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PEROXYNITRITE ACTIVATES GLUCOSE UPTAKE IN 3T3-L1 ADIPOCYTES THROUGH A PI3-K-DEPENDENT MECHANISM

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TABLE OF CONTENTS

1. ABSTRACT

Peroxynitrite, the product of the reaction between $^{\cdot}NO$ and $O_2^{\cdot-}$, is a strong oxidant and nitrating molecule, and it has been recently considered as a component of some important signaling pathways. Herein, we report the effect of peroxynitrite on glucose uptake in 3T3-L1 adipocytes. Peroxynitrite stimulated glucose uptake and this effect was inhibited by citochalasin B, indicating the participation of facilitated GLUT transporters. Peroxynitrite-induced glucose uptake was not related to intracellular ATP, nor to external or internal calcium, but it was inhibited by the phosphatidylinositol 3-kinase (PI3-K) inhibitor, wortmannin. Additionally, we also found that peroxynitrite did not activate the insulin receptor nor the PI3-K downstream signaling protein kinase B (PKB/Akt). The dose-dependent inhibitory action of wortmannin suggests that peroxynitrite activates glucose transport without affecting GLUT transporters translocation.

2. INTRODUCTION

Superoxide anion ($O_2^{\cdot-}$) overproduction causes an oxidative stress related to several pathological conditions, such as endothelial dysfunction (1), septic shock (2), and diabetes (3). $O_2^{\cdot-}$ and nitric oxide ($^{\cdot}NO$) react together to form peroxynitrite ($ONOO^-$), in a diffusion-controlled radical-radical reaction which is almost three fold faster than the $O_2^{\cdot-}$ dismutation catalyzed by superoxide dismutase (4). Peroxynitrite is a powerful oxidant and nitrating compound that reacts over a wide range of biomolecules (4,5). Tyrosine nitration, commonly used as a marker of peroxynitrite production, is one of chemical reactions produced by peroxynitrite on proteins. Consequently, peroxynitrite may affect the phosphorylation-dephosphorylation reactions that regulate the biological activity of many proteins (6). Indeed, it has been reported that tyrosine nitration by peroxynitrite (at 3-position of tyrosine) diminishes phosphorylation of proteins catalyzed by tyrosine kinases (7, 8). However, other reports have shown the opposite effect, that is, that peroxynitrite stimulates protein-tyrosine phosphorylation (9-12). Stimulation of tyrosine phosphorylation by peroxynitrite has been found in nonreceptor src tyrosine kinases (9), the tyrosine kinase receptor and the epidermal growth factor receptor, including its downstream signaling protein kinases, Raf-1, MEK (11,12), and the PI3K/PKB(Akt) pathway (10). In addition, peroxynitrite can oxidize thiols and metal-containing proteins that regulates multiple redox-sensitive pathways inducing expression of stress genes (13) and apoptosis (8,13,14). All these characteristics raise the possibility that peroxynitrite might operate as a signaling molecule (reviewed in 15,16).

Glucose transport is a complex process regulated by tyrosine phosphorylation signaling pathways and

therefore, peroxynitrite-dependent nitration reactions can affect it. Glucose is transported across cell membranes by several mechanisms, but the most ubiquitously distributed is the facilitative transport, mediated by different GLUT transporters (17). In insulin responsive cells, this hormone induces an increase of glucose uptake over the basal condition by stimulation of GLUT4 translocation from intracellular vesicles to the plasma membrane (18). This process is mediated by a sequence of tyrosine phosphorylation-dependent signaling pathways that occur after insulin-insulin receptor binding, such as the activation of the insulin receptor substrates (IRS's) and the phosphatidylinositol 3-kinase (PI3-K) (18). Additionally, peroxynitrite could modify the glucose transport in cells endowed with phosphoinositide 3-kinase/PKB(Akt) since in human skin fibroblasts, peroxynitrite can activate this pathway (10).

In this paper we show that peroxynitrite produces a significant stimulation of glucose transport in 3T3-L1 adipocytes, and that this effect is dependent of PI3-K activation but independent of both insulin receptor activation and PKB activation. The possible molecular mechanism is discussed.

3. MATERIALS AND METHODS

3.1. Materials

Peroxynitrite was prepared by azide-ozone reaction as reported (19). Dulbecco modified Eagle´s medium (DMEM) and trypsin were obtained from GIBCO BRL and Life Technologies (Grand Island, N.Y., USA). Bovine and fetal sera were purchased from HyClone Laboratories, Inc (Logan, UT, USA). Dexamethasone 21-acetate (Decadronal) was obtained from Merck Sharp & Dohme (México). 2-deoxy-D-[2,6-^{3}H] glucose and enhanced chemioluminiscence (ECL) kit were from Amersham Pharmacia Biotechnology (England). Rabbit anti-IRβ antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA), rabbit polyclonal anti-phospho-Akt (Ser 473) and anti-Akt antibodies were obtained from Cell Signaling Tech., monoclonal anti-PY antibody was from Zymed, HRP-conjugated goat anti-rabbit IgG secondary antibody was from Dako and Protein A-Sepharose was from Pharmacia (Uppsala, Sweden). All other chemicals were obtained from Sigma Chemical (St. Louis, MO). 3T3-L1 fibroblast cell line was kindly provided by Dr. Walid Kuri-Harcuch.

3.2. Cell culture and peroxynitrite treatment

3T3-L1 fibroblasts were cultured in DMEM medium with 10% of bovine serum, and differentiated in 6-wells cell culture clusters as previously reported (20). Before glucose transport measurements, cell monolayers were treated with 1% BSA in Krebs-Ringer-Phosphate Buffer (KRP) in order to remove bound insulin according to the debinding procedure of Ronnett et al. (21). Cell monolayers were treated with peroxynitrite as follows. After debinding, cell monolayers were washed tree times with KRP buffer alone and then peroxynitrite was added. This addition did not modify the pH of the KRP buffer. After one minute incubation, the later buffer was replaced by fresh KRP buffer. As a control, cells were incubated for 1 minute with inactivated peroxynitrite. Peroxynitrite inactivation was achieved by mixing peroxynitrite with KRP buffer 5 minutes before addition to the cells. The mixture did not absorb at 301 nm, confirming peroxynitrite inactivation (19).

3.3. Glucose transport and cell viability

Glucose transport was measured in KRP buffer using 2-deoxy-[2,6-^{3}H] glucose according to (20). Cell viability was determined in 3T3-L1 confluent fibroblasts and 3T3-L1 adipocytes after peroxynitrite treatment, by Trypan Blue exclusion test following detachment with Trypsin-EDTA.

3.4. Intracellular ATP determination

ATP was quantified by a bioluminescence assay, with slight modifications to the method previously described (22). 3T3-L1 fibroblasts were differentiated to adipocytes in 6-wells cell culture clusters and treated with peroxynitrite as indicated above. After rinsed with KRP buffer, the cell monolayers were rapidly extracted over ice with 250 µl of cold 5% trichloroacetic acid, and an aliquot of 10 µl was used to quantify ATP with an ATP detection Kit (1243 Bio-Orbit, Turku, Finland), utilizing a Bio-Orbit 1250 Luminometer (Turku, Finland).

3.5. Immunoprecipitations and Western Blotting

3T3-L1 fibroblasts were cultured and differentiated to adipocytes in 10-cm diameter cell culture dishes. After the indicated treatments, adipocytes were solubilized over ice by scraping and passing 10 times through a 25-gauge needle in lysis buffer (50 mM Hepes pH 7.4, 150 mM NaCl, 1 mM EDTA, 1mM PMSF, 5 mM NaF, 1 mM Na_3VO_4, 10 mM β-glycerophosphate, 10 µg/ml aprotinin, 1 µg/ml leupeptin, 1 µg/ml pepstatin A and 1% Triton X-100). The lysate was clarified by centrifugation at 10,000g for 15 min at 4^0C and the supernatant saved. For insulin receptor (IR) activation studies, extracts aliquotes (500 µg protein) were immunoprecipitated by incubation with 2 µg rabbit anti-IR-beta antibody for 2 h at 4^0C before 20 µl of 30 % (vol/vol). Protein A-Sepharose was added, the mixture was brought to 300 µl with lysis buffer and mixed overnight at 4^0C on a rotating mixer. The mixtures were centrifuged and the pellet was washed once with TNET buffer (20 mM Tris pH 7.4, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100), once with TNET buffer without Triton X-100 and once more with deionized H_2O at 4^0C. Immunoprecipitates were treated (96^0C, 5 min) with Laemmli solution, resolved by SDS-PAGE and electroblotted to polyvinildiene difluoride membranes. Membranes were blocked (1 hour, room temperature) with 3% bovine serum albumin (for phosphorylated proteins detection) or 3% milk powder (non-phosphorylated proteins detection) in TBST buffer (50 mM Tris pH 7.4, 150 mM NaCl, 0.1% Tween 20). IR-beta and tyrosine-phosphorylated proteins were detected by incubation (overnight, 4^0C) with the appropriate primary and horseradish-peroxidase conjugated secondary (1.5 hours, room temperature) antibodies in TBST, using an Enhanced Chimioluminiscence (ECL) kit. Protein kinase B (PKB) activation was assessed by Akt-S^{473} phosphorylation. Cell

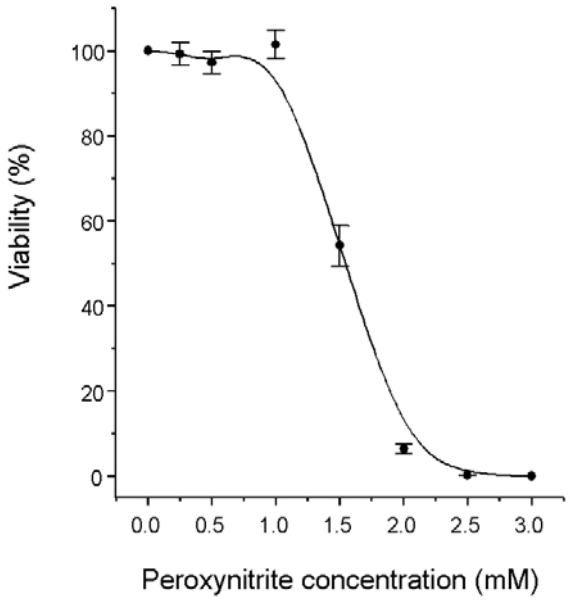

Figure 1. Effect of peroxynitrite on cell viability. 3T3-L1 adipocytes cultured in 6-wells cell culture clusters were treated with peroxynitrite at the indicated concentration for 1 min, and then cell viability was measured as indicated in Methods. The values are means ± SE of 4 independent experiments.

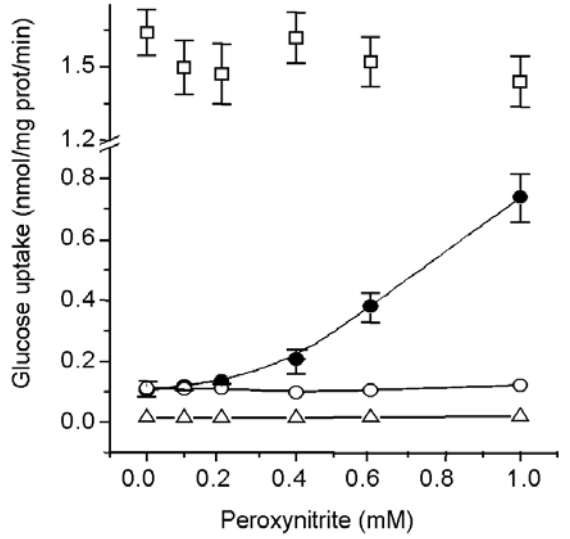

Figure 2. Effect of peroxynitrite on glucose transport. 3T3-L1 adipocytes were treated with peroxynitrite (●), inactivated peroxynitrite (○), or peroxynitrite plus 10 μM cytochalasin B (△) for 5 minutes. The cells were washed and the glucose uptake measured as described in Methods. In addition, glucose uptake was measured in cells treated with peroxynitrite before stimulation with 100 nM insulin (□). Values are means ± SE of 7 independent experiments.

extracts (without immunoprecipitation) were resolved and immunoblotted with rabbit polyclonal anti-phospho-Akt (Ser 473) as described above.

3.6. Statistical analysis

All values are presented as mean ± SE. Comparisons between groups were determined by analysis of variance (ANOVA). p values < 0.01 were considered significant.

4. RESULTS

4.1. Effect of peroxynitrite on cell viability

3T3-L1 fibroblast and adipocytes viability was not affected by peroxynitrite treatment at concentrations up to 1 mM; at higher concentrations, peroxynitrite produced cell loss viability, with a 50% lethal concentration (LC_{50}) of 1.5 mM (Figure 1). In addition, 3T3-L1 fibroblasts monolayers treated with increasing peroxynitrite concentrations (up to 1 mM) retained their proliferative characteristics after subcultured. 3T3-L1 adipocytes were also able to increase glucose uptake in response to insulin after peroxynitrite treatment (Figure **2**). Thus, a peroxynitrite concentration range of 0.1 to 1 mM was selected for further experiments.

4.2. Effect of peroxynitrite on glucose transport

As shown in Figure 2, peroxynitrite increased glucose uptake in a dose-dependent manner in adipocytes. At 1 mM, peroxynitrite significantly stimulated glucose transport, from 0.106 ± 0.011 nmol/mg protein/min to 0.737 ± 0.072 nmol/mg protein/min. As expected, inactivated peroxinitrite did not increase basal glucose transport in 3T3-L1 adipocytes (Figure 2). The glucose transporter inhibitor, citochalasin B (23), blocked activation of glucose transport by peroxynitrite (Figure 2). This indicated that the peroxynitrite-induced increase in glucose transport involved facilitated GLUT transporters and was not related to unspecific permeation through the plasma membrane (23). Consistently, insulin induced glucose uptake in peroxynitrite-treated cells (Figure 2), indicating that cells were in a good condition. The biochemical mechanism involved in the peroxynitrite-induced glucose uptake was further investigated.

4.3. Lack of effect of ATP and calcium ions on peroxynitrite-induced glucose uptake

It has been shown that a low intracellular ATP content increases glucose transport (24, 25) and, on the other hand, that peroxynitrite inhibits mitochondrial respiration (26) thus, peroxynitrite could increase basal glucose transport via peroxynitrite-induced intracellular ATP decrease. In this regard, we have found that intracellular ATP was not significantly changed after 1 min of peroxynitrite treatment. The ATP content in cells treated with peroxynitrite was 14.9 ± 0.67 nmol/mg whereas that in intact cells was 15.53 ± 0.57 nmol/mg protein (n= 6, mean ± SE) .This result indicated that the stimulating effect of peroxynitrite on glucose transport was not related to ATP depletion.

Furthermore, peroxynitrite induces increase in intracellular calcium in rat liver (27) and mouse cortical neurons (28) and it has been reported that GLUT1 activation may be related to an increase in intracellular calcium (29). In order to test if calcium is involved in the

Table 1. Effect of calcium on glucose transport in 3T3-L1 adipocytes

Additions	**+ Ca^{2+}**	**- Ca^{2+}**
None	0.176 ± 0.010 n=5	0.163 ± 0.012 n=5
Peroxynitrite 1 mM	1.384 ± 0.07* n=8	1.428 ± 0.036* n=8
A23187 5μM	0.211 ± 0.016 n=5	ND

2-deoxy-[^{3}H]-glucose uptake was measured in regular (+ Ca^{2+}) or calcium-free (- Ca^{2+}) KRP buffer, with the indicated additions. The values are in nmol/mg prot/min (mean ± SE). ND: not determined. *p< 0.001 vs control without peroxynitrite.

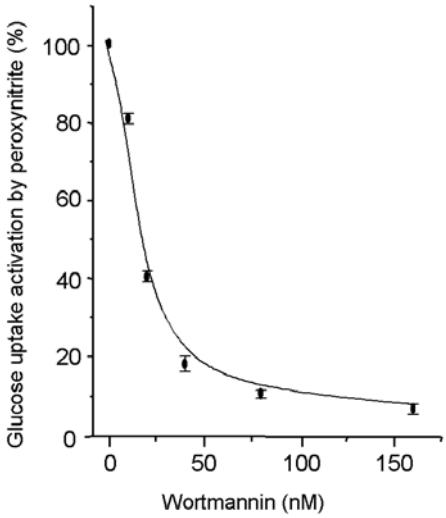

Figure 3. Inhibition of peroxynitrite-activated glucose transport by wortmannin. 3T3-L1 adipocytes were incubated with the indicated wortmannin concentrations in KRP buffer, 10 minutes before 1 mM peroxynitrite treatment, then glucose transport was measured as described in Methods. The values are means ± SE of 5 independent experiments.

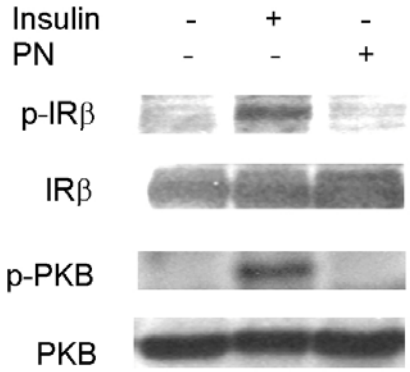

Figure 4. Effect of peroxynitrite and insulin on the posphorylation status of insulin receptor and PKB. 3T3-L1 adipocytes were treated with 100 nM insulin or 1 mM peroxynitrite (PN), then the phosphorylation status of IR (p-IR-beta) and PKB (p-PKB) were assessed by immunoprecipitation and Western Blott analysis as described in Methods. Non-phosphorylated proteins (IR-beta and PKB) are also shown as controls of protein loaded into the gels.

activation of glucose transport by peroxynitrite, we studied the effect of peroxynitrite on glucose transport in calcium-free KRP buffer and in presence of the calcium ionophore A23187 in normal medium. As shown in Table 1, the absence of calcium did not modify the glucose transport in medium without peroxynitrite nor the peroxynitrite-activated glucose transport. In the presence of the calcium ionophore A23187, a slight, non significant increase was produced. Thus, intracellular calcium did not participate in the peroxynitrite-stimulated glucose transport.

4.4. Effect of PI3-K inhibition on the peroxynitrite-induced glucose uptake

In insulin responsive cells, insulin stimulates glucose transport through PI3-K activation (18). We explored if PI3-K participates in the mechanism underlying the activation of glucose transport by peroxynitrite, by assessing the effect of the PI3-K inhibitor, wortmannin (30). When 3T3-L1 adipocytes were incubated with wortmannin 10 minutes prior peroxynitrite treatment, we found a dose-dependent inhibition of the peroxynitrite-activated glucose uptake, with an IC_{50} of 18 nM and 95% inhibition at 160 nM (Figure 3).

Given that the activation of insulin receptor (IR) is upstream of IP3-K, the effect of peroxynitrite on the insulin receptor was explored. We examined if IR undergoes tyrosine phosphorylation in response to peroxynitrite. The IR was immunoprecipitated and examined by Western blotting. As shown in Figure 4, IR was not phosphorylated after peroxynitrite treatment; whereas that, as expected, IR was phosphorylated by insulin. This result indicated that the IR was not involved in the peroxynitrite-induced glucose uptake stimulation.

The effect of peroxynitrite on the protein kinase B (PKB), that is activated by PI3-K, was also studied. We found that PKB was phophorylated in 3T3-L1 adipocytes treated with insulin (positive control), but no PKB phosphorylation was found after peroxynitrite treatment (Figure 4). Hence, glucose transport activation by peroxynitrite was not related to IR nor to PKB activation. This suggested that the peroxynitrite-activated IP3-K stimulated glucose transporters located at the plasma membrane.

5. DISCUSSION

In this paper we show evidence that peroxynitrite treatment produces a significant and dose-dependent

increase in glucose uptake in 3T3-L1 adipocytes, that involves facilitated (GLUT´s) transporters.

Other conditions that stimulate basal glucose transport have been reported. For instance, glucose deprivation increases the rate of glucose uptake by a slow and reversible process in 3T3-L1 adipocytes and in other cell types. The mechanism involved in this phenomenon seems be related to GLUT1 synthesis (31), and/or to "GLUT1 activation", with no requirement of GLUT translocation toward plasma membrane. Additionally, hypoxia and several inhibitors of mitochondrial oxidative phosphorylation, also enhance glucose uptake by "activation" or "unmasking" of preexisting GLUT1 transporters in plasma membrane (24). The mechanism(s) involved in these processes is not well understood, but it has been related to ATP depletion and stimulation of AMP-activated protein kinase (25). In our study, we found that peroxynitrite treatment did not modify the intracellular ATP in 3T3-L1 adipocytes, consequently, the mechanism involved in the peroxynitrite-induced glucose transport increase must be independent of ATP content. Besides, in rat liver epithelial cells, the activation of GLUT1 induced by mitochondrial inhibitors could be related to an increase in intracellular calcium (29). However, we did not find effect neither in the absence of extracellular calcium nor in the presence of the calcium ionophore A23187 in the glucose transport activated by peroxynitrite. This indicates that the peroxynitrite effects observed here are not related to calcium mobilization.

There is a great deal of evidence that links phosphatidyl inositol-3 kinase-dependent Protein kinase B activation (PI3-K/PKB(Akt) and the stimulation of glucose transport by insulin through GLUT4 translocation to the plasma membrane (reviewed in 18,31). Since the peroxynitrite-induced glucose transport activation was inhibited by wortmannin, a PI3-K inhibitor, peroxynitrite could also produce GLUT4 translocation to the plasma membrane by activation of PI3-K/PKB(Akt) pathway. Furthermore, PI3-K/PKB(Akt) activation by peroxynitrite has been demonstrated in human skin fibroblasts (10), and H4IIE cells (32). We found that although peroxynitrite increased glucose uptake in a PI3-K-dependent fashion, unexpectedly it did not induce PKB activation (detected by its phosphorylation status) in 3T3-L1 adipocytes (Figure 4). This result could be explained if peroxynitrite inhibited PKB, as reported in bovine retinal endothelial cells (33) and in cultured bovine aortic endothelial cells (34). Regardless the mechanism, the fact that peroxynitrite activates glucose uptake in 3T3-L1 adipocytes without PKB activation (this paper) shows that the mechanism of peroxynitrite-induced glucose uptake is different than that induced by insulin, despite that PI3-K is involved in both processes.

The difference between glucose uptake activation induced by insulin and by peroxynitrite may be related to two postulated and distinct events, namely, glucose transporters activation and glucose transporters translocation. The hypothesis of glucose transporters activation (increase in intrinsic activity) has been supported by the finding that under some conditions (for example, inhibiting mitogen-activated protein kinase, MAPK), glucose uptake is stimulated without glucose transporters translocation to plasma membrane (reviewed in 35).Thus, a mechanism involving glucose transporter activation without affecting transporter translocation could reasonably explain the peroxynitrite-induced glucose uptake reported here. Consistently, the IC_{50} for peroxynitrite-induced glucose uptake inhibition by wortmannin (18 nM this work) was much lower than that required to inhibit the insulin-induced glucose transporter translocation in 3T3-L1 adipocytes (IC_{50} = 80 nM, reference 36). This evidence suggests that peroxynitrite does not induce glucose uptake through glucose transporters translocation, but through transporters activation.

In summary, this work provides evidence suggesting that peroxynitrite activates glucose transporter at the plasma membrane and that this activation involves PI3-K activity but it is independent on PKB. The mechanisms downstream PI3-K remains to be elucidated.

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7. REFERENCES

1. Griendling K, D. Sorescu & M. Ushio-Fukai: NAD(P)H Oxidase: Role in cardiovascular Biology and Disease. *Circ Res* 86, 494-501 (2000)

2. Fukuyama N & S. Takebayashi: Clinical evidence of peroxynitrite formation in chronic renal failure with septic shock. *Free Radic Biol Med* 22, 771-774 (1997)

3. Nishikawa T, D. Edelstein, D.X. Liang, S. Yamagishi, T. Matsumura, Y. Kaneda, M.A. Yorek, D. Beebe, P.J. Oates, H.P. Hammes, I. Giardino & M. Browlee: Normalizing mitochondrial superoxide production blocks three pathways of hyperglycemic damage. *Nature* 404, 787-790 (2000)

4. Squadrito G.L & W.A. Pryor: Oxidative chemistry of nitric oxide: the roles of superoxide, peroxynitrite, and carbon dioxide. *Free Radic Biol Med* 25, 392-403 (1998)

5. Beckman J.S & W.H. Koppenol: Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and the ugly. *Am J Physiol* 271, C1424-C1437 (1996)

6. Schlessinger J: Cell signaling by receptor tyrosine kinases. *Cell* 103, 211-225 (2000)

7. Gow A.J, D. Duran, S. Malcom & H. Ischiropoulos: Effects of peroxynitrite-induced protein modification on tyrosine phosphorylation and degradation. *FEBS Lett* 385, 63-66 (1996)

8. Brito C, M. Naviliat, A.C. Tiscornia, F. Vuillier, G. Gualco, G. Dighiero, R. Radi & A.M. Cayota:

Peroxynitrite inhibits T lymphocyte activation and proliferation by promoting impairment of tyrosine phosphorylation and peroxynitrite-driven apoptotic death. *J Immunol* 162, 3356-3366 (1999)

9. Mallozzi C, A.M. Di Stasi & M. Minetti: Activation of src tyrosine kinases by peroxynitrite. *FEBS Lett* 456, 201-206 (1999)

10. Klotz L.O, S.M. Scieke, H. Sies & N.J. Holbrook: Peroxynitrite activates the phosphoinositide 3-kinase/Akt pathway in human skin primary fibroblasts. *Biochem J* 352, 219-225 (2000)

11. Bapat S, A. Verkleij & J.A.Post: Peroxynitrite activates mitogen-activated protein kinase (MAPK) via a MEK-independent pathway: a role of protein kinase C. *FEBS Lett* 499, 21-26 (2001)

12. Zouki C, S.L. Zhang, J.S. Chang & J.G. Filep: Peroxynitrite induces integrin-dependent adhesion of human neutophils to endothelial cells via activation of the Raf-1/MEK/Erk pathway. *FASEB J* 15, 25-27 (2001)

13. Foresti R, S. Chandre, J.E. Clark, C.J. Green & R. Motterlini: Peroxynitrite induces haem oxygenase-1 in vascular endothelial cells: a link to apoptosis. *Biochem J* 339, 729-736 (1999)

14. Zhuang S & G. Simon: Peroxynitrite-induced apoptosis involves activation of multiple caspases in HL-60 cells. *Am J Physiol* 279, C341-C351 (2000)

15. Klotz L.O, P. Schroeder & H. Sies: Peroxynitrite signalling: receptor tyrosine kinases and activation of stress-responsive pathways. *Free Radic Biol Med* 33, 737-743 (2002)

16. Minetti M, C. Mallozzi & M. Di Stasi: Peroxynitrite activates kinases of the src family and upregulates tyrosine phosphorylation signalling. *Free RadicBiol Med* 33, 744-754 (2002)

17. Olson A.L & J.E. Pessin: Structure, function, and regulation of the mammalian facilitative glucose transporter gene family. *Annu Rev Nutr* 16, 235-256 (1996)

18. Shepherd P.R & B.B. Kahn: Glucose transporters and insulin actions. *N Engl J Med* 341, 248-257 (1999)

19. Uppu R.M, G.L. Squadrito, R. Cueto & W.A. Pryor: Synthesis of peroxynitrite by azide-ozone reaction. *Methods Enzymol* 269, 311-331 (1996)

20. Frost S.C & M.D. Lane: Evidence for the involvement of vicinal sulfhydryl groups in insulin-activated hexose transport by 3T3-L1 adipocytes. *J Biol Chem* 260, 2646-2652 (1985)

21. Ronnett G.V, V.P. Knutson & M.D. Lane: Insulin-induced down-regulation of insulin receptors in 3T3-L1 adipocytes. *J Biol Chem* 257, 4285-4291 (1982)

22. Guzman-Grenfell A.M, M.A. Bonilla-Hernandez & M.T. Gonzalez-Martinez: Glucose induces a Na^+,K^+-ATPase-dependent transient hyperpolarization in human sperm. I. Induction of changes in plasma membrane potential by the proton ionophore CCCP. *Biochim Biophys Acta* 1464, 188-198 (2000)

23. Bloch R: Inhibition of glucose transport in the human erythrocyte by cytochalasin B. *Biochemistry* 12, 4799-4801 (1973)

24. Khayat Z.A., T. Tsakiridis, A. Ueyama, R. Somwar, Y. Ebina & A. Klip: Rapid stimulation of glucose transport by mitochondrial uncoupling depends in part on cytosolic Ca^{2+} and cPKC. *Am J Physiol* 275, C1487-C1497 (1998)

25. Hayashi T., M.F. Hirshman, N. Fujii, S.A. Habinowski, L.A. Witters & L.J. Goodyear: Metabolic stress and altered glucose transport: activation of AMP-activated protein kinase as a unifying coupling mechanism. *Diabetes* 49, 527-531 (2000)

26. Radi R., M. Rodriguez, L. Castro & L. Telleri: Inhibition of mitochondrial electron transport by peroxynitrite. *Arch Biochem Biophys* 308, 89-95 (1994)

27. Muriel P & G. Sandoval: Hepatic basolateral plasma high-affinity Ca^{2+}-ATPase is inhibited by nitric oxide and peroxynitrite anion. *J Appl Toxicol* 20, 435-439 (2000)

28. Ohkuma S., M. Katsura, A. Higo, K. Shirotani, A. Hara, C. Tarumi & T. Ohgi: Peroxynitrite affects Ca^{2+} influx through voltage-dependent calcium channels. *J Neurochem* 76, 341-350 (2001)

29. Quintanilla R.A., O.H. Porras, J. Castro & L.F. Barros: Cytosolic [Ca^{2+}] modulates basal GLUT1 activity and plays a permissive role on its activation by metabolic stress and insulin in epithelial cells. *Cell Calcium* 28, 97-106 (2000)

30. Ui M., T. Okada, K. Hazeki, O & Hazeki: Wortmannin as a unique probe for an intracellular signaling protein, phosphoinositide 3-kinase. *Trends Biochem Sci* 20, 303-307 (1995)

31. Hajduch E., G.J. Litherland & H.S. Hundal: Protein kinase B (PKB/Akt) a key regulator of glucose transport? *FEBS Lett* 492, 199-203 (2001)

32. Kang K.W, S.H. Choi & S.G. Kim: Peroxynitrite activates NF-E2-related factor 2/antioxidant response element through the pathway of phosphatidylinositol 3-kinase: the role of nitric oxide synthase in rat glutathione S-transferase A2 induction. *Nitric Oxide* 7, 244-53 (2002)

33. Gu X, A.B. EI-Remessy, S.E. Brooks, M. AI-Shabrawey, N.T. Tsai & R.B. Caldwell: Hyperoxia induces retinal vascular endothelial cell apoptosis through formation of peroxynitrite. *Am J Physiol Cell Physiol* 285, C546-54 (2003)

34. Zou M.H., X.Y. Hou, C.M. Shi, D. Nagata, K. Walsh & R.A. Cohen: Modulation by peroxynitrite of Akt- and

AMP-activated kinase-dependent Ser1179 phosphorylation of endothelial nitric oxide synthase. *J Biol Chem* 277, 32552-7 (2002)

35. Furtado L.M., R. Somewar, G. Sweeney, W. Niu & A. Klip: Activation of the glucose transporter GLUT4 by insulin. *Biochem Cell Biol* 80, 569-78 (2002)

36. Hausdorff S.F., D.C. Fingar, K. Morioka, L.A. Garza, E.L. Whitman, S.A. Summers & M.J. Birnbaum: Identification of wortmannin-sensitive targets in 3T3-L1 adipocytes. Dissociation of insulin-stimulated glucose uptake and GLUT4 translocation. *J Biol Chem* 274, 24677-24684 (1999)

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