulin were obtained from the tail tip capillary at 0 (baseline), 30, 60, and 120 min after glucose. Values are expressed as means ± SE. *P < 0.05 vs. VEH rats.
Body weight and food and water intake. Although RTX-treated rats lost weight and food intake the day after the first treatment and were less active for the 1st h after treatment than control rats, no particular differences were observed in the long-term clinical appearance of the two rat groups. The immediate reaction may be due to the acute proinflammatory and pain-evoking effects of RTX through activation of sensory nerves (20). Most of the weight loss, as well as the activity level, was regained within 1 or 2 days following RTX treatment. The average food intake during the experimental period (8 wk) was lower in the RTX-treated rats, which was mainly caused by the marked decrease in food intake seen in direct proximity to the RTX treatments. However, a food intake similar to that of VEH rats was regained within 2–3 days following RTX treatment. No detailed investigations on changes in food intake patterns were performed.

OGTT. The VEH rats developed mild hyperglycemia, impaired glucose tolerance, and marked hyperinsulinemia during the 3 wk between the two OGTTs, whereas the RTX-treated rats had improved oral glucose tolerance, indicating improved glucose disposal, which is concordant with capsaicin desensitization studies (15, 17). At the time of the OGTT after RTX, fasting PI levels had increased in both groups but less in RTX-treated rats. The fasting PI level reflects changes in insulin sensitivity and in the fasting glucose level, but probably not changes in β-cell mass or β-cell function (9). The PI level decreased to a lower level after 60 min in RTX-treated rats, which might also indicate increased peripheral insulin sensitivity.

Pancreas perfusion and incubation of isolated pancreatic islets. Insulin secretion in the whole isolated perfused pancreas was reduced at basal glucose levels (7 mM). The reduced basal secretion probably reflects the adaptation of the pancreas to the improved insulin sensitivity. Also, the chronically reduced glucose level in the RTX group is likely to have had an effect on the results of the pancreas perfusions. No differences in
Hyperinsulinemic euglycemic clamp. To assess whether RTX treatment had improved insulin sensitivity, a hyperinsulinemic euglycemic clamp was performed at the peak of spontaneous insulin resistance in the obese Zucker rat (i.e., 14- to 15-wk-old rats) (52). During the clamp, insulin was infused at a physiological level (5 mU·kg⁻¹·min⁻¹), because a previous study had shown that a moderate insulin level yielded the most prominent difference in insulin sensitivity (26). Despite significantly different levels of PI at the beginning of the clamp (PIclamp: 0 min), the rise in PI levels during the clamp (ΔPIclamp) did not differ between the two groups. Steady-state PG conditions were reached 60 min after glucose infusion initiation. The PG coefficient of variation for the last 60 min of the clamp was 10% indicated an acceptable maintenance of normoglycemia despite the presence of hyperinsulinemia (27). The GIR is a measure of whole body insulin sensitivity (10, 27). RTX-treated rats needed a markedly higher GIR than VEH rats to maintain PG steady state. Because both reduced PG levels and increased body weights influence insulin sensitivity and the increase in insulin sensitivity after RTX treatment may in part be accounted for by the reduced body weight (27), a test of repeated measurements taking PG level and body weight into consideration as covariates was performed. This analysis

Table 2. Characteristics of PIclamp

<table>
<thead>
<tr>
<th>Rat Group</th>
<th>PIclamp, 0 min</th>
<th>PIclamp, 120 min</th>
<th>ΔPIclamp</th>
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<tbody>
<tr>
<td>VEH</td>
<td>1.015±110</td>
<td>1.761±268</td>
<td>714±191</td>
</tr>
<tr>
<td>RTX</td>
<td>0.621±96*</td>
<td>1.537±111</td>
<td>922±121</td>
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Data are expressed as means ± SE. *PIclamp, plasma insulin levels (pM) during euglycemic hyperinsulinemic clamp. *P < 0.01 vs. VEH rats.
showed that RTX treatment had improved insulin sensitivity substantially and that, although the increased body weight also affected insulin sensitivity, the effect of RTX treatment alone was still highly significant.

Further studies are required to evaluate the site of improved insulin sensitivity (e.g., liver vs. peripheral tissues) following desensitization by infusion of tracer-labeled glucose (27). Desensitization has been shown to result in complete or partial depletion of CGRP- and substance P-containing sensory nerves in many rodent tissues, such as pancreas, skeletal muscle, and adipose tissue (7, 22, 29). Furthermore, CGRP has been shown to modulate glucose homeostasis by antagonizing insulin action in both skeletal muscle and liver (8, 31, 33).

Other clamp studies have investigated the effect of capsaicin treatment on hepatic glucose metabolism in normal rats (8, 34). Capsaicin desensitization has been shown to inhibit hepatic glucose production (26). However, a recent study investigated the effect of chronic regional capsaicin treatment of fibers in the anterior plexus of the liver on insulin sensitivity in normal rats (34). This selective denervation of the liver induced insulin resistance, whereas insulin sensitivity was increased following acute intraportal administration of capsaicin (34). It is therefore more likely that the improved insulin resistance seen in the present study was caused by an improvement of insulin sensitivity in peripheral tissue.

A clamp study on the effect of capsaicin desensitization in normal rats demonstrated that insulin sensitivity was improved mainly due to increased insulin sensitivity of skeletal muscle glycogen synthesis through increased glycogen synthase activity (26). Leighton and Foot (29) performed in vitro experiments comparing the acute effect of RTX and capsaicin in muscle preparations and found that both vaniloids have an immediate excitatory effect on the sensory nerves, leading to a release of the sensory neuropeptide CGRP and an inhibition of the insulin-stimulated rates of glycogen synthesis. Thus depletion of CGRP through RTX desensitization may influence whole body glucose homeostasis by amelioration of insulin-stimulated glycogen synthesis and thus insulin sensitivity. Consequently, at least part of the improvement of insulin sensitivity seen in the present study is likely due to the lack of CGRP binding to skeletal muscle receptors and increased glycogen synthesis. This could be of relevance in the animal model used in this study, since a decrease in insulin-mediated skeletal muscle glycogen synthesis has a dominant role in insulin resistance (18, 48).

Besides improvement in skeletal muscle sensitivity to insulin, it is possible that adipose tissue was also affected by RTX sensory nerve desensitization. Reduced insulin sensitivity in adipose tissue is also a cause of insulin resistance. This is especially relevant in the obese Zucker rat, which has markedly increased fat deposits (36) and furthermore an increased level of CGRP in plasma (35). Circulating free fatty acids (FFAs) are elevated in many insulin-resistant states and may contribute to the insulin resistance by inhibiting glucose uptake and glycogen synthesis by increasing hepatic glucose output (6, 25). FFA levels were not measured in the present study, although they may provide an index of adipose insulin sensitivity that may help explain the improvement of insulin sensitivity seen after RTX desensitization. Capsaicin-treated normal rats with increased insulin sensitivity have been shown to have reduced levels of nonesterified FFAs as well as an enhanced whole body lipogenesis (26, 38). This might be connected to reduced levels of epinephrine, norepinephrine, glucagon, and corticosterone, which are all lipolytic (23, 26) and should be further studied.

Insulin sensitivity can be achieved in animal models of type 2 diabetes as well as in type 2 diabetes patients by drugs activating PPAR-γ (5, 47). In addition, several aspects of lipid metabolism are regulated by PPAR receptors, which can lead to improvement of insulin resistance (16, 51), and it was reasoned that RTX might act as a PPAR agonist. In the present study, it was therefore tested whether RTX functions as a PPAR ligand. However, no induction of PPAR transactivation activity was found following application of RTX to PPAR assays specific for either α-, δ-, or γ-subtypes, whereas control compounds induced significant activity. The basic mechanism underlying the significant improvement of insulin resistance following RTX desensitization is therefore very likely independent of PPARs and remains unknown. Further studies are needed to clarify the mechanism of action.

In conclusion, whole body insulin sensitivity, insulin secretion, and oral glucose tolerance were all markedly improved in obese Zucker rats following RTX sensory nerve desensitization. The study also demonstrates that repeated small doses of RTX effectively desensitize sensory nerves in the obese Zucker rat, an insulin-resistant and obese animal model. These results support further exploitation of sensory nerve involvement in the development of insulin resistance and impaired glucose tolerance.

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Sensory nerve inactivation improves insulin sensitivity

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Current address of S. G. Møesgaard: Department of Basic Animal and Veterinary Sciences, The Royal Veterinary and Agricultural University, Frederiksberg, Denmark.

GRANTS

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