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Improved glucose tolerance and insulin secretion by inhibition of dipeptidyl peptidase IV in mice

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Abstract

We explored whether inhibition of the enzyme dipeptidyl peptidase IV (DPP IV) increases endogenous levels of glucagon-like peptide-1 (GLP-1) and improves glucose tolerance and insulin secretion in mice. Glucose (150 mg) was administered through a gastric gavage with or without the inhibitor of dipeptidyl peptidase IV, valine-pyrrolidide (100 μmol/kg), in high-fat fed glucose intolerant or control C57BL/6J mice. The increase in plasma GLP-1 after gastric glucose was potentiated by dipeptidyl peptidase IV inhibition ($P < 0.05$). Valine-pyrrolidide also potentiated the plasma insulin response to gastric glucose and improved the glucose tolerance in both groups of mice ($P < 0.001$). In contrast, valine-pyrrolidide did not affect glucose-stimulated insulin secretion from isolated islets. This suggests that valine-pyrrolidide improves insulin secretion and glucose tolerance through indirect action, probably through augmentation of levels of GLP-1 and other incretin hormones. Therefore, inhibition of dipeptidyl peptidase IV activity is feasible to exploit as a treatment for glucose intolerance and type 2 diabetes.

Keywords: Dipeptidyl peptidase IV; Glucagon-like peptide-1 (GLP-1); Insulin secretion; Glucose tolerance; Diabetes, type 2; Glucose tolerance, impaired

1. Introduction

Glucagon-like peptide-1 (GLP-1 or GLP-1-(7-36)amide) is a gut hormone, which is released from the L-cells of the small intestine after oral ingestion of nutrients (Kreymann et al., 1987; Ørskov, 1992; Sugiyama et al., 1994; Ahrén, 1998). GLP-1 potentiates glucose-stimulated insulin secretion (Holst et al., 1987; Kreymann et al., 1987; Fridolf et al., 1991; Ørskov, 1992; Ahrén, 1998; Ahrén and Pacini, 1999), and is thought to be an incretin hormone, i.e., a gut hormone participating in the potent insulin response to food intake (Ørskov, 1992; Ahrén, 1998). GLP-1 also inhibits glucagon secretion (Fridolf et al., 1991; Larsson et al., 1997; Ahrén, 1998) and slows gastric emptying (Willms et al., 1996; Nauck et al., 1997). These actions in concert result in lowering of circulating glucose, and GLP-1 has therefore been explored as a treatment for diabetes (Ahrén, 1998). In support of this concept, several studies have shown that administration of the peptide exerts antidiabetogenic action in subjects with type 2 diabetes (Gutniak et al., 1992; Juntti-Berggren et al., 1996; Gutniak et al., 1997; Ahrén et al., 1997a). However, a main drawback in this concept is the fast elimination of GLP-1 as an active peptide from the circulation, which is instituted by rapid removal of two residues from the N-terminal end of the peptide (Mentlein et al., 1993; Deacon et al., 1995). The enzyme responsible for the degradation of GLP-1 is the exopeptidase dipeptidyl peptidase IV (DPP IV or CD26) (Mentlein et al., 1993; Kieffer et al., 1995; Deacon et al., 1995; Mentlein 1999), and due to its action, the circulating half-life of active GLP-1 is only 1–1.5 min (Deacon et al., 1996).

As recently reviewed (Holst and Deacon, 1998), one possibility for the use of the spectrum of beneficial actions of GLP-1 in the treatment of type 2 diabetes is to inhibit the activity of dipeptidyl peptidase IV. This was initially suggested by the results of a study in pigs showing that the inhibitor of dipeptidyl peptidase IV, valine-pyrrolidide, prevented the inactivation of GLP-1, which was accompa-

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nied by augmented insulin response to glucose (Deacon et al., 1998). Similarly, two other inhibitors of dipeptidyl peptidase IV, isoleucine thiazolidide (Pederson et al., 1998) and NVP-DPP728 (Balkan et al., 1999), have recently been demonstrated to improve glucose tolerance in obese Zucker rats. In the present study, we have explored the effects of inhibition of dipeptidyl peptidase IV on glucose tolerance and insulin secretion in glucose intolerant high-fat fed C57BL/6J mice and their controls fed a normal diet. The high-fat fed C57BL/6J mouse model shows a similarity to the metabolic syndrome in humans, since it displays modest glucose intolerance and modest hyperlipidemia (Ahrén et al., 1997b). As inhibitor of dipeptidyl peptidase IV, we used valine-pyrrolidide, which has previously been demonstrated to be a stable and highly selective competitive inhibitor of dipeptidyl peptidase IV activity (Deacon et al., 1998; Hansen et al., 1999). The substance was administered through gastric gavage, together with glucose, in both high-fat fed and control mice, and glucose tolerance, insulin and GLP-1 levels were determined.

2. Materials and methods

2.1. Animals

Female mice of the C57BL/6J mice (Bomholtgaard Breeding and Research Centre, Ry, Denmark) were given either a high-fat diet or an ordinary rodent chow diet (both diets from Research Diets, N Brunswick, NJ, USA) from the age of 5 weeks. On a caloric base, the high-fat diet consisted of 16.4% protein, 25.6% carbohydrates and 58.0% fat (total 23.4 kJ/g), whereas the control diet consisted of 25.8% protein, 62.8% carbohydrates and 11.4% fat (total 12.6 kJ/g). The studies were performed after 3–4 months on the respective diets. Throughout the study period, the mice had free access to food and water. Four to five mice were kept per cage in a temperature-controlled (22°C) room with a 12-h light–dark cycle with lights on at 06:00 am. The study was approved by the Animal Ethics Committee at Lund University.

2.2. GLP-1 elimination test

Non-fasted mice were anesthetized with an intraperitoneal injection of midazolam (Dormicum^{R}, Hoffman-La-Roche, Basel, Switzerland, 0.4 mg/mouse) and a combination of fluanison (0.9 mg/mouse) and fentanyl (0.02 mg/mouse; Hypnorm^{R}, Janssen, Beerse, Belgium). At 30 min after induction of anesthesia, valine-pyrrolidide (Novartis, Summit, NJ, USA; 100 μmol/kg dissolved in saline) was given through a gavage tube (outer diameter 1.2 mm) placed in the stomach; controls were given saline. Ten minutes later, a blood sample (75 μl) was taken from the retrobulbar, intraorbital, capillary plexus, whereafter synthetic GLP-1 (Peninsula Laboratories, Merseyside, England; 300 pmol/kg) was rapidly injected intravenously in a tail vein (volume load 10 μl/g body weight). New blood samples were taken at 1, 5, 10, 20 and 50 min. The samples were taken in heparinized tubes and stored on ice. Following centrifugation, plasma was separated and stored at −20°C until analysis for GLP-1. Mice from four animals were pooled to obtain sufficient plasma volume for analysis.

2.3. Gastric glucose tolerance test

The mice were fasted for 2 h and anesthetized as above and a blood sample was taken. Thereafter, D-glucose (150 mg/mouse dissolved in 0.5 ml saline) was administered alone or together with valine-pyrrolidide (100 μmol/kg) through a gavage tube as above. New blood samples were taken after 10, 30, 60 and 120 min and handled as above until analysis for insulin, glucose and GLP-1. In the experimental series with determination of plasma GLP-1, plasma from four mice was pooled as above.

2.4. Insulin secretion in vitro

Pancreatic islets were isolated from eight mice with the collagenase isolation technique. In brief, the pancreas was filled retrogradely through the pancreatic duct with 3 ml of Hank's Balanced Salt Solution (Sigma), supplemented with 0.3 mg/ml of Collagenase P (activity 1.86 U/mg;

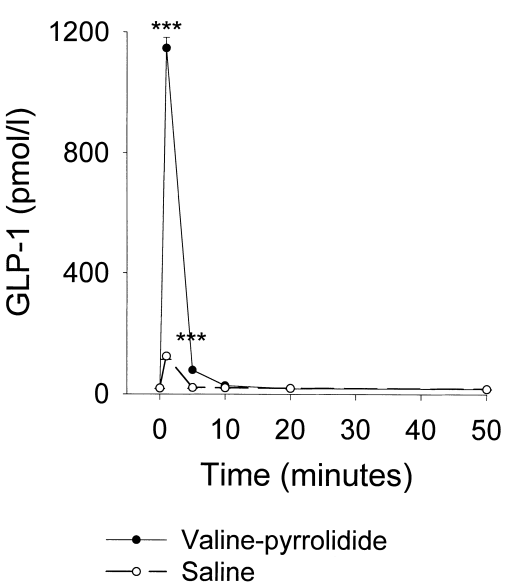

Fig. 1. Plasma levels of GLP-1 immediately before and at 1, 5, 10, 20 and 50 min after intravenous administration of synthetic GLP-1 (300 pmol/kg) or saline in anesthetized normal fed C57BL/6J mice fed a normal diet. The inhibitor dipeptidyl peptidase IV, valine-pyrrolidide (100 μmol/kg) or saline was given by gastric gavage 10 min before GLP-1. Means ± S.E.M. are shown. There were eight observations in each group; each observation consisting of four animals. Asterisks indicate the probability level of random difference between the groups, $^{***}P < 0.001$.

Boehringer Mannheim, Mannheim, Germany), and after removal, incubated in the same solution for 20 min at 37°C. After rinsing, the islets were handpicked under a stereomicroscope and incubated overnight in RPMI 1640 medium supplemented with 10% fetal calf serum, 2.05 mmol/l L-glutamine, 2.5 μg/ml amphotericin B (GIBCO, Paisley, Scotland), 100 IU/ml penicillin and 100 μg/ml streptomycin (Biol Ind, Beit Haemek, Israel) at 37°C in humidified air equilibrated with 5% CO_2. Following the overnight incubation, the islets were washed three times and then pre-incubated for 60 min at 37°C in a HEPES medium (pH 7.36) supplemented with 0.1% human serum albumin (Sigma) and 3.3 mmol/l glucose. The medium consisted of (in mmol/l): 125 NaCl, 5.9 KCl, 1.2 $MgCl_2$,

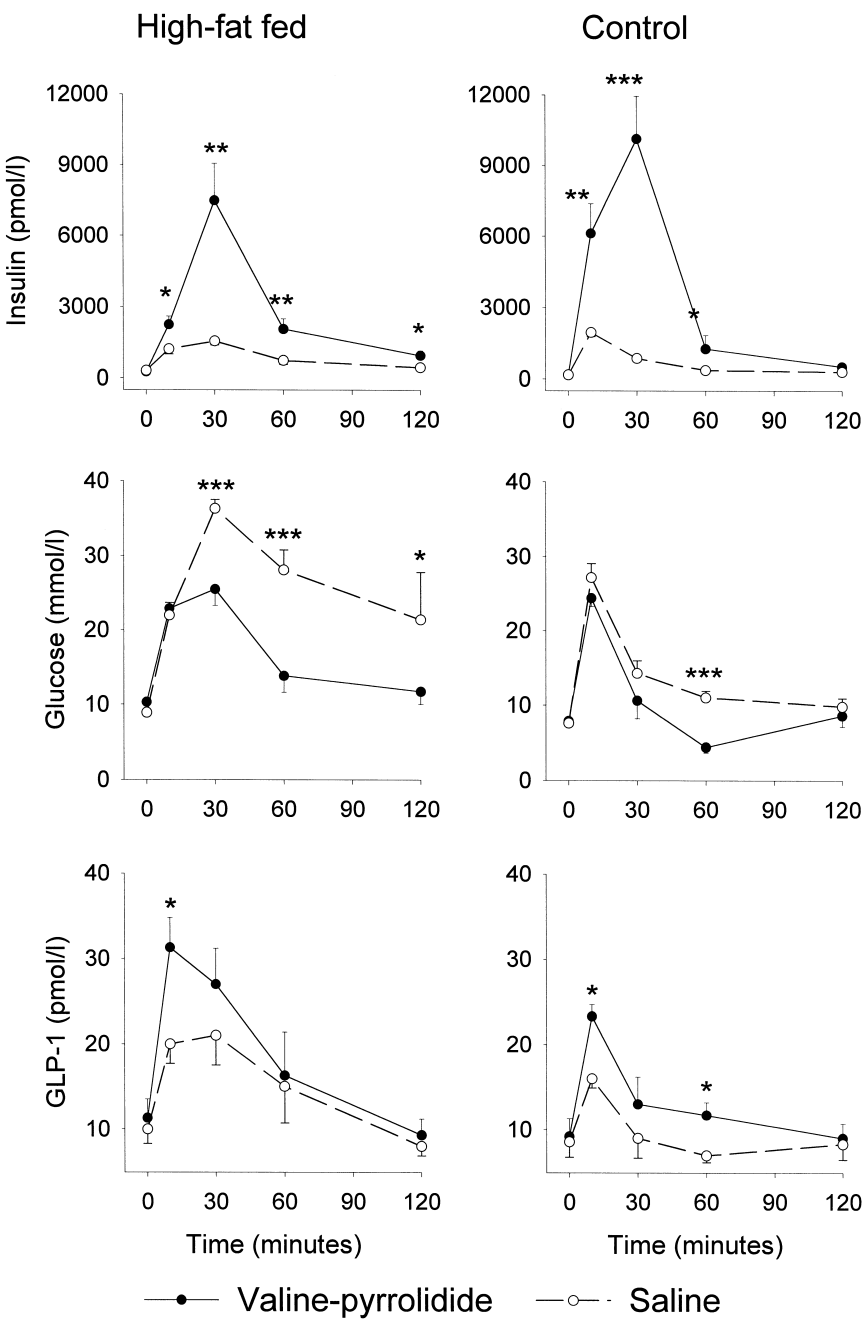

Fig. 2. Plasma levels of insulin (upper panels), glucose (middle panels) and biologically active intact GLP-1 (lower panels) immediately before and at 10, 30, 60 and 120 min after gastric administration of glucose (150 mg/mouse) with or without the inhibitor of dipeptidyl peptidase IV, valine-pyrrolidide (100 μmol/kg) in anesthetized high-fat fed C57BL/6J mice (left panels) or normal C57BL/6J given a control diet (right panels). Means ± S.E.M. are shown. There were 12 observations in each group, each observation consisting of one animal in the experimental series examining insulin and glucose and four animals in the series examining GLP-1. Asterisks indicate the probability level of random difference between the groups, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

1.28 $CaCl_2$ (all Sigma) and 25 HEPES (Boehringer Mannheim). After the pre-incubation, groups of three islets were transferred into separate chambers containing 200 μl of the medium supplemented with glucose at different concentrations and valine-pyrrolidide at 0.1, 10 or 1000 μmol/l. Following incubation at 37°C for 60 min, 25 μl of the medium were collected from each chamber and stored at −20°C until analysis.

2.5. Analysis

Insulin was determined radioimmunochemically with the use of a guinea pig anti-rat insulin antibody, ^{125}I-labelled porcine insulin as tracer, and rat insulin as standard (Linco Research, St. Charles, MO, USA). Free and bound radioactivity was separated by use of an anti-IgG (goat anti-guinea pig) antibody (Linco). The sensitivity of the assay is 12 pmol/l and the coefficiency of variation is less than 3% at both low and high levels. Plasma glucose was determined with the glucose oxidase method. Plasma GLP-1 was measured by a radioimmunoassay after extraction of plasma samples with ethanol. Four hundred microliters 0.05 mol/l sodium phosphate buffer, pH 7.5, containing 6% albumin and 0.1 mol/l NaCl was added to 100 μl mouse plasma on ice and mixed well. The mixture was then extracted with 70% ethanol (vol/vol, final dilution), and after vacuum centrifugation, the residue was reconstituted in assay buffer. Standard and ^{125}I-labelled tracers were GLP-1 (7-36 amide) and separation of antibody-bound peptide from antibody-free peptide was achieved with plasma-coated charcoal. The antiserum used (code no. 93242), has an absolute requirement of the free and unmodified N-terminal of GLP-1 for binding and, therefore, recognizes intact mouse GLP-1, but not the N-terminally truncated and inactive metabolites generated by dipeptidyl peptidase IV or N-terminally extended molecular forms (also inactive) secreted from, e.g. the pancreas. The sensitivity using this procedure was approximately 5 pmol/l and the intra-assay coefficient of variation was approximately 10%.

2.6. Statistical analysis

Means ± S.E.M. are shown. Areas under the curve (AUC) for plasma insulin levels ($AUC_{insulin}$) and plasma glucose levels ($AUC_{glucose}$) were calculated by the trapezoid rule. Statistical analyses were performed with the Statistical Package for Social Sciences (SPSS) for Windows system. Statistical comparisons between groups in vivo were performed with Students *t*-test and the statistical comparisons of the in vitro studies were performed with analysis of variance.

3. Results

3.1. Baseline body weight and plasma levels of glucose, insulin and GLP-1

Body weight was higher in the mice given the high-fat diet than in the controls (27.1 ± 1.1 g, $n = 12$, vs. 23.8 ± 0.7 g, $n = 12$; $P < 0.001$). Also, baseline glucose (9.6 ± 0.7 vs. 7.8 ± 0.3 mmol/l; $P < 0.001$) and insulin levels (290 ± 47 vs. 174 ± 26 pmol/l; $P < 0.001$) were higher in high-fat fed mice than in controls, whereas baseline levels of intact GLP-1 were not different between the two groups (10.7 ± 1.1 vs. 7.7 ± 4.0 pmol/l; NS).

3.2. GLP-1 elimination test

Fig. 1 shows that intravenous administration of GLP-1 rapidly increased plasma GLP-1 levels within 1 min, and after 5 min, the levels were back to baseline. Valine-pyrrolidide enhanced plasma GLP-1 levels after GLP-1 administration, both at 1 and 5 min ($P < 0.001$).

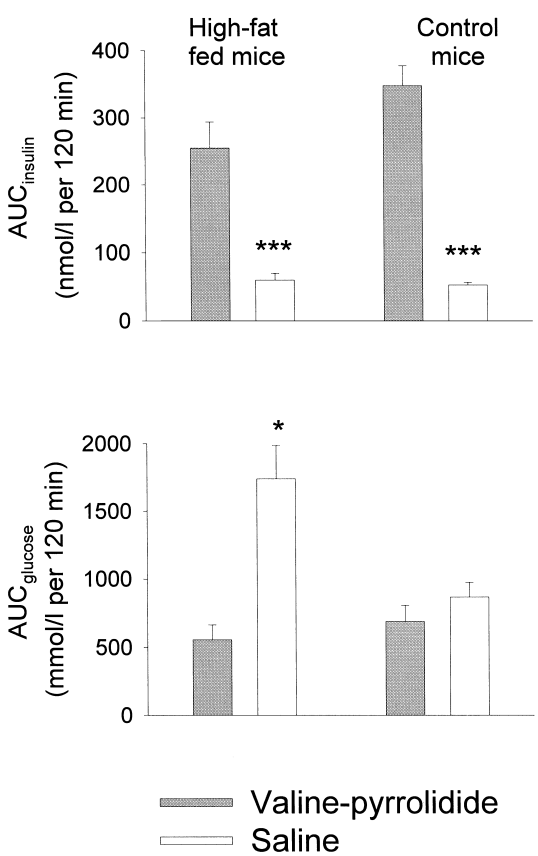

Fig. 3. $AUC_{insulin}$ and $AUC_{glucose}$ during gastric administration of glucose (150 mg/mouse) with or without the inhibitor of dipeptidyl peptidase IV, valine-pyrrolidide (100 μmol/kg) in anesthetized high-fat fed C57BL/6J mice (left panels) or normal C57BL/6J given a control diet (right panels). Means ± S.E.M. are shown. There were 12 animals in each group. Asterisks indicate the probability level of random difference between the groups, $^{*}P < 0.05$; $^{***}P < 0.001$.

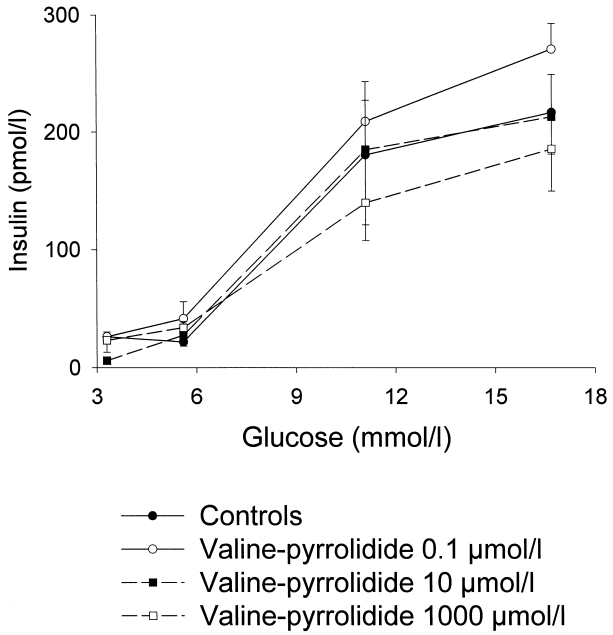

Fig. 4. Insulin secretion from islets from normal fed C57/Bl/6J mice incubated for 60 min in the presence of various concentrations of glucose with or without valine-pyrrolidide at 0.1, 10 or 1000 μmol/l. Means ± S.E.M. of eight incubations each consisting of three islets in each data point are shown. There was no statistically significant difference between the groups.

3.3. Insulin, glucose and GLP-1 response to gastric glucose

Gastric glucose gavage increased plasma levels of insulin, glucose and GLP-1. Both in high-fat fed mice and in controls, the insulin and GLP-1 responses were augmented by valine-pyrrolidide whereas the glucose responses were reduced (Fig. 2). Fig. 3 shows that $AUC_{insulin}$ was increased by valine-pyrrolidide 4.2-fold in high-fat fed mice ($P < 0.001$) and 6.6-fold in mice fed a control diet ($P < 0.001$). Furthermore, the $AUC_{glucose}$ in valine-pyrrolidide treated high-fat fed mice was 31% of that in saline-treated mice ($P = 0.021$), whereas the difference in mice fed a control diet did not reach significance.

3.4. Insulin secretion in vitro

Fig. 4 shows that valine-pyrrolidide did not affect glucose-stimulated insulin secretion from isolated islets at either of the concentrations 0.1, 10 or 1000 μmol/l.

4. Discussion

As recently reviewed, dipeptidyl peptidase IV is ubiquitously distributed with highest activity in kidney, lung, adrenals, small intestine, liver and spleen; the enzyme is also located in endothelial cells and occurs as free activity in plasma, enabling contact with circulating factors (Mentlein, 1999). The enzyme is a glycoprotein consisting of two subunits, each of a size of 120 kDa and its cDNA codes for a polypeptide of 766 residues in man and 767 residues in rodents (Mentlein, 1999). It has a short cytoplasmic domain of seven residues and an anchoring domain of 22 transmembraneously located residues, whereas the remaining 737 or 738 residues are extracellular containing the C-terminally located catalytic region (Mentlein, 1999). In 1993, it was demonstrated that the main incretin hormones, GLP-1 and gastric inhibitory polypeptide (GIP), are inactivated by the enzyme (Mentlein et al., 1993; Mentlein, 1999). For both hormones, N-terminally truncated peptides are formed [GLP-1-(9-36)amide and GIP-(3-42)], which are either inactive or antagonistic for the hormones (Kieffer et al., 1995; Knudsen and Pridal, 1996; Holst and Deacon, 1998; Mentlein, 1999). The rapidity of this inactivation yields a short half-life of the circulating hormones; for circulating GLP-1, the half-life is only 1–1.5 min (Deacon et al., 1996). To optimize the use of GLP-1 in the treatment of diabetes, inhibition of this inactivation has been proposed which would prolong the half-life of GLP-1 (Holst and Deacon, 1998). In this study, we used valine-pyrrolidide to explore this potential. Valine-pyrrolidide has previously been shown to be an inhibitor of dipeptidyl peptidase IV (Deacon et al., 1998). We used valine-pyrrolidide in a dose of 100 μmol/kg, which is above the previously demonstrated IC_{50} dose in rats after oral gavage, being 18 μmol/kg (Balkan, unpublished). We found that the substance prolongs the elimination of GLP-1 in mice as evident from the GLP-1 elimination test (Fig. 1), which confirms previous results in the pig (Deacon et al., 1998). Furthermore, we also found that valine-pyrrolidide potentiated the rise of plasma levels of active GLP-1 in response to enteral glucose, both in glucose intolerant and normal mice. This was accompanied by potentiation of the insulin response to gastric administration of glucose and improved glucose tolerance. Since the potentiation of insulin release (seen at 10 min after glucose administration) preceded the reduction in glucose levels (seen at 20–30 min), it is likely that a main mechanism underlying the improved glucose tolerance is potentiation of insulin secretion. Since we also found that valine-pyrrolidide was without effect on glucose-stimulated insulin secretion in isolated islets, our results suggest that valine-pyrrolidide, through the inhibition of dipeptidyl peptidase IV, has potentiated glucose-stimulated insulin secretion through an indirect action which has improved glucose tolerance. This is analogous to the improved glucose tolerance by other inhibitors of dipeptidyl peptidase IV in the severely insulin resistant diabetic Zucker rats (Pederson et al., 1998; Balkan et al., 1999) and that valine-pyrrolidide increases the insulin response to glucose in pigs (Deacon et al., 1998).

Besides influences on islet function, GLP-1 also inhibits gastric emptying (Willms et al., 1996; Nauck, 1999). It may therefore be argued that valine-pyrrolidide would

inhibit gastric emptying, which would slow the glucose absorption, which could explain the improved glucose tolerance. However, this is an unlikely explanation in the present study because the rate of increase in plasma glucose after the administration of gastric glucose was not affected by valine-pyrrolidide. It is also unlikely that gastric emptying itself would significantly contribute to glucose homeostasis after administration of glucose through a liquid gastric gavage in the mice, because the stomach is probably already empty at the time when glucose reaches the distal portion of the small intestine to liberate GLP-1. Therefore, the improved glucose tolerance after valine-pyrrolidide is best explained by the increased insulin secretion.

It is well-known that dipeptidyl peptidase IV inactivates a number of biologically active peptides and factors within the immune system through cleavage of the N-terminal dipeptide containing either proline or alanine as residue number 2 (for review see Mentlein, 1999). Of relevance for glucose homeostasis, not only GLP-1, but also GIP, neuropeptide Y, peptide YY and peptide histidine methionine or its counterpart peptide histidine isoleucine are degraded by dipeptidyl peptidase IV (Mentlein, 1999). It is therefore expected that in the mice treated with valine-pyrrolidide in the present study, circulating levels of these biologically active peptides are raised. Besides GLP-1, GIP and peptide histidine isoleucine are also known to stimulate insulin secretion in mice and, therefore, might contribute to the improved insulin secretion in the high-fat fed mice treated with valine-pyrrolidide, whereas it is unlikely that neuropeptide Y or peptide YY contributes since these two peptides inhibit insulin secretion (Ahrén and Lundquist, 1988; Ahrén, 1999). In our study, the potentiation by valine-pyrrolidide of the insulin response to gastric glucose was much more potent than its augmentation of the plasma levels of GLP-1. In fact, it is unlikely that the modest increase in plasma GLP-1 obtained by valine-pyrrolidide by itself may explain the large insulinotropic action, as based on previous dose–response relationships between administration of exogenous GLP-1 and insulin secretion in normal mice (Ahrén and Pacini, 1999). Therefore, it is possible that other peptides add to the effects of GLP-1 in explaining the beneficial effect of inhibition by dipeptidyl peptidase IV of glucose tolerance. The relative involvement of these regulatory peptides for improvement of glucose tolerance by inhibition of dipeptidyl peptidase IV in glucose intolerant mice remains to be established.

In conclusion, our study shows that inhibition of dipeptidyl peptidase IV activity increases the circulating level of active GLP-1 in mice and that this is accompanied by markedly increased insulin secretion and improved glucose tolerance. The results demonstrate the usefulness of developing inhibitors of dipeptidyl peptidase IV for the treatment of glucose intolerance and type 2 diabetes (cf. Holst and Deacon, 1998). However, whether inhibition of dipeptidyl peptidase IV activity is feasible as a treatment of type 2 diabetes in humans remains to be established.

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