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Sensory nerve desensitization by resiniferatoxin improves glucose tolerance and increases insulin secretion in Zucker Diabetic Fatty rats and is associated with reduced plasma activity of dipeptidyl peptidase IV

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Abstract

Sensory nerve desensitization by capsaicin has been shown to improve the diabetic condition in Zucker Diabetic Fatty rats. However, administration of capsaicin to adult rats is associated with an increased mortality. Therefore, in this experiment, we examined the influence of resiniferatoxin, a tolerable analogue of capsaicin suitable for in vivo use, on the diabetic condition of Zucker Diabetic Fatty rats. A single subcutaneous injection of resiniferatoxin (0.01 mg/kg) to these rats was tolerable, with no mortality. When administered to early diabetic rats at 15 weeks of age, the further deterioration of glucose homeostasis was prevented by resiniferatoxin. Further, when administered to overtly diabetic rats at 19 weeks of age, resiniferatoxin markedly improved glucose tolerance at two weeks after administration and this was accompanied by an increased insulin response to oral glucose as well as a reduction in the plasma levels of dipeptidyl peptidase IV. Therefore, resiniferatoxin is a safe alternative to capsaicin for further investigations of the role of the sensory nerves in experimental diabetes.

Keywords: Resiniferatoxin; Diabetes; Sensory nerve; Dipeptidyl peptidase IV; (Zucker Diabetic Fatty rat)

1. Introduction

Type 2 diabetes is strongly related to the development of obesity (Mokdad et al., 2000; Kopelman, 2000; Steppan et al., 2001; Unger, 1997). The mechanism of the deterioration of glucose metabolism in obese subjects developing type 2 diabetes has not been established. Although it is probably multifactorial, recent studies have suggested involvement of the sensory nerves, since capsaicin desensitization improves glucose tolerance and the glucose stimulated insulin response (Gram et al., 2000a,b) in the Zucker Diabetic Fatty rat, which is a spontaneous model of type 2 diabetes mellitus (Peterson, 2000). Capsaicin specifically binds to small non-myelinated C-fibres resulting in sensory desensitization at high doses (Holzer, 1991). A problem is, however, that capsaicin is toxic when given to adult diabetic rats at high doses (unpublished personal findings). A better tool to investigate the potential role of sensory nerves in obesity-related type 2 diabetes might be resiniferatoxin, which is an ultra potent capsaicin analogue (Szallasi et al., 1999), that is more tolerable in vivo than capsaicin (Szolcsanyi et al., 1991). However, no previous studies have investigated the influence of resiniferatoxin on rodent type 2 diabetes.

The aim of the present study was, therefore, to evaluate the use of resiniferatoxin as a substitute for capsaicin to

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prevent or to revert the development of diabetes in Zucker Diabetic Fatty rats. We examined the potential mechanisms by studying the insulin response to glucose, both in vivo after an oral glucose challenge, and in vitro in isolated islets, as well as studying the circulating levels of the enzyme dipeptidyl peptidase IV, which has been shown to degrade several biologically active peptides of importance for insulin secretion and action (Mentlein et al., 1993; Mentlein, 1999) and for which inhibitors of dipeptidyl peptidase IV are expected to be potential drug candidates for the prevention of type 2 diabetes. Therefore, alterations in the level of this enzyme might affect the diabetic state of the Zucker Diabetic Fatty rats.

2. Materials and methods

2.1. Laboratory animals

Two groups of diabetic male Zucker Diabetic Fatty rats (Genetic Models Inc., US), were kept in the Bagsvaerd Animal Unit, Novo Nordisk. The rats were housed 2 per cage, in a normal day light cycle (light on at 6 am) at a fixed room temperature (22±2 °C), and had free access to tap water and Purina chow no. 5008 (Formula Diet, US). Rats for the first experiment (study 1, n=6) were studied between 15 and 21 weeks of age. Rats for the second experiment (study 2, n=7) were studied between 17 and 19 weeks of age. 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).

2.2. Desenisitization of sensory nerves by resiniferatoxin

After an acclimatization period of 2 weeks, the rats were first submitted to 5% halothane anesthesia, whereafter they were given 5 mg/kg carprofen s.c. (Rimadyl®, Pfizer Animal Health, Denmark), 0.2 ml (0.06 mg) buprenorphine s.c. (Anorfin®, GEA, Denmark) for analgesic relief, insulin s.c. (Actrapid®, Novo Nordisk, Denmark) according to their blood glucose levels the previous day and 5 ml isotonic sodium chloride SAD s.c. (SD, Denmark). The rats were then given a single injection of 0.1 mg/kg resiniferatoxin s.c. (Sigma-Aldrich, Denmark) or vehicle in the scruff of the neck at 15 weeks of age (study 1) or at 17 weeks of age (study 2). Resiniferatoxin (1 mg) was dissolved in the vehicle, consisting of 0.5 ml 99.9% ethanol diluted in 4.5 ml 10% Tween 80 (Sigma-Aldrich Chemie, Germany) in isotonic saline (SD, Denmark). Ten to fifteen minutes after dosing, rats were gradually taken off anesthesia and, for the first hour after sedation, were kept at 24 °C in a heated chamber to avoid hypothermia, where after they were transferred to the general housing area. Animals were inspected four times per day for the following 2 days and then given 5 mg/kg carprofen s.c. (Rimadyl®, Pfizer Animal Health, Denmark) twice daily to assure adequate analgesic relief during the acute painful and proinflammatory phase following resiniferatoxin administration.

2.3. Oral glucose tolerance test

Oral glucose tolerance tests were performed 1, 4 and 7 weeks after resiniferatoxin in study 1 and 2 weeks after resiniferatoxin in study 2. Rats were fasted for 2 h. At 9 a.m., 2 h fasting plasma insulin and blood glucose levels were assessed, where after the animals were given an oral glucose load of 2 g/kg by gavage. Samples for glucose and insulin were obtained from the tail tip of freely moving rats at time 0, 30, 60 and 120 min following test start. The Area Under the Curve (AUC) was calculated by the trapezoidal method.

2.4. Analysis of blood glucose

Tail-tip blood (10 μl) was obtained and immediately diluted in 500 μl EBIO buffer solution (Eppendorf, Germany) and kept on ice until analysis by the immobilized glucose oxidase method (EBIO Plus auto analyzer, Eppendorf, Germany).

2.5. Analysis of plasma insulin

Approximately 70 μl tail tip blood was collected in glass capillary tubes containing heparin and kept on ice until centrifugation (8000 rpm/6 min/4 °C) after which 15 μl plasma was transferred to cooled micronic racks containing 60 μl bovine calf serum, and stored at −20 °C until analysis by an in house Enzyme-Linked Immuno Sorbant Assay (ELISA) method. In brief, the assay for measuring insulin in rat and mouse serum or plasma is a two-site immunoassay using two polyclonal guinea pig antibodies raised against rat insulin (GP114 and GP116). Micro titer plates (Nunc, Immunoplates) were coated with GP114 serum diluted 1:1000 in PBS (Phosphate Buffered Saline) overnight. After careful washing with PBS, Tween 100 μl peroxidase labeled GP116 (1:1000 dilution in assay buffer) and 10 μl sample (serum or EDTA (ethylene diamine tetraacidic acid) stabilised plasma), calibrator or quality control were added to each well and allowed to incubate for 2 h at ambient temperature. Peroxidase was measured using TMB (3, 3′ , 5, 5′ -tetramethylbenzidine peroxidase substrate, KEM-EN-TEC, Denmark) as substrate. Purified rat insulin (Novo Nordisk batch 220891) was used as calibrator. Calibrator concentrations in heat inactivated bovine adult serum range from 3000 pM to 4.1 pM. The detection limit of the assay was 3 pM. Both insulin type 1 and type 2 are measured equally. The variation at 3 concentration levels (1650 pM, 330 pM and 55 pM) was below 10%. The AUC was calculated by the trapezoidal method.

2.6. Isolation and incubation of islets

A day after the oral glucose tolerance test and thus 2 weeks after resiniferatoxin in study 2, islets of Langerhans were isolated by the collagenase digestion technique after bleeding the rat from the abdominal aorta. In short, the common bile duct was cannulated and ligated at the papilla Vateri. The pancreas was perfused through the cannula with 8 ml of cold Hank's balanced salt solution (Sigma, St. Louis, MO), supplemented with 1.0 mg/ml collagenase P (Boehringer Mannheim, Mannheim, Germany). The pancreas was then removed and incubated for 24 min at 37 °C. After rinsing in Hank's solution (Sigma St. Louis, MO), all islets were handpicked under a stereomicroscope and cultured overnight in 10 ml of RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 2.05 mmol/l L-glutamine, 2.5 μg/ml amphotericin B (all GIBCO BRL; Paisley, Scotland), 100 IU/ml penicillin, and 100 μg/ml streptomycin (both Biological Industries, Beit Haemek, Israel) at 37 °C (pH 7.4) in a humid atmosphere of 95% air and 5% CO_2. After overnight incubation, the islets were rinsed in modified HEPES medium (pH 7.4) consisting of 125 mM NaCl, 5.9 mM KCl, 1.2 mM $MgCl_2$, 1.28 mM $CaCl_2$, and 25 mM HEPES (Boehringer Mannheim), supplemented with 0.1% human serum albumin (Behringwerke, Marburg, Germany) and 3.3 mM glucose (Fluka Chemie, Buchs, Switzerland). The islets were then preincubated for 60 min in modified HEPES medium at 3.3 mM glucose in an atmosphere of 5% CO_2 at 37 °C (pH 7.4). Thereafter, groups of islets were transferred into separate chambers for 60 min of incubation in air equilibrated with 5% CO_2 at 37 °C (pH 7.4) in the HEPES medium supplemented with 0.1% human serum albumin and varying glucose concentrations of 3.3, 5.6, 8.3, 11.1, 16.7 and 22.2 mM glucose (8 groups of 3 islets per glucose concentration). The HEPES medium was continuously gassed in an atmosphere of 95% air and 5% CO_2. After incubation, 2×25 μl of the medium surrounding the islets were removed and frozen at −18 °C for subsequent analysis of insulin, using a guinea pig anti-rat insulin antibody, mono ^{125}I-labeled human insulin as a 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.

2.7. Terminal arterial blood sampling

At the end of study 2, the rats were anaesthetized with 5% halothane, the abdominal cavity opened, and the rats were bled by collecting as much blood as possible from the abdominal aorta into 10 ml K_3-EDTA vacutainer glasses (Becton Dickinson Vacutainer Systems, UK) using a butterfly cannula. Blood for the analysis of dipeptidyl peptidase IV-activity (500 μl) was immediately removed, centrifuged (10 000 rpm/10 min/4 °C), and plasma was referred to aliquots and stored at −20 °C until analysis. To the remainder of the blood sample, the specific dipeptidyl peptidase IV inhibitor, valine pyrrolidide, (final concentration: 0.3 mmol/l) was added. The sample was remixed and centrifuged (10 000 rpm/10 min/4 °C), and plasma aliquoted and stored at −20 °C until analysis for glucagon-like peptide-1.

2.8. Analysis of plasma dipeptidyl peptidase IV

Plasma samples were obtained 5 and 7 weeks after resiniferatoxin in study 1 (after blood sampling from the retroorbital plexus during anaesthesia) and 2 weeks after resiniferatoxin in study 2 (as described above) and the activity of dipeptidyl peptidase IV was estimated by the ability to degrade glucagon-like peptide-1 (7–37) added to the sample. The method is based upon the fact that dipeptidyl peptidase IV is the sole enzyme responsible for N-terminal degradation of glucagon-like peptide-1 in plasma. In brief glucagon-like peptide-1 (7–37) (5 μl, 100 fmol) was added to plasma samples (95 μl), which were then incubated for 1 hour at 37 °C. Samples were put on an ice bath immediately after incubation and the amount of glucagon-like peptide-1 determined by an ELISA method specific for N-terminally intact peptide. A reference sample with glucagon-like peptide-1 added to heat inactivated plasma containing in addition valine pyrrolidide (0.01 mM) and aprotinin (500 KIE/ml) was processed in parallel. The dipeptidyl peptidase IV activity estimate was then calculated as Activity (%)=$(1-C_{\text{unknown}}/C_{\text{reference}})\times 100$ where C_{unknown} and $C_{\text{reference}}$ are the calculated concentrations of the sample and the reference, respectively. The minimum detectable difference was determined to be 3.2%.

2.9. Analysis of plasma glucagon-like peptide-1

C-terminal glucagon-like peptide-1 immunoreactivity was measured using antiserum 89390 (Orskov et al., 1994), which has an absolute requirement for the intact amidated C-terminus of glucagon-like peptide-1 (7–36) amide, and cross-reacts 83% with glucagon-like peptide-1 (9–36) amide, but less than 0.01% with the glycine extended form, glucagon-like peptide-1 (7–37), or with C-terminally truncated fragments. N-terminal glucagon-like peptide-1 immunoreactivity was measured using the method described by Gutniak (Gutniak et al., 1996), except for the use of antiserum Her4 (D Bataille, Montpellier, France). This assay is specific for the intact N-terminus of glucagon-like peptide-1, and cross-reacts less than 0.1% with glucagon-like peptide-1 (9–36) amide, and approximately 2% the structurally related peptides glucagon-like peptide-1 (1–36) amide and the major proglucagon fragment (proglucagon (72–158)) secreted from the pancreas. Valine pyrrolidide (0.01 mmol/l final concentration) was added to the assay buffer to prevent N-terminal degradation of glucagon-like peptide-1 during the assay incubation. High Pressure Liquid Chromatography (HPLC) supports the use of Radio

Immuno Assays (RIAs) with this specificity for determination of intact glucagon-like peptide-1 (Deacon et al., 1995). For both assays, the intra-assay coefficient of variation was less than 6%. Plasma samples were extracted with 70% ethanol (v v^{-1}, final concentration) before assay, giving recoveries of 75% (Orskov et al., 1991). For both assays, standard and ^{125}I-labelled tracers were glucagon-like peptide-1 (7–36) amide, and separation of bound from free peptide was achieved using plasma-coated charcoal (Orskov and Holst, 1987).

2.10. Statistical analysis

Data are presented as mean±S.E.M. Kruskal-Wallis nonparametric statistical method was used to compare the group means when there was a difference between the variances of the two groups. When equal variances were found, Student's *t*-test was used.

3. Results

Since the diabetic condition is known to vary quite extensively between individual Zucker Diabetic Fatty rats, an oral glucose tolerance test was performed prior to treatment, and based upon the results, the rats were allocated to two groups with matching glucose tolerance.

Capsaicin and its analogues induce desensitization of sensory nerves, but this feature is preceded by an acute activation of the nerves which causes transient pain and general malaise. In Zucker Diabetic Fatty rats, the acute reaction to neurotoxic doses of capsaicin has been associated with mortality (personal unpublished findings). In these experiments, however, we found that after 0.1 mg/kg resiniferatoxin, the acute reaction was less pronounced and had a shorter duration than after capsaicin. In these studies, no mortality was observed after resiniferatoxin. In addition, we found this dose of resiniferatoxin to be effective in that eye-wipe response was eliminated in all rats receiving the compound. In study 1, the efficacy and time course of resiniferatoxin were evaluated with regard to its ability to improve glucose tolerance in early diabetic 15 week old Zucker Diabetic Fatty rats. It was found that resiniferatoxin improved glucose tolerance (AUC for glucose) at 1 week after administration and was still effective at 4 weeks and at 7 weeks a tendency for improved glucose tolerance, although not significant, was observed (Fig. 1).

Simultaneously, plasma dipeptidyl peptidase IV activity was reduced by resiniferatoxin (Table 1).

In study 2, the efficacy of resiniferatoxin to improve glucose tolerance through augmented insulin secretion was addressed by administering the compound to 17-week-old Zucker Diabetic Fatty rats. It was again demonstrated

Table 1
The effect of resiniferatoxin on plasma dipeptidyl peptidase IV activity in Zucker Diabetic Fatty rats

Treatment	Resiniferatoxin	Vehicle
5 weeks (Study 1)	38±5[a]	59±5
7 weeks (Study 1)	62±6	69±5
2 weeks (Study 2)	68±13[a]	87±7

The plasma dipeptidyl peptidase IV activity (%) at varying timepoints after resiniferatoxin. In study 1, resiniferatoxin was given as a single subcutaneous dose at the age of 15 weeks and the rats were followed for 7 weeks. Plasma dipeptidyl peptidase IV activity was measured at 5 and 7 weeks after treatment. In study 2, resiniferatoxin was given at the age of 17 weeks and the rats were followed for 2 weeks. Plasma dipeptidyl peptidase IV activity was measured 2 weeks after treatment (the day after the oral glucose tolerance test).
Values are expressed as means±S.E.M.
[a] $P<0.03$ versus vehicle.

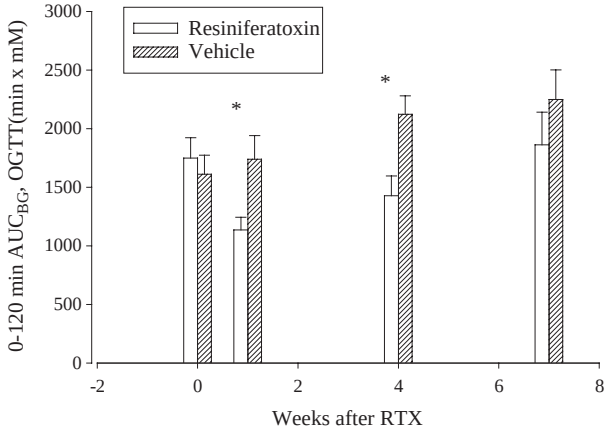

Fig. 1. The effect of resiniferatoxin on oral glucose tolerance in Zucker Diabetic Fatty rats over time. Resiniferatoxin or vehicle was given at week 0. The Area under the curve for glucose (AUC$_{BG}$) during a 2 h oral glucose tolerance test (2 g glucose/kg given by gavage right after time 0) was measured after resiniferatoxin (open bars) or vehicle (filled bars). Values are expressed as means±S.E.M. *$P<0.03$ versus vehicle.

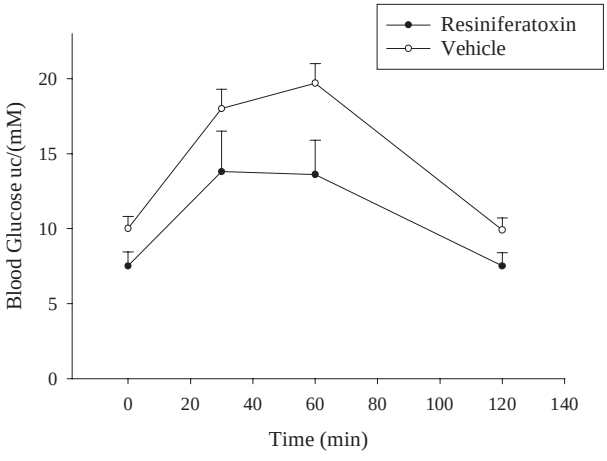

Fig. 2. The effect of resiniferatoxin on oral glucose tolerance in severely diabetic Zucker Diabetic Fatty rats. The blood glucose levels during a 2 h oral glucose tolerance test (2 g glucose/kg given by gavage right after time 0). Resiniferatoxin (closed circles) or vehicle (open circles) had been administered to 17 week old Zucker Diabetic Fatty rats 2 weeks prior to the oral glucose tolerance test. Values are expressed as means±S.E.M.

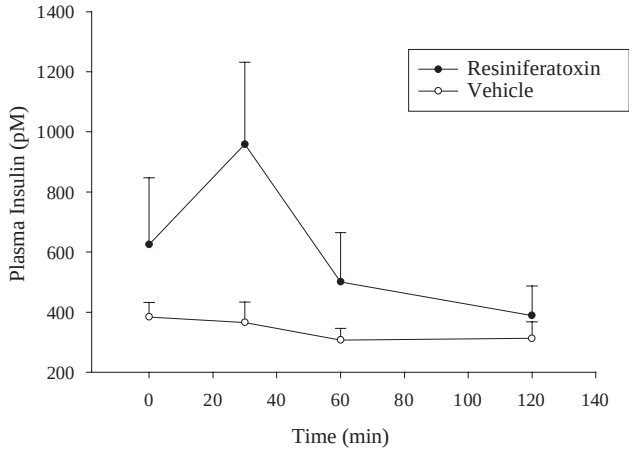

Fig. 3. The effect of resiniferatoxin on glucose stimulated insulin secretion in vivo in severely diabetic Zucker Diabetic Fatty rats. Plasma insulin levels during an oral glucose tolerance test (2 g glucose/kg given by gavage right after time 0) 2 weeks after resiniferatoxin desensitization (closed circles) or vehicle (open circles) in the 17 week old Zucker Diabetic Fatty rat. Values are expressed as means±S.E.M.

that resiniferatoxin improved oral glucose tolerance (AUC_{BG}: 0–120 min: 1366±218 (resiniferatoxin) vs. 1873±121 (vehicle) min mM, P=0.055, Fig. 2). This was accompanied by an augmented insulin response to glucose (Fig. 3). Thus, the 30-min glucose stimulated insulin-response was potentiated (AUC_{INS}: 0– 30 min: 23 750±7090 (resiniferatoxin) vs. 11 248±1585 (vehicle) min pM, P=0.025). Furthermore, also in isolated islets insulin secretion was also increased after resiniferatoxin ($AUC_{INS\text{-}in\ vitro}$: 8368±749 (resiniferatoxin) vs. 5552±610 (vehicle) pM mM, P=0.011), although the glucose sensitivity in the beta cells was not different between the groups. In study 2, plasma dipeptidyl peptidase IV activity was also reduced following resiniferatoxin (68±13 (resiniferatoxin) vs. 87±7 (vehicle) %, P=0.011), thus replicating the findings of study 1. In this study we simultaneously assessed the levels of glucagon-like peptide-1 in the plasma and found that neither total nor active glucagon-like peptide-1 was changed following resiniferatoxin (data not shown).

4. Discussion

This study examined the use of resiniferatoxin as an alternative to capsaicin, as a pharmacological tool, in experimental diabetes. It was found that the compound was well tolerated at a dose of 0.1 mg/kg s.c. in Zucker Diabetic Fatty rats. In parallel to what has been found for resiniferatoxin in relation to its effects in neurogenic inflammation in normal rats (Szallasi et al., 1989), an optimal time course of 1–2 weeks following a single subcutaneous injection was found to improve glucose tolerance in the Zucker Diabetic Fatty rat. It was further established that a significant effect lasted for 4 weeks after the dose, but after 7 weeks, the effect, although still visible, was insignificant. The efficacy of the compound was further investigated in overtly diabetic Zucker Diabetic Fatty rats during the optimal time span following dosing. It was found that resiniferatoxin was able to improve glucose tolerance and that this improvement was accompanied by augmented insulin secretion in response to glucose. Finally, the improved glucose homeostasis was accompanied by a reduction in plasma dipeptidyl peptidase IV activity.

Sensory afferent nerves of the C-fibre subset are small diameter, unmyelinated nerve fibres of the peripheral nervous system. The thin fibres are mainly nociceptors responding to noxious stimuli of mechanical, chemical or thermal origin (Holzer, 1991). Capsaicin or resiniferatoxin are plant derived vanilloids (exogenous) which bind specifically to a subset of these fibers (Szallasi et al., 1999) and induce nociception (Szolcsanyi, 1987; Caterina et al., 1999, 2000) as well as neurogenic inflammation (Jancso et al., 1967). Capsaicin and resiniferatoxin have been used as tools to accomplish either acute stimulation of the nerve fibres, or to abolish the presence of the fibres (Jancso et al., 1987; Abdel-Salam et al., 1995). Resiniferatoxin is active in micromolar concentrations (Szallasi and Blumberg, 1989; Blumberg et al., 1993), and capsaicin-sensitive nerves are inactivated by a lower dose of resiniferatoxin than capsaicin (Xu et al., 1997). Following resiniferatoxin, the desensitization begins to diminish after 1 week (Szallasi et al., 1989) or 2 weeks (Broberger et al., 2000). The improvement of glucose tolerance after resiniferatoxin confirms previous findings after using capsaicin in Zucker Diabetic Fatty rats (Gram et al., 2000a,b), in the streptozotocin-diabetic rat (Guillot et al., 1996) and normal rodents (Guillot et al., 1996; Karlsson et al., 1994). Furthermore, the potentiation of insulin secretion to oral glucose confirmed previous studies after capsaicin in Zucker Diabetic Fatty rats (Gram et al., 2000b).

A mechanism for the improved insulin secretion could be through reduced dipeptidyl peptidase IV activity.

Dipeptidyl peptidase IV is a peptidase which degrades several biologically active peptides in vitro of importance for insulin secretion and glucose homeostasis (Mentlein et al., 1993). In this study we found that sensory desensitization consistently reduced the level of dipeptidyl peptidase IV in the plasma. We also found that the level of dipeptidyl peptidase IV activity in the vehicle rats was increasing over time, but that this increase was delayed by desensitization. Further, desensitizing at an early age resulted in a greater reduction of dipeptidyl peptidase IV plasma actity. To our knowledge, the involvement of the sensory nerves in the endogenous regulation of dipeptidyl peptidase IV plasma activity has not previously been shown. However, inhibition of dipeptidyl peptidase IV by means of chemical inhibitors has been known for some time. It has also been shown that the inhibition of the enzyme activity is

associated with improved glucose tolerance, both in laboratory animals (Balkan et al., 1999) as well as in humans (Ahren et al., 2002, 2004). The inhibition of dipeptidyl peptidase IV plasma activity is, therefore, considered to be a promising future target for pharmacological intervention in glucose intolerance in obesity and type 2 diabetes. The beneficial effect of inhibiting dipeptidyl peptidase IV is thought to be mediated through the prevention of degradation of the gut incretins glucagon-like peptide-1 (Ahren et al., 2002; Balkan et al., 1999) and gastric inhibitory polypeptide. These gut derived peptides are incretin hormones released during food ingestion, which, stimulate insulin secretion. Glucagon-like peptide-1 is the incretin of main importance for a normal islet response to meal intake (Holst et al., 1988). The effect of resiniferatoxin on glucose tolerance remains unknown, therefore we investigated whether dipeptidyl peptidase IV activity and incretin levels might mediate the effect of resiniferatoxin—i.e. that their levels are modulated by sensory nervous activity. In study 1, we demonstrated a reduction of dipeptidyl peptidase IV activity in the plasma following resiniferatoxin accompanied by improved glucose tolerance. In study 2, we replicated these results but could not demonstrate any change in the plasma levels of glucagon-like peptide-1, neither active nor total. This could be related to the fact that the Zucker Diabetic Fatty rat is not a good model for studying endogenous levels of glucagon-like peptide-1 although the Zucker Diabetic Fatty rat has been shown to respond well to exogenous glucagon-like peptide-1 (Shen et al., 1998). Another explanation is that the reduction of dipeptidyl peptidase IV activity might not have been reduced enough to effectively affect incretin levels. Thirdly, it could be that the plasma levels do not correlate to the local levels in proximity to the glucagon-like peptide-1 producing L-cells in the gut, and that the local levels might be altered. A fourth explanation to the unaltered levels of plasma glucagon-like peptide-1 despite decreased plasma levels of dipeptidyl peptidase IV could be that other peptides, which are also degraded by dipeptidyl peptidase IV and have insulinotrophic properties, might be of importance and might be affected by resiniferatoxin, such as Pituitary Adenylate Cyclase-Activating Polypeptide (Filipsson et al., 2000) or Gastrin Releasing Peptide (Nausch et al., 1990). These topics should be investigated in future studies.

In conclusion, it has been shown that resiniferatoxin is tolerable and improves glucose tolerance and insulin secretion in adult Zucker Diabetic Fatty rats. In addition resiniferatoxin is better tolerated than capsaicin in Zucker Diabetic Fatty rats. Resiniferatoxin therefore represents an attractive alternative to capsaicin for the study of sensory nervous function in experimental diabetes.

Further, the improvement of glucose tolerance by resiniferatoxin is accompanied by a reduced plasma dipeptidyl peptidase IV activity, which might suggest the involvement of biologically active peptides, which are substrates of this enzyme, in the beneficial effects of resiniferatoxin.

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