Naunyn-Schmiedeberg's Arch Pharmacol (2000) 362 : 1–6
Digital Object Identifier (DOI) 10.1007/s002100000234

ORIGINAL ARTICLE

Jan Krützfeldt · Walter Raasch · Harald H. Klein

Ramipril increases the protein level of skeletal muscle IRS-1 and alters protein tyrosine phosphatase activity in spontaneously hypertensive rats

Received: 19 October 1999 / Accepted: 26 January 2000 / Published online: 14 April 2000

Abstract To investigate mechanisms by which angiotensin converting enzyme (ACE)-inhibition increases insulin sensitivity, spontaneously hypertensive (SH) rats were treated with or without ramipril (1 mg/kg per day) for 12 weeks. Insulin binding and protein levels of insulin receptor substrate-1 (IRS-1), p85-subunit of phosphatidylinositol 3'-kinase (p85) and Src homology 2 domain-containing phosphatase-2 (SHP2) were then determined in hindlimb muscle and liver. Additionally, protein tyrosine phosphatase (PTPase) activities towards immobilized phosphorylated insulin receptor or phosphorylated IRS-1 of membrane (MF) and cytosolic fractions (CF) of these tissues were measured. Ramipril treatment increased IRS-1-protein content in muscle by 31±9% (P<0.05). No effects were observed on IRS-1 content in liver or on insulin binding or protein expression of p85 or SHP2 in both tissues. Ramipril treatment also increased dephosphorylation of insulin receptor by muscle CF (22.0±1.0%/60 min compared to 16.8±1.5%/60 min; P<0.05), and of IRS-1 by liver MF (37.2±1.7%/7.5 min compared to 33.8±1.7%/7.5 min; P<0.05) and CF (36.8±1.0%/7.5 min compared to 33.2±1.0%/7.5 min; P<0.05). We conclude that the observed effects of ACE-inhibition by ramipril on the protein expression of IRS-1 and on PTPase activity might contribute to its effect on insulin sensitivity.

Key words ACE-inhibition · Ramipril · Spontaneously hypertensive rats · IRS-1 · Insulin receptor · Protein tyrosine phosphatases

J. Krützfeldt · H.H. Klein (✉)
Medizinische Klinik 1, Medical University of Lübeck,
Ratzeburger Allee 160, D-23538 Lübeck, Germany
e-mail: klein@medinf.mu-luebeck.de,
Fax: +49-451-5006483

W. Raasch
Institut für Experimentelle und Klinische Pharmakologie
und Toxikologie, Medical University of Lübeck,
Lübeck, Germany

Introduction

Angiotensin converting enzyme (ACE)-inhibition has been shown to increase insulin sensitivity in animals (Tomiyama et al. 1994; Uehara et al. 1994; Chow et al. 1995; Leighton et al. 1996; Caldiz and de Cingolani 1999) and may have similar effects in humans (Vuorinen-Markkola and Yki-Jarvinen 1995; Valensi et al. 1996; Galletti et al. 1999). Cellular insulin action is initiated by hormone binding to its heterotetrameric cell surface receptors, which is followed by autophosphorylation of multiple tyrosine residues on the receptor β-subunit. This results in activation of the receptor kinase and tyrosine phosphorylation of insulin receptor substrates. One of these substrates is the insulin receptor substrate-1 (IRS-1). Several other proteins bind to the phosphorylated IRS-1 and are thought to mediate the effects of insulin. Phosphatidylinositol 3' (PI3)-kinase is a lipid kinase, which upon binding to IRS-1 is activated, and which is important for the signaling that leads to the translocation of glucose transporter 4 (GLUT4) to cell surface and the initiation of glucose uptake (Virkamäki et al. 1999). Another protein that binds to phosphorylated IRS-1 is the Src homology 2 domain-containing phosphatase-2 (SHP2), a protein tyrosine phosphatase (PTPase) that on the one hand appears to be important for the dephosphorylation of IRS-1 (Myers et al. 1998), and on the other hand also for downstream insulin signaling (Milarski and Saltiel 1994; Ugi et al. 1996). Short-term (6 h) ACE-inhibition has been shown to increase insulin-stimulated phosphorylation of the insulin receptor and IRS-1 in muscle and liver of insulin-resistant aged (Carvalho et al. 1997) or obese wistar rats (Carvalho et al. 1998), but not in insulin-sensitive young wistar rats (Carvalho et al. 1997).

Little is known about the effects of long-term ACE-inhibition on insulin signaling, except that ACE-inhibition for 12 (Katayama et al. 1997) or 2 weeks (Jacob et al. 1996) was associated with an increased GLUT4-protein amount in skeletal muscle of spontaneously hypertensive (SH) and obese Zucker (fa/fa) rats, respectively. One goal

of the present study was therefore to investigate whether long-term treatment with ramipril (12 weeks) also affects the expression of other proteins that are important for initial insulin signaling [insulin receptor, IRS-1, p85-subunit of PI3-kinase (p85) or SHP2].

Another mechanism by which ACE-inhibition could modulate insulin signaling is by alteration of PTPase activities directed towards phosphorylated insulin receptor or its substrates (Goldstein 1993; Klein et al. 1997). There is evidence that ACE-inhibition might influence the activity of such PTPases. ACE-inhibition results in increased bradykinin and reduced angiotensin II (Campbell et al. 1994), and these peptides have been shown to alter PTPase activity, at least towards synthetic substrates (Brechler et al. 1994; Takahasi et al. 1994; Tsuzuki et al. 1996; Fleming and Busse 1997). An additional goal of the present study was therefore to investigate whether ramipril alters PTPase activities towards phosphorylated recombinant insulin receptor or IRS-1.

SH rats were chosen in our study because this animal model combines essential hypertension and insulin resistance (Reaven et al. 1989; Hulman et al. 1993), and because long-term ACE-inhibition has been shown to increase insulin sensitivity in these animals (Tomiyama et al. 1994; Chow et al. 1995; Leighton et al. 1996; Caldiz and de Cingolani 1999).

Materials and methods

Animals. Male SH rats were purchased at an age of 6 weeks from Charles River (Sulzfeld, Germany) and fed ad libitum. Twenty-four rats were divided randomly into two groups that received 1 mg/kg per day of ramipril (1 mg/ml in distilled water) or distilled water (1 ml/kg per day), respectively, once daily by gavage for 12 weeks. Animals were then sacrificed by decapitation, and liver and hindlimb muscle rapidly frozen in liquid nitrogen. The frozen tissues were broken into pieces of approximately 0.3 g by 3–5 hammer strokes and stored at –80°C. Blood was collected for the measurement of glucose (Beckman Glucose Analyzer; Beckman Instruments, Munich, Germany) and insulin (rat-insulin-RIA-kit; Linco Research, St. Charles, USA). At the end of the treatment period serum glucose was 147±1 mg% and 150±4 mg% (not significant = n.s.), serum insulin 2.45±0.18 ng/ml and 2.46±0.29 ng/ml (n.s.), body weight 341±2 g and 343±5 g (n.s.), and systolic blood pressure (tail cuff method; Bunag et al. 1982) 221±2 mmHg and 178±5 mmHg ($P<0.05$) in control and ramipril-treated animals, respectively. The study was carried out in accordance with the guide for the care and use of laboratory animals as adopted by the Ministerium für Natur und Umwelt des Landes Schleswig-Holstein, Deutschland, animal protocols nos. 9/p/93 and 9/r/95.

Insulin binding. Frozen muscle or liver tissue was homogenized at 4°C in buffer A [20 mM HEPES, 1% Triton X-100, 800 kallikrein inhibitor units/ml aprotinin, 2.5 mM phenylmethylsulfonylfluoride (PMSF), 2.5 mg/ml benzamidine, 2.5 μg/ml leupeptin, 2.5 μg/ml pepstatin A; pH 7.4; 1 g/4 ml] with a motor-driven Elvehjem homogenizer. After centrifugation at 40,000 *g* for 30 min, binding of insulin was performed essentially as described (Kotzke et al. 1995). Insulin receptors from muscle and liver extracts were immobilized to microwells coated with anti-insulin receptor antibody (αIR), and [^{125}I]Tyr-A^{14}-labeled insulin (0.06 nmol/l) was then added in the absence or presence of unlabeled insulin (0–3460 nmol/l). After 16 h at 4°C wells were washed to remove unbound insulin. [^{125}I]insulin that had bound to the immobilized receptors was then collected by twice adding a solution of 2% SDS to the wells for 30 min (at room temperature), and the radioactivity determined by gamma-counting.

Immunoblots. Frozen muscle or liver tissue was homogenized in Laemmli sample buffer (1 g/4 ml; Laemmli 1970) that contained 100 mM dithiothreitol (DTT) and was boiled for 5 min. After centrifugation at 40,000 *g* for 30 min supernatants were stored at –80°C. Extracts were then adjusted for the same protein content by dilution in Laemmli sample buffer, boiled, subjected to SDS-PAGE (7.5%), and proteins transferred to polyvinylidene difluoride membranes. Proteins were then detected with anti-IRS-1 (0.75 μg/ml; Upstate Biotechnology, Lake Placid, USA), anti-p85 (0.2 μl/ml; Upstate Biotechnology), and anti-SHP2 antibodies (0.5 μg/ml; Transduction Laboratories, Lexington, USA), peroxidase-labeled second antibodies (BioRad, Munich, Germany), and chemiluminescence using the enhanced chemiluminescence detection kit (Amersham, Braunschweig, Germany).

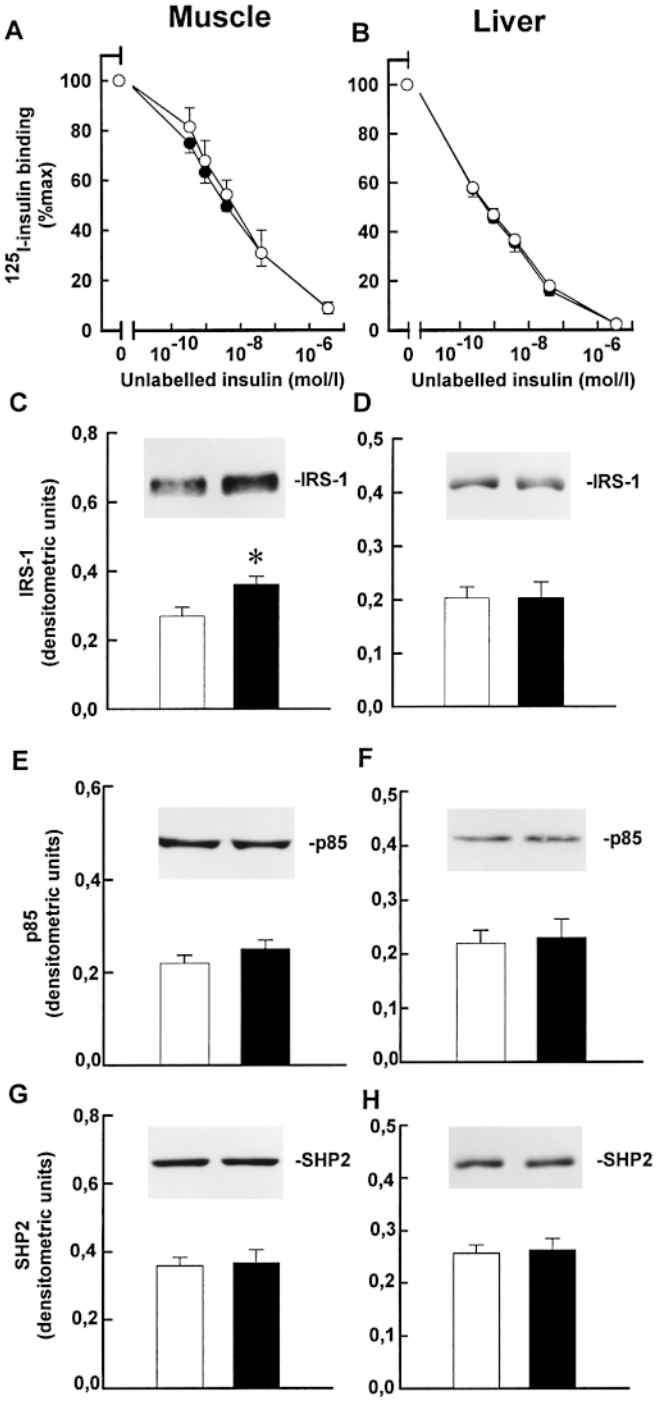
Dephosphorylation assays. Frozen muscle or liver tissue was homogenized in buffer B (25 mM HEPES, 1 mM PMSF, 800 kallikrein inhibitor units/ml aprotinin, 5 mM EDTA, 250 mM sucrose, 2.5 μg/ml leupeptin, 5 mM benzamidine, 1 mM DTT; pH 7.4; 1 g muscle/8 ml, 1 g liver/10 ml) and separated into membrane (MF) and cytosolic fractions (CF) as described earlier (Ahmad and Goldstein 1995). Assays were performed in microwells with immobilized recombinant phosphorylated insulin receptor or IRS-1 as previously described (Krützfeldt et al. 1999). For the measurement of insulin receptor dephosphorylation, insulin receptors from Chinese hamster ovary cells that overexpress human insulin receptors were first immobilized to microwells coated with αIR and then subjected to in vitro autophosphorylation (Krützfeldt et al. 1999). For the measurement of IRS-1-dephosphorylation, recombinant IRS-1 (Upstate Biotechnology) was first phosphorylated by immobilized, activated insulin receptor kinase and then immobilized to wells that had been coated with polyclonal antibody against IRS-1 (Krützfeldt et al. 1999). The wells with the immobilized substrates were then preincubated with buffer B for 30 min at 30°C. Dephosphorylation reactions were initiated by replacing buffer B by 30 μl of tissue fractions (muscle MF and CF: 0.35 mg/ml; liver MF and CF: 0.2 mg/ml) and continued for 60 min (insulin receptor dephosphorylation by muscle MF and CF), 30 min (insulin receptor dephosphorylation by liver MF and CF), 15 min (IRS-1-dephosphorylation by muscle MF and CF), or for 7.5 min (IRS-1-dephosphorylation by liver MF and CF). These protein concentrations and incubation times had been shown to be in the linear range for the dephosphorylation reactions (Krützfeldt et al. 1999; and data not shown). Parallel incubations were performed without tissue fractions (buffer B) or with the respective muscle or liver samples to which 10 mM sodium orthovanadate (final concentration) had been added. After the end of the incubations, wells were washed and the phosphotyrosine (PY)-content of the immobilized insulin receptors or IRS-1 measured using biotinylated anti-PY antibody and colorimetrical detection with streptavidin-horseradish peroxidase conjugate and ABTS (Boehringer, Mannheim, Germany) oxidation (Krützfeldt et al. 1999).

Immunodepletion of SHP2. Protein G-sepharose (30 μl; Pierce, Rockford, USA) was incubated with SHP2-antibody (5 μg; Santa Cruz Biotechnology, Santa Cruz, USA) for 2 h at room temperature and washed. Then 120 μl of muscle cytosolic fraction (3 mg/ml) was added to the sepharose. After 16 h at 4°C the sepharose was removed by centrifugation, and immunoblots and IRS-1-dephosphorylation assays performed with the supernatants. Actin immunoblots were carried out with anti-actin antibody (3.3 μg/ml; Boehringer, Mannheim, Germany) to demonstrate that no other proteins were lost during the immunodepletion procedure.

Protein determination. Protein determinations were performed by the Bradford dye method (Bradford 1976) using the Bio-Rad reagent (Munich, Germany) and BSA as a standard.

Statistical analysis. For blood pressure, body weight, serum insulin and glucose, normal distribution was verified by the Kol-

mogorov-Smirnov test and differences analyzed by the two-tailed Student's *t*-test for unpaired data. Immunoblots for IRS-1, p85 and SHP2 were performed for all rats in a single experiment per parameter, bands analyzed by scanning densitometry, and densitometric units compared between the groups treated with or without ramipril by the Mann-Whitney rank sum test for unpaired data. PTPase activities could not be measured in a single experiment for all groups and conditions. Since considerable interassay variation may occur with this assay procedure, rats treated with or without ramipril were randomly paired, and dephosphorylation measured in pairs. Paired data were compared by the Wilcoxon signed rank test. *P*-values <0.05 were considered statistically significant.



Results

Effect of ramipril on insulin binding and on protein levels of IRS-1, p85 and SHP2

In muscle and liver no ramipril effects were observed on insulin binding or on the amount of p85 or SHP-2 (Fig. 1). IRS-1 was, however, significantly increased by 31% in muscle of ramipril-treated rats as compared to control rats (Fig. 1C). No such difference in the amount of IRS-1 was observed in liver (Fig. 1D).

Effect of ramipril on PTPase activities

Dephosphorylation of immobilized phosphorylated insulin receptors or IRS-1 by tissue fractions was expressed as a decrease of the PY-signal in the presence of tissue fractions as compared to wells without added tissue fractions (absolute decrease; Fig. 2, open columns) or as compared to wells with added tissue fractions and vanadate (vanadate-inhibitable decrease; Fig. 2, filled columns). Liver CF of ramipril-treated rats resulted in significantly higher IRS-1-dephosphorylation than liver CF of controls, irrespective of how the results were expressed. If dephosphorylation was expressed as the absolute decrease of the PY-signal (Fig. 2, open columns), ramipril treatment also resulted in a significantly increased dephosphorylation of the insulin receptor by muscle CF and of IRS-1 by liver MF. A similar tendency was observed if dephosphorylation was expressed as vanadate-inhibitable decrease of the PY-signal, but statistical significance was not reached.

Effect of SHP2 on IRS-1-dephosphorylation

To evaluate the contribution of SHP2 to IRS-1-dephosphorylation, muscle CF was depleted from SHP2 (Fig. 3A).

Fig. 1A–H Effect of ramipril on insulin binding and protein levels of IRS-1, p85 and SHP2. Rats were treated with (*filled circles, filled columns*) or without ramipril (*open circles, open columns*) for 12 weeks. For the measurement of insulin binding, **A** skeletal muscle and **B** liver were solubilized as described in Materials and methods, and extracts adjusted to 13 mg/ml and 3.3 mg/ml, respectively. Insulin receptors were immunoimmobilized to microwells coated with αIR, and binding determined in the presence of the indicated insulin concentrations as described. For the measurement of the amounts of IRS-1, p85 and SHP2 in muscle (**C,E,G**) and liver (**D,F,H**), equal amounts of protein from rats treated with or without ramipril (muscle: 45, 120 and 50 μg for IRS-1, p85 and SHP2, respectively; liver: 20 μg for IRS-1, p85 and SHP2) were loaded to SDS-polyacrylamide gels. Immunoblots were then performed with the respective antibodies and bands analyzed by scanning densitometry. **A,B** Insulin binding. Shown is the percentage of the radioactivity bound in the absence of unlabelled insulin. Results represent means ± SEM (*n*=4 for muscle; *n*=6 for liver). **C–H** Amounts of IRS-1, p85 and SHP2. Shown are means ± SEM (*n*=11; **P*<0.05)

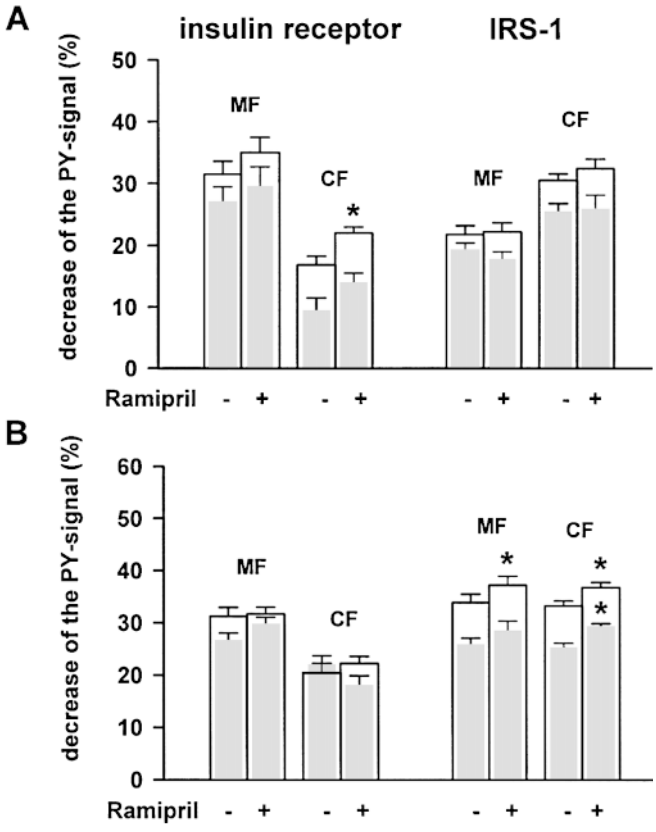


Fig. 2A,B Effect of ramipril on PTPase activities directed against insulin receptor or IRS-1. Rats were treated as described in Fig. 1. Muscle (**A**) or liver tissue (**B**) was then separated into membrane fractions (*MF*) and cytosolic fractions (*CF*) as described in Materials and methods. These fractions were added to wells with immobilized phosphorylated insulin receptor or IRS-1 in the presence or absence of vanadate, and wells incubated as described. Wells were washed and the phosphotyrosine (*PY*)-signal detected with anti-PY antibody. Shown is the reduction of the PY-signal in the presence of tissue fractions as compared to the signal in the absence of tissue fractions (buffer only; *open columns*) or as compared to the signal in the presence of tissue fractions that contained 10 mM vanadate (*filled columns*). Results represent means ± SEM (n=12). *P<0.05 (ramipril compared to control)

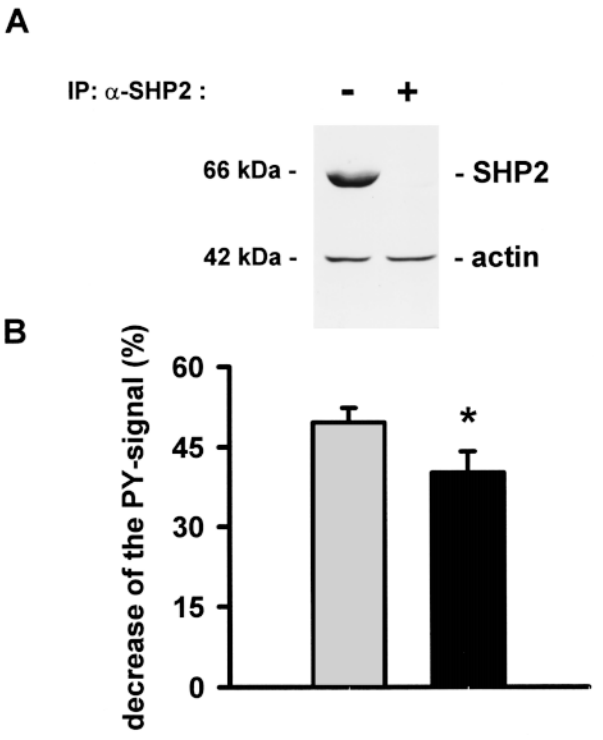


Fig. 3A,B Contribution of SHP2 to dephosphorylation of IRS-1. Protein G-sepharose was incubated with (*black column*) or without (*gray column*) SHP2-antibody (α-SHP2) for 2 h at room temperature and washed. Muscle cytosolic fractions (3 mg/ml) from rats that had not been treated with ramipril were then added for 16 h at 4°C. Sepharose was removed by centrifugation, and immunoblots and IRS-1-dephosphorylation assays performed with the supernatants. **A** SHP2 and actin immunoblots of a representative experiment. In all experiments immunodepletion of SHP2 evaluated by scanning densitometry was >95%. **B** IRS-1-dephosphorylation. Shown is the reduction of the phosphotyrosine (*PY*)-signal in the presence of tissue fractions as compared to the signal in the absence of tissue fractions. Results represent means ± SEM (n=8; *P<0.05)

This depletion decreased the ability of the extracts to dephosphorylate IRS-1 by 20±5% (Fig. 3B).

Discussion

An important finding of the present study is that long-term (12 weeks) ACE-inhibition with ramipril was associated with a 30% increase of skeletal muscle IRS-1 content. This may, at least partially, explain the increased whole-body insulin sensitivity that was observed in this animal model after ACE-inhibition for the same period of time (Chow et al. 1995). It is conceivable that an increased protein expression of this important insulin receptor substrate results in amplified insulin signaling, and in fact a correlation between expression levels of IRS-1 and insulin sensitivity has been observed in 3T3-L1-adipocytes (Rice and Garner 1994) and IRS-1-deficient mice (Araki et al. 1994).

Carvalho et al. have previously investigated effects of ACE-inhibition with captopril in aged (1997) or obese wistar rats (1998), and, in contrast to our study, did not observe alterations in skeletal muscle IRS-1 content. This is most likely explained by the fact that they investigated short-term effects (6 h) whereas in our study long-term effects (12 weeks) of ACE-inhibition were studied. It is conceivable that the mechanisms that contribute to increased insulin sensitivity following ACE-inhibition differ, at least partially, between short- and long-term treatment.

At present it is not clear how ACE-inhibition influences the protein amount of IRS-1. It has previously been reported that bradykinin (El-Dahr et al. 1998) and angiotensin II (Kohara et al. 1992) can influence gene regulation, and therefore increased bradykinin and/or decreased angiotensin II levels may be responsible. A decreased sympathetic activity following ACE-inhibition

that has been described by Grassi et al. (1997) may also be of importance. This would be consistent with a recent study that demonstrated increased IRS-1-protein levels in skeletal muscle of SH rats after inhibition of sympathetic activity with the selective imidazoline receptor agonist moxonidine for 12 weeks (Ernsberger et al. 1999).

The observed ramipril effects on PTPase activity towards the insulin receptor and IRS-1 might also contribute to the effect of ACE-inhibitors on insulin sensitivity. PTPase activity was measured as the effect of tissue extracts on the decrease of the PY-signal of tyrosine-phosphorylated immobilized recombinant insulin receptor or IRS-1. This decrease was either expressed as compared to the PY-signal obtained without addition of tissue extracts (absolute decrease of the PY-signal), or as compared to the PY-signal obtained with added tissue extracts and vanadate (vanadate-inhibitable decrease of the PY-signal). The vanadate-inhibitable decrease of the PY-signal was only slightly smaller than the absolute decrease (Fig. 2), which suggests that most of the decrease of the PY-signal was due to PTPases, and that other factors, i.e. degradation of substrates, only played a minor role. Ramipril treatment resulted in a more pronounced absolute decrease of the PY-signal of the insulin receptor by muscle CF and of IRS-1 by liver MF and CF. A similar tendency was observed for the vanadate-inhibitable decrease of the PY-signal, but statistical significance was only obtained for the dephosphorylation of IRS-1 by liver CF. These differences between the results for the absolute and vanadate-inhibitable decrease of the PY-signal may be due to a slightly higher variation of the results for the vanadate-inhibitable PTPase activity. Alternatively, it is possible that the ACE-inhibition affected PTPases that were not (completely) inhibited by vanadate, or that substrate degradation contributed to the ramipril effect on the absolute decrease of the PY-signal. In any case, our results demonstrate that ACE-inhibition increases rather than decreases PTPase activity. They thus do not support the hypothesis that ACE-inhibition increases insulin signaling by increasing tyrosine phosphorylation of the insulin receptor or IRS-1 by reducing their in-situ dephosphorylation by PTPases.

The observed alteration in PTPase activity after ACE-inhibition might nevertheless be related to increased insulin sensitivity. There is evidence that the activation of certain PTPases increases downstream insulin signaling. The cytosolic PTPase SHP2 has been shown to have a stimulatory effect on insulin-mediated activation of mitogen-activated protein kinase (Milarski and Saltiel 1994) and PI3-kinase (Ugi et al. 1996). Moreover, in several previous studies a reduction of PTPase activity in the cytosolic fraction, which is opposite to the ramipril effect observed in the present study, has been shown to be associated with insulin resistance (Olichon-Berthe et al. 1994; Worm et al. 1996a, 1996b).

The reason for the increase of PTPase activity following ramipril treatment is not clear. It is possible that stimulation of bradykinin B2 receptors influences phosphatase activity towards the insulin receptor and/or IRS-1. This notion that bradykinin might influence PTPase activity is supported by the finding that bradykinin increased PTPase activity, at least towards a synthetic peptide in endothelial cells (Fleming and Busse 1997). Another mechanism by which ACE-inhibition might influence PTPase activities towards the insulin receptor and IRS-1 is decreased stimulation of angiotensin II AT2-receptors. AT2-receptors are present at least in adult rat liver (Regitz-Zagrosek et al. 1995), and the hypothesis that AT2-receptor stimulation can be associated with alterations of PTPase activities is supported by previous data that showed inhibition (Takahasi et al. 1994) or stimulation (Brechler et al. 1994; Tsuzuki et al. 1996) of PTPase activities towards synthetic substrates in different cell models.

Muscle MF was more potent than CF with respect to insulin receptor dephosphorylation and muscle CF was more potent than MF with respect to IRS-1-dephosphorylation (Fig. 2A). We explored whether these differences in substrate specificity were caused by a predominant role of the cytosolic PTPase SHP2, which binds to phosphorylated IRS-1 via SH2 domains (Kuhne et al. 1994), for IRS-1-dephosphorylation. Our finding that SHP2 contributed only to about 20% of the IRS-1-dephosphorylation showed that SHP2 was not a predominant PTPase for dephosphorylation of IRS-1 in our assay system, and that the different substrate specificities of MF and CF cannot, at least not solely, be explained by the presence of SHP2 in the cytosolic fractions.

In conclusion, our data suggest that a 3-month treatment of SH rats with ramipril increases the protein expression of IRS-1 in skeletal muscle, and enhances PTPase activity. Both effects might contribute to the increased insulin sensitivity observed in this rat model after ACE-inhibition.

Acknowledgements This work was supported by Deutsche Forschungsgemeinschaft (Kl-503/7-3).

References

Ahmad F, Goldstein BJ (1995) Alterations in specific protein-tyrosine phosphatase accompany insulin resistance of streptozotocin diabetes. Am J Physiol 268:E932–E940

Araki E, Lipes MA, Patti ME, Bruning JC, Haag B 3rd, Johnson RS, Kahn CR (1994) Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. Nature 372:186–190

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Brechler V, Reichlin S, De Gasparo M, Bottari SP (1994) Angiotensin II stimulates protein tyrosine phosphatase activity through a G-protein independent mechanism. Receptors Channels 2:89–98

Bunag RD, Tomita T, Sasaki S (1982) Streptozotocin diabetic rats are hypertensive despite reduced hypothalamic responsiveness. Hypertension 4:556–565

Caldiz CI, Cingolani GE de (1999) Insulin resistance in adipocytes from spontaneously hypertensive rats: effect of long-term treatment with enalapril and losartan. Metabolism 48:1041–1046

Campbell DJ, Kladis A, Duncan AM (1994) Effects of converting enzyme inhibitors on angiotensin and bradykinin peptides. Hypertension 23:439–449

Carvalho CR, Thirone AC, Gontijo JA, Velloso LA, Saad MJ (1997) Effect of captopril, losartan, and bradykinin on early steps of insulin action. Diabetes 46:1950–1957

Carvalho DS, Villaca V, Brenelli SL, Carvalho CR, Saad MJ (1998) Angiotensin-converting enzyme inhibitor increases insulin-induced pp 185 phosphorylation in liver and muscle of obese rats. Biochem Mol Biol Int 46:259–266

Chow L, De Gasparo M, Levens N (1995) Improved glucose metabolism following blockade of angiotensin converting enzyme but not angiotensin AT1 receptors. Eur J Pharmacol 282:77–86

El-Dahr SS, Dipp S, Baricos WH (1998) Bradykinin stimulates the ERK→Elk-1→Fos/AP-1 pathway in mesangial cells. Am J Physiol 275:F343–F352

Ernsberger P, Ishizuka T, Liu S, Farrell CJ, Bedol D, Koletsky RJ, Friedman JE (1999) Mechanisms of antihyperglycemic effects of moxonidine in the obese spontaneously hypertensive Koletsky rat (SHROB). J Pharmacol Exp Ther 288:139–147

Fleming I, Busse R (1997) Tyrosine phosphorylation and braykinin-induced signaling in endothelial cells. Am J Cardiol 80: 102A–109A

Galletti F, Strazzullo P, Capaldo B, Carretta R, Fabris F, Ferrara LA, Glorioso N, Semplicini A, Mancini M (1999) Controlled study of the effect of angiotensin converting enzyme inhibition versus calcium-entry blockade on insulin sensitivity in overweight hypertensive patients: Trandolapril Italian Study (TRIS). J Hypertens 17:439–445

Goldstein BJ (1993) Regulation of insulin receptor signaling by protein-tyrosine dephosphorylation. Receptor 3:1–15

Grassi G, Cattaneo BM, Seravalle G, Lanfranchi A, Pozzi M, Morganti A, Carugo S, Mancia G (1997) Effects of chronic ACE inhibition on sympathetic nerve traffic and baroreflex control of circulation in heart failure. Circulation 96:1173–1179

Hulman S, Falkner B, Freyvogel N (1993) Insulin resistance in the conscious spontaneously hypertensive rat: euglycemic hyperinsulinemic clamp study. Metabolism 42:14–18

Jacob S, Henriksen EJ, Fogt DL, Dietze GJ (1996) Effects of trandolapril and verapamil on glucose transport in insulin-resistant rat skeletal muscle. Metabolism 45:535–541

Katayama S, Inaba M, Maruno Y, Morita T, Awata T, Oka Y (1997) Glucose intolerance in spontaneously hypertensive and Wistar-Kyoto rats: enhanced gene expression and synthesis of skeletal muscle glucose transporter 4. Hypertens Res 20:279–286

Klein HH, Kowalewski B, Drenckhan M, Fehm HL (1997) Insulin stimulation of human adipocytes activates the kinase of only a fraction of the insulin receptors. Am J Physiol 272:E576–E583

Kohara K, Brosnihan KB, Ferrario CM, Misted A (1992) Peripheral and central angiotensin II regulates expression of the renin-angiotensin system. Am J Physiol 262:E651–E657

Kotzke G, Schutt M, Missler U, Moller DE, Fehm HL, Klein HH (1995) Binding of human, porcine and bovine insulin to insulin receptor from human brain, muscle and adipoctytes and to expressed recombinant alternatively spliced insulin receptor isoforms. Diabetologia 38:757–763

Krützfeldt J, Grünweller A, Raasch W, Drenckhan M, Klein HH (1999) Microtiter well assays for protein tyrosine phosphatase activities directed against phosphorylated insulin receptor or insulin receptor substrate-1. Anal Biochem 271:97–99

Kuhne MR, Zhao Z, Rowles J, Lavan BE, Shen SH, Fischer EH, Lienhard GE (1994) Dephosphorylation of insulin receptor substrate 1 by the tyrosine phosphatase PTP2C. J Biol Chem 269:15833–15837

Laemmli UK (1970) The cleavage of T4-Phage. Nature 227: 680–685

Leighton B, Sanderson AL, Young ME, Radda GK, Boehm EA, Clark JF (1996) Effects of treatment of spontaneously hypertensive rats with the angiotensin-converting enzyme inhibitor trandolapril and the calcium antagonist verapamil on the sensitivity of glucose metabolism to insulin in rat soleus muscle in vitro. Diabetes 45:120–124

Milarski KL, Saltiel AR (1994) Expression of catalytically inactive Syp phosphatase in 3T3 cells blocks stimulation of mitogen-activated protein kinase by insulin. J Biol Chem 269: 21239–21243

Myers MG Jr, Mendez R, Shi P, Pierce JH, Rhoads R, White MF (1998) The COOH-terminal tyrosine phosphorylation sites on IRS-1 bind SHP-2 and negatively regulate insulin signaling. J Biol Chem 273:26908–26914

Olichon-Berthe C, Hauguel-De Mouzon S, Peraldi P, Van Obberghen E, Le Marchand-Brustel Y (1994) Insulin receptor dephosphorylation by phosphotyrosine phosphatases obtained from insulin-resistant obese mice. Diabetologia 37:56–60

Reaven GM, Chang H, Hoffman BB, Azhar S (1989) Resistance to insulin-stimulated glucose uptake in adipocytes isolated from spontaneously hypertensive rats. Diabetes 39:1155–1160

Regitz-Zagrosek V, Auch-Schwelk W, Hess B, Klein U, Duske E, Steffen C, Hildebrandt AG, Fleck E (1995) Tissue- and subtype-specific modulation of angiotensin II receptors by chronic treatment with cyclosporin A, angiotensin-converting enzyme inhibitors and AT1 antagonists. J Cardiovasc Pharmacol 26: 66–72

Rice KM, Garner CW (1994) Correlation of the insulin receptor substrate-1 with insulin-responsive deoxyglucose transport in 3T3-L1 adipocytes. Biochem Biophys Res Commun 28:523–530

Takahasi K, Bardhan S, Kambayashi Y, Shirai H, Inagami T (1994) Protein tyrosine phosphatase inhibition by angiotensin II in rat pheochromocytoma cells through type 2 receptor, AT2. Biochem Biophys Res Commun 198:60–66

Tomiyama H, Kushiro T, Abeta H, Ishii T, Takahashi A, Furukawa L, Asagami T, Hino T, Saito F, Otsuka Y (1994) Kinins contribute to the improvement of insulin sensitivity during treatment with angiotensin converting enzyme inhibitor. Hypertension 23:450–455

Tsuzuki S, Eguchi S, Inagami T (1996) Inhibition of cell proliferation and activation of protein tyrosine phosphatase mediated by angiotensin II type 2 (AT2) receptor in R3T3 cells. Biochem Biophys Res Commun 228:825–830

Uehara M, Kishikawa H, Isami S, Kisanuki K, Ohkubo Y, Mitamura N, Miyata Y, Yano T, Shichiri M (1994) Effect on insulin sensitivity of angiotensin converting enzyme inhibitors with or without a sulphydryl group: bradykinin may improve insulin resistance in dogs and humans. Diabetologia 37:300–307

Ugi S, Maegawa H, Kashiwagi A, Adachi M, Olefski JM, Kikkawa R (1996) Expression of dominant negative mutant SHPTP2 attenuates phosphatidylinositol 3'-kinase activity via modulation of phosphorylation of insulin receptor substrate-1. J Biol Chem 271:12595–12602

Valensi P, Derobert E, Genthon R, Riou JP (1996) Effect of ramipril insulin sensitivity in obese patients. Time-course study of glucose infusion rate during euglycaemic hyperinsulinaemic clamp. Diabetes Metab 22:197–200

Virkamäki A, Ueki K, Kahn CR (1999) Protein-protein interaction in insulin signaling and the molecular mechanisms of insulin resistance. J Clin Invest 103:931–943

Vuorinen-Markkola H, Yki-Jarvinen H (1995) Antihypertensive therapy with enalapril improves glucose storage and insulin sensitivity in hypertensive patients with non-insulin-dependent diabetes mellitus. Metabolism 44:85–89

Worm D, Handberg A, Hoppe E, Vinten J, Beck-Nielsen H (1996a) Decreased skeletal muscle phosphotyrosine phosphatase (PTPase) activity towards insulin receptors in insulin-resistant Zucker rats measured by delayed Europium fluorescence. Diabetologia 39:142–148

Worm D, Vinten J, Staehr P, Henriksen JE, Handberg A, Beck-Nielsen H (1996b) Altered basal and insulin-stimulated phosphotyrosine phosphatase (PTPase) activity in skeletal muscle from NIDDM patients compared with control subjects. Diabetologia 39:1208–1214