Ligand-independent Activation of Peroxisome Proliferator-activated Receptor-γ by Insulin and C-peptide in Kidney Proximal Tubular Cells

DEPEN<on phosphatidylinositol 3-kinase activity*

Peroxisome proliferator-activated receptor γ (PPARγ) has key roles in the regulation of adipogenesis, inflammation, and lipid and glucose metabolism. C-peptide is believed to be inert and without appreciable biological functions. Recent studies suggest that C-peptide possesses multiple functions. The present study investigated the effects of insulin and C-peptide on PPARγ transcriptional activity in opossum kidney proximal tubular cells. Both insulin and C-peptide induced a concentration-dependent stimulation of PPARγ transcriptional activity. Both agents substantially augmented thiazolidinedione-stimulated PPARγ transcriptional activity. Neither insulin nor C-peptide had any effect on the expression levels of PPARγ, GW9662, a PPARγ antagonist, blocked PPARγ activation by thiazolidinediones but had no effect on either insulin- or C-peptide-stimulated PPARγ transcriptional activity. Co-transfection of opossum kidney cells with dominant negative mitogen-activated protein kinase kinases significantly depressed basal PPARγ transcriptional activity but had no effect on that induced by either insulin or C-peptide. Both insulin- and C-peptide-stimulated PPARγ transcriptional activity were attenuated by wortmannin and by expression of a dominant negative phosphatidylinositol (PI) 3-kinase p85 regulatory subunit. In addition PI 3-kinase-dependent phosphorylation of PPARγ was observed after stimulation by C-peptide or insulin. C-peptide effects but not insulin on PPARγ transcriptional activity were abolished by pertussis toxin pretreatment. Finally both C-peptide and insulin positively control the expression of the PPARγ-regulated CD36 scavenger receptor in human THP-1 monocytes. We concluded that insulin and C-peptide can stimulate PPARγ activity in a ligand-independent fashion and that this effect is mediated by PI 3-kinase. These results support a new and potentially important physiological role for C-peptide in regulation of PPARγ-related cell functions.

Connecting peptide (C-peptide) is a 31-amino-acid enzymatic cleavage product derived from proinsulin during the biosynthesis and release of insulin from pancreatic beta cells. Although insulin and C-peptide are released into the circulation in equimolar amounts, it has become generally accepted that C-peptide does not possess biological activity of its own. This view has been challenged, however, with recent studies suggesting that C-peptide may possess multiple and significant functions.

Published, JBC Papers in Press, September 16, 2004, DOI 10.1074/jbc.M408268200

*The costs of publication of this article were deferred in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†To whom correspondence should be addressed: Dept. of Cell Physiology and Pharmacology, Medical Sciences Bldg., University of Leicester, University Rd., Leicester, LE1 9HN, United Kingdom. Tel.: 44-116-258-8043; Fax: 44-116-258-4764; E-mail: njb18@le.ac.uk.

© 2004 by The American Society for Biochemistry and Molecular Biology, Inc.
Within the promoter regions of target genes (14). Each PPAR isoform exerts distinct actions on cellular function and exhibits different specificity in ligand-binding properties (15–17).

Glitazones or thiazolidinediones (TZDs) are synthetic high affinity PPARγ ligands that act as insulin-sensitizing agents and are currently used for the treatment of type 2 diabetes mellitus (18). They improve lipid profiles together with insulin sensitivity and glucose tolerance in patients with type 2 diabetes and thus control hyperglycemia (19, 20). These metabolic effects of TZDs are mediated via PPARγ and thus control hyperglycemia (19, 20). These metabolic effects of TZDs are mediated via PPARγ and thus control hyperglycemia.

Several lines of evidence indicate convergence between insulin and PPARγ signaling pathways. Firstly the TZD, pioglitazone, augments insulin-stimulated tyrosine phosphorylation of IR/IRS-1 (19), secondly insulin participates in the control of PPARγ-regulated transcription of genes involved in glucose and lipid homeostasis. Similarly, insulin also promotes an altered expression of important genes regulating glucose and fatty acids metabolism (21–23). Several lines of evidence indicate convergence between insulin and PPARγ signaling pathways. Firstly the TZD, pioglitazone, augments insulin-stimulated tyrosine phosphorylation of IR/IRS-1 (19), secondly insulin participates in the control of PPARγ expression (23, 24), and finally insulin mediates ligand-independent activation of PPARγ in a phosphorylation-dependent manner (25).

Materials—Human 31-amino-acid scrambled C-peptide and a 31-amino-acid scrambled C-peptide were generously provided by Dr. John Wahren, Karolinska Institute (Stockholm, Sweden). Ciglitazone was from Biomol (Manheim, UK). GW9662 was purchased from Cayman Chemical Co (Nottingham, UK). Bovine insulin was obtained from Sigma. Wortmannin and PTX were obtained from Calbiochem. Anti-PPARγ, anti-PPARβ, anti-PPARγ, and anti-PI 3-kinase p85-α were from Santa Cruz Biotechnology Inc. The plasmid, pCMX-PPARγ encoding mouse PPARγ-1 was kindly provided by Dr. R. Evans (Salk Institute, San Diego, CA). The reporter plasmid pPRE-TK-luc was kindly provided by Dr. M. Lazar (University of Pennsylvania, Philadelphia, PA). The plasmid pFC-MEK1 encoding constitutively active MAP kinase kinase (MEK-1) (pMEK1-CA), and the kinase-dead mutant of MEK-1 in pCMV5 (pMEK1-KD) were provided by Dr. J. Blank (University of Leicester, Leicester, UK). Luciferase assays were performed using the LucLite kit (Packard, Pangbourne, England). β-Galactosidase assay...
versus each plasmid DNA was used in each well, and in experiments involving PPAR addition of 200 nM ciglitazone. Membranes were stripped and reprobed for C-peptide or insulin for 24 h, and PPAR expression was determined. Normalized luciferase activity is expressed as a percentage compared with the control in non-stimulated cells. Results are expressed as means ± S.E. of three experiments. *, p < 0.05; **, p < 0.01 versus ciglitazone alone.

**Fig. 5.** Effects of C-peptide and insulin on protein levels of PPARγ in OK cells. Cells were treated with indicated concentrations of C-peptide or insulin for 24 h, and PPARγ was detected by immunoblotting. Membranes were stripped and reprobed for β-actin as a loading control. This is a representative blot of four independent experiments.

![PPARγ immunoblot](image)

**Fig. 4.** Synergistic stimulation of PPARγ activity by both C-peptide and insulin with TZD. Cells were transiently transfected with pPPRE-TK-luc and pSVβgal to normalize transfection efficiency to β-galactosidase activity. Transiently transfected OK cells were treated for 24 h with the indicated concentrations of C-peptide, insulin, ciglitazone, or combination. Cells were lysed, and luciferase activity in the lysates was determined. Normalized luciferase activity is expressed as a percentage compared with control in non-stimulated cells. Results are expressed as the means ± S.E. of three experiments. *, p < 0.05 relative to ciglitazone alone.

Where appropriate native and recombinantly expressed proteins were detected in cell lysates by Western blotting using anti-PPARγ, anti-PPARβ, anti-PPARγ, anti-PI 3-kinase p85α, anti-CD36, or anti-β-actin primary antibodies. In all cases, primary antibodies were visualized using peroxidase-conjugated secondary antibodies and enhanced chemiluminescence (Amersham Biosciences). Luciferase Reporter Assays—24 h after transient transfection with pPPRE-TK-luc and/or other plasmids, cells were incubated in serum-free medium for 18 h before stimulation with various potential PPARγ agonists. Where applicable 100 ng/ml PTX was added at this stage to the serum-free medium. In some experiments inhibitors were applied to cells 30 min prior to the addition of agonists, and the cells were subsequently incubated with agonists with or without inhibitors for a further 24 h. Medium was then removed, and cells were lysed in a buffer containing 500 mM HEPES, 2% Triton X-100, 1 mM CaCl₂, 1 mM MgCl₂, pH 7.8. Cell lysis was allowed to proceed for 10 min, and luciferase activity was measured using the LucLite assay kit in a LumiCount luminometer (Packard, Pangbourne, England). In all experiments a 50-μl aliquot of lysate was removed for β-galactosidase assay, and luciferase activity was normalized to β-galactosidase content.

Orthophosphate Labeling of OK Cells and Immunoprecipitation of Phospho-PPARγ—Confluent wild-type OK cells in 6-well plates were serum-starved overnight, incubated with or without 100 nM wortmannin for 30 min, and then washed three times with phosphate-free Dulbecco’s modified Eagle’s medium-Ham’s F12 mix. Cells were then incubated with serum-free medium containing 200 μCi/nmol ([³²P]Orthophosphate) and 5 mM C-peptide or 100 mM insulin for 4 h at 37 °C. Cell monolayers were then washed three times with ice-cold phosphate-buffered saline, pH 7.4, and incubated for 30 min with ice-cold lysis buffer composed of 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, and 200 μM sodium vanadate made up in phosphate-buffered saline, pH 7.4. The lysates were clarified by 5 min of centrifugation, and the supernatant was precleared with protein A-Sepharose for 45 min at 4 °C and then incubated with 10 μl of anti-PPARγ overnight at 4 °C. Immune complexes were collected using protein A-Sepharose beads. The beads were then washed three times with lysis buffer and then 35 μl of Laemmli buffer (60 mM Tris, pH 6.8, 10% glycerol, 2% SDS, 100 mM dithiothreitol, and 0.01% bromophenol blue) was added. Immunoprecipitated proteins were then separated on 12% polyacrylamide gels, and radiolabeled proteins were detected by autoradiography. Densitometry of phosphorylated protein bands was performed using Scion Image Beta 4.02 image analysis software, Scion, Frederick, MD.

**THP-1 Cell Differentiation Experiments**—THP-1 cells were plated in 6-well plates in serum-free medium containing potential stimulators of differentiation, including as a positive control 1 μM phorbol myristate.
acetate, for either 24 or 48 h. Cells were collected by centrifugation, washed twice with serum-free medium, and then lysed in polyacrylamide gel electrophoresis loading buffer. Expression of the differentiation marker CD36 (27, 28) was examined in whole cell lysates by Western blotting using anti-H9252-actin as a loading control.

Statistical Analyses—Data are presented as the mean ± S.E. Data were analyzed using unpaired Student’s t test for comparison between two experimental groups. For multiple comparisons, one-way analysis of variance with Tukey’s correction was used. Differences were considered significant at p < 0.05.

RESULTS

Western blotting was used to determine PPAR isoform expression in OK cells whole cell lysates. Our previous PPRE-luciferase reporter studies in OK cells (29) indicated transcriptional activation only by PPARγ or PPARβ ligands. In agreement with this observation both PPARγ and PPARβ but not PPARα proteins were detected in OK cells by Western blotting in the current study (Fig. 1). A concentration-dependent increase in luciferase expression in pPPRE-TK-luc-transfected OK cells was observed after stimulation with the TZD ciglitazone (maximum 207 ± 2.8% of control, EC₅₀ 80 nM), C-peptide (maximum 170 ± 6.5% of control, EC₅₀ 4 nM), and insulin (maximum 250 ± 4.5% of control, EC₅₀ 10 nM) (Fig. 2).

To confirm that the observed effects of C-peptide or insulin were mediated via activation of PPARγ cells were transfected with pPPRE-TK-luc (Fig. 3A). Overexpression of PPARγ enhanced both basal PPRE expression and that induced by 5 nM C-peptide, 100 nM insulin, or 5 μM ciglitazone by 11, 20, and 14-fold, respectively, above that observed in non-stimulated, wild-type pPPRE-TK-luc-transfected OK cells. Increased levels of PPARγ protein in pCMX-PPARγ-transfected cells were confirmed by Western blotting (Fig. 3B). Combining either 5 nM C-peptide or 100 nM insulin with 5 μM ciglitazone resulted in an augmented luciferase response (Fig. 4) compared with that seen in cells treated with each agonist alone (270 ± 6 and 400% ± 8, respectively, compared with 190 ± 3% for ciglitazone alone). However the combination of insulin and C-peptide was not synergistic in terms of luciferase activity (Fig. 4). The effects of C-peptide and insulin on the PPARγ-mediated activation of PPRE were not related to changes in expression of PPARγ itself (Fig. 5).

To determine whether C-peptide or insulin per se are PPARγ ligands, or whether treatment with these agents caused the release of intracellular PPARγ ligands, cells were pretreated with 0.5 μM GW9662, a selective irreversible PPARγ antagonist, for 30 min before the application of 100 nM insulin, 5 nM C-peptide, or 5 μM ciglitazone. GW9662 treatment significantly inhibited the effects of ciglitazone on PPRE activity to 60% of that observed compared with non-GW9662-treated cells. However, neither C-peptide nor insulin-induced PPRE activity was affected by GW9662 treatment (Fig. 6).

Insulin has been shown previously to activate PPARγ via a ligand-independent mechanism involving PPARγ phosphorylation by ERK MAP kinase. To determine whether this was the operative mechanism in the current experiments OK cells were

---

**FIG. 7.** ERK MAP kinase is not required for C-peptide- or insulin-induced PPARγ activity. Cells were transiently transfected with pPPRE-TK-luc and pSVgal to normalize for transfection efficiency. A, transiently transfected cells were treated with 5 μM PD98059 for 30 min prior to stimulation and during treatment for 24 h with the indicated concentrations of C-peptide and insulin. B, cells were co-transfected with either constitutive active MEK1, dominant negative MEK1, or empty vector. Transiently transfected cells were treated for 24 h with the indicated concentrations of C-peptide or insulin. Cells were lysed, and the luciferase activity in lysates was determined. Normalized luciferase activity is expressed as a percentage compared with control in non-stimulated cells. Results are expressed as the means ± S.E. of three experiments.
pretreated with or without 5 μM PD98059, a specific inhibitor of MEK1, for 30 min before stimulation with 5 nM C-peptide or 100 nM insulin, and the antagonist was kept during the entire stimulation period. The effect of C-peptide or insulin on PPRE activation was not attenuated to any significant level by the PD98059 treatment (Fig. 7A). In cells transfected with pMEK1-CA basal unstimulated PPRE-driven luciferase expression was increased by 270 ± 10%, and no further significant stimulation by agonists was observed (Fig. 7B). Transfection with pMEK1-KD markedly inhibited basal transactivation
Activation of PPARγ by Insulin and C-peptide

FIG. 9. Phosphorylation of PPARγ in response to C-peptide and insulin. Wild-type OK cells were labeled with [32P]orthophosphate, treated with the indicated concentrations of C-peptide and insulin with or without wortmannin pretreatment, and then immunoprecipitated with anti-PPARγ. Immunoprecipitates were collected on Protein A-Sepharose and then subjected to PAGE followed by autoradiography. A representative gel is shown in the upper panel where the labeled band represents phosphorylated PPARγ. The lower panel depicts the combined results of densitometric analysis from three identical experiments. *, p < 0.05 relative to non-stimulated, non-wortmannin-inhibited control condition.

FIG. 10. PTX inhibits C-peptide but not insulin-induced activation of PPARγ. Cells were transiently transfected with pPPRE-TK-luc and pSVβgal to normalize for transfection efficiency. A, transiently transfected wild-type OK cells were treated with or without 100 ng/ml PTX for 18 h prior to stimulation for 24 h with the indicated concentrations of C-peptide or insulin. Cells were lysed, and the luciferase activity in lysates was determined. Normalized luciferase activity is expressed as a percentage compared with control in non-stimulated cells. Results are expressed as means ± S.E. of three experiments. *, p < 0.05 relative to non-PTX-treated, C-peptide-stimulated cells.

A growing body of evidence challenges the generally accepted dogma that C-peptide is devoid of biological function (1, 30). Particularly interesting are the insulinomimetic effects of C-peptide whereby cross-talk is observed between C-peptide and insulin signaling pathways (11), C-peptide stimulates glucose uptake and glycogen synthesis in muscle cells (3), and C-peptide increases whole body glucose utilization in streptozotocin diabetic rats (2). In view of our developing interests in the function of the insulin sensitizer PPARγ in the kidney (29, 31), and primarily using OK PT cells as a model, we elected to determine whether C-peptide and/or insulin were able to activate the transcriptional activity of PPARγ.

Building on our earlier work examining C-peptide signaling in kidney PT cells (7) we now provide for the first time unequivocal confirmation that treatment of OK PT cells with C-peptide at physiologically relevant concentrations results in significant transactivation of PPRE. Likewise we also demonstrated a similar, but less potent, dose-dependent effect of insulin itself. With respect to the PPAR isoform responsible for this effect, PPARβ is not expressed in these cells and accordingly shows no response to PPARβ agonists (29). Furthermore OK cells demonstrate only very modest responses to PPARβ agonists (29), and thus we reasoned that PPARβ α and β were unlikely to be involved. We therefore hypothesized that PPARγ was the subtype mediating PPRE transactivation and confirmed this by demonstrating augmentation of C-peptide- and insulin-evoked PPRE activity in cells overexpressing recombinant PPARγ. In adipocytes insulin treatment leads to increased expression of both PPARγ mRNA and protein (23, 24). Enhanced expression of the PPARγ protein was not induced by insulin or C-peptide in OK cells in the current study, and thus changes in PPARγ expression levels cannot explain the demonstrated increase in PPRE transactivation by insulin and C-peptide in wild-type OK cells.

The TZD ciglitazone is a prototypic PPARγ ligand that also transactivates PPRE in OK cells. Combining ciglitazone with C-peptide or insulin resulted in synergistic PPARγ-mediated transactivation of PPRE whereas combining C-peptide with insulin did not, suggesting distinct means of PPARγ activation. Although it seemed unlikely that either insulin or C-peptide themselves could be ligands for PPARγ we considered the possibility that an endogenous intracellular PPARγ ligand may be produced as a result of their respective signaling capabilities. GW9662 binds to the ligand-binding domain of PPARγ resulting in the covalent modification of Cys384 thus conferring upon GW9662 properties of a full PPARγ antagonist because of irreversible loss of ligand binding (32). Interestingly in the current study GW9662 was able to inhibit ciglitazone but not C-pep-
Activation of PPARγ by Insulin and C-peptide

Despite the previous reports that ERK MAP kinase is a key regulator of ligand-independent PPARγ activity, our results provided several lines of evidence to suggest that the ERK MAP kinase pathway is not involved in C-peptide- or insulin-mediated alterations in PPARγ activity in OK cells. Firstly, although we have demonstrated previously a robust ERK MAP kinase activation by C-peptide in OK cells (7), the stimulatory effects of C-peptide and insulin on PPRE transactivation were not affected by a chemical ERK MAP kinase inhibitor. Secondly, although overexpression of a dominant negative MEK1 significantly depressed basal transactivation of PPARγ, activity the effects of C-peptide and insulin on PPRE transactivation were preserved. In OK cells, rather than inhibiting PPARγ transcriptional activity as demonstrated by others, expression of constitutively active MEK1 leads to a maximally enhanced basal activation of endogenous PPARγ activity such that ligand-dependent and -independent activation can no longer be detected.

In view of our earlier observations that C-peptide activated PI 3-kinase in OK cells (7), a capacity also shared by insulin (26), we studied the reliance of ligand-independent PPARγ activity by these agents on the activity of PI 3-kinase. Wortmannin-induced attenuation of the PPRE transactivation stimulated by both C-peptide and insulin strongly implicates PI 3-kinase in this effect, and the concordant results of the experiments using Δp85 confirm the involvement of type 1A PI 3-kinase. Furthermore, ligand-independent activation of PPARγ by C-peptide and insulin is clearly associated with PI 3-kinase-dependent phosphorylation of PPARγ, although the precise kinase mediating the phosphorylation is not established by these studies.

These observations raise a major question as to whether the PPRE activation induced by C-peptide and insulin observed using luciferase reporter constructs is mirrored by transcriptional activation of established PPARγ-regulated genes in native cells. Although data regarding PPARγ function in PT cells is beginning to accrue, little information is available regarding specific PPARγ-regulated genes in these cells. A variety of
PPARγ regulated genes have been identified in macrophages, and co-localization of C-peptide with macrophages in atherosclerotic lesions described in diabetic patients suggests a potential role for C-peptide in regulation of function in this cell type (38, 39). The type B scavenger receptor CD36 is expressed and co-localization of C-peptide with macrophages in athero-

tial for the actions of insulin sensitizing actions of TZDs (41–

49) in the protection against insulin resistance and is essential for the actions of insulin sensitizing actions of TZDs (41–

49).

In summary our results demonstrated C-peptide- and insul

in-evoked, phosphorylation-associated, ligand-independent ac

tivation of PPARy with the requirement for PI 3-kinase activity in OK cells. These effects measured using reporter constructs are mirrored by C-peptide- and insulin-stimulated expression of PPARγ-regulated genes in a monocyte cell line. The findings indicate a potentially major new role for C-peptide in the reg

ulation of cell biology, insulin sensitivity, glucose homeostasis, and PPARγ function.

REFERENCES

1. Wahren, J., Ekberg, K., Johansson, J., Henriksson, M., Jonsson, B.-L. (2000) Am. J. Physiol. 278, E759–E768

2. Wu, W., Oshida, Y., Yang, W.-F., Ohzawa, I., Sato, J., Iwao, S., and Johansson, B.-L. (1996) Acta Physiol. Scand. 157, 253–258

3. Zierath, J., Galuska, D., Johansson, B.-L., and Wallberg-Henriksson, H. (1991) Diabetes 34, 899–901

4. Zierath, J., Hauberg, A., Tally, M., and Wallberg-Henriksson, H. (1996) Diabetes 35, 306–313

5. Sima, A. A. F. (2001) Diabetologia 44, 889–897

6. Ido, Y., Vindigni, A., Chang, K., Stramm, L., Chance, R., Heath, W. F., Dima-

richi, R. D., Di Cera, E., and Williamson, J. R. (1997) J. Biol. Chem. 272, 563–566

7. Al-Rasheed, N. M., Meakin, F., Royal, E. L., Lewington, A. J., Brown, J., Stahl, S., Ekberg, K., Johansson, B., Uhlen, M., Jornvall, H., and Wahren, J., Ekberg, K., Johansson, B., Uhlen, M., Jornvall, H., and Wahren, J. (1997) Science 277, 563–566

8. Kuchel, J., Schaller, J., and Brunskill, N. (2004) Biochem. Biophys. Res. Commun. 2081–2086

9. Adams, M., Regnato, M. J., Shao, D., Lazar, M. A., and Chatterjee, V. K. (1997) J. Biol. Chem. 272, 5128–5132

10. Camp, H. S., and Tafuri, S. R. (1997) J. Biol. Chem. 272, 10811–10816

11. Lesnitzer, L. M., Parks, D. J., Bledsoe, R. K., Cobb, J. E., Collins, J. L., Consr, T. G., Davis, R. G., Hull-Ryde, E. A., Lenhard, J. M., Patel, L., Bontke, K. D., Shenk, L., Stimmel, J. B., Thierampont, C., Willson, T. M., and Blanchard, S. G. (2000) Biochemistry 39, 8177–8184

12. Johansson, J., Ekberg, K., Shafigat, J., Henriksson, M., Chabalin, A., Wahren, J., and Jornvall, H. (2002) Biochem. Biophys. Res. Commun. 293, 1035–1040

13. Adams, M., Regnato, M. J., Shao, D., Lazar, M. A., and Chatterjee, V. K. (1997) J. Biol. Chem. 272, 5128–5132

14. Camp, H. S., and Tafuri, S. R. (1997) J. Biol. Chem. 272, 10811–10816

15. Lesnitzer, L. M., Parks, D. J., Bledsoe, R. K., Cobb, J. E., Collins, J. L., Consr, T. G., Davis, R. G., Hull-Ryde, E. A., Lenhard, J. M., Patel, L., Bontke, K. D., Shenk, L., Stimmel, J. B., Thierampont, C., Willson, T. M., and Blanchard, S. G. (2000) Biochemistry 39, 8177–8184

16. Johansson, J., Ekberg, K., Shafigat, J., Henriksson, M., Chabalin, A., Wahren, J., and Jornvall, H. (2002) Biochem. Biophys. Res. Commun. 293, 1035–1040

17. Adams, M., Regnato, M. J., Shao, D., Lazar, M. A., and Chatterjee, V. K. (1997) J. Biol. Chem. 272, 5128–5132

18. Camp, H. S., and Tafuri, S. R. (1997) J. Biol. Chem. 272, 10811–10816

19. Lesnitzer, L. M., Parks, D. J., Bledsoe, R. K., Cobb, J. E., Collins, J. L., Consr, T. G., Davis, R. G., Hull-Ryde, E. A., Lenhard, J. M., Patel, L., Bontke, K. D., Shenk, L., Stimmel, J. B., Thierampont, C., Willson, T. M., and Blanchard, S. G. (2000) Biochemistry 39, 8177–8184

20. Rangwala, S. M., and Lazar, M. A. (2004) Trends Cardiovasc. Med. 14, 8–12

21. Chana, R., Lewington, A. J., and Brunskill, N. J. (2004) Kidney Int. 65, 2081–2090

22. Wahren, J. (2004) Clin. Pharmacol. Therapeut. 75, 771–774

23. Wahren, J. (2004) Clin. Pharmacol. Therapeut. 75, 771–774

24. Arici, M., Chana, R., Lewington, A. J., Brown, J., and Brunskill, N. J. (2003) J. Am. Soc. Nephrol. 14, 2316–2322

25. Wahren, J. (2004) Diabetologia 47, 987–997

26. Kuchel, J., Schaller, J., and Brunskill, N. (2004) Biochem. Biophys. Res. Commun. 306, 313–319

27. Chana, R. S., Lewington, A. J., and Brunskill, N. J. (2004) Kidney Int. 65, 2081–2090

28. Wahren, J. (2004) Clin. Pharmacol. Therapeut. 75, 771–774

29. Arici, M., Chana, R., Lewington, A. J., Brown, J., and Brunskill, N. J. (2003) J. Am. Soc. Nephrol. 14, 2316–2322

30. Wahren, J. (2004) Diabetologia 47, 987–997

31. Chana, R. S., Lewington, A. J., and Brunskill, N. J. (2004) Kidney Int. 65, 2081–2090

32. Wahren, J. (2004) Clin. Pharmacol. Therapeut. 75, 771–774

33. Wahren, J. (2004) Clin. Pharmacol. Therapeut. 75, 771–774

34. Arici, M., Chana, R., Lewington, A. J., Brown, J., and Brunskill, N. J. (2003) J. Am. Soc. Nephrol. 14, 2316–2322

35. Wahren, J. (2004) Diabetologia 47, 987–997

36. Kuchel, J., Schaller, J., and Brunskill, N. (2004) Biochem. Biophys. Res. Commun. 306, 313–319

37. Chana, R. S., Lewington, A. J., and Brunskill, N. J. (2004) Kidney Int. 65, 2081–2090

38. Wahren, J. (2004) Clin. Pharmacol. Therapeut. 75, 771–774

39. Arici, M., Chana, R., Lewington, A. J., Brown, J., and Brunskill, N. J. (2003) J. Am. Soc. Nephrol. 14, 2316–2322

40. Chana, R. S., Lewington, A. J., and Brunskill, N. J. (2004) Kidney Int. 65, 2081–2090

41. Wahren, J. (2004) Clin. Pharmacol. Therapeut. 75, 771–774