

Abstract

When insulin binds to the alpha subunit of the insulin receptor the intracellular beta subunit, autophosphorylates, and then binds to a specific subunit within the insulin receptor substrate-1 (IRS-1) triggering its phosphorylation and a series of down stream signals. Tyrosine and serine residues within the beta subunit of the insulin receptor IR- β exhibit a competitive mechanism of phosphorylation resulting in different outcomes. Insulin mediated signals (resulting in activated glucose transporters) are transduced by tyrosine phosphorylation. This research was designed to investigate the effect of nitric oxide (NO) released from S-nitroso-N-acetyl-D,L-penicillamine (SNAP) and S-nitrosoglutathione (GSNO) on the phosphorylation of serine and tyrosine residues in adipose, liver and skeletal muscle of rats.

The tissues adipose, liver and skeletal muscle were excised from 6-8 week old Sprague-Dawley rats and were then subjected to collagenase digestion and then exposed to drugs SNAP and GSNO (25 and 50 ng/ml), in the presence or absence of glucose (25 mM) and insulin (100 and 1000 nM). NO released from the drugs was determined as nitrite (NO_2^-) using the Greiss test and phosphorylation of IRS-1, IR- β , tyrosine and serine was measured using a specific dot blot analysis. These experiments were repeated in the presence of a NO scavenger, carboxy-2-phenyl-4,4,5,5-tetramethyl-imidazoline-1-oxyl-3-oxide (carboxy-PTIO).

Results were obtained for insulin receptor- β (IR- β) in the three tissues (adipose, liver and muscle). IR- β phosphorylation was significantly reduced by 92.12 - 99.24 % in adipocytes treated with glucose and insulin treated with GSNO (25 and 50 ng/ml) and similar observations were made for SNAP (25 and 50 ng/ml), 84.82 - 97.40 %. IR- β phosphorylation was less reduced in hepatocytes and myocytes treated with GSNO at 25 and 50 ng/ml (37.67 - 76.20 % and 50.75 - 94.25 %) and SNAP at 25 and 50 ng/ml (84.82 - 97.40 % and 47.59 - 99.19 %), respectively ($p < 0.05$).

Results were obtained for IRS -1 phosphorylation in hepatocytes in which, IRS-1 phosphorylation was significantly reduced by 59.67 - 86.50 % when treated with GSNO (25 and 50 ng/ml) and 59.90-80 % treated with SNAP (25 and 50 ng/ml). A greater reduction was observed in myocytes, in which IRS-1 phosphorylation was significantly reduced by 58.52 - 95.63 % treated with GSNO and 87.30 - 93.97 % treated with SNAP, respectively ($p < 0.05$).

Serine phosphorylation was significantly increased in hepatocytes to a maximum of 828.90 % when treated with GSNO and 728.00 % treated with SNAP, ($p < 0.05$). A greater increase was observed in myocytes to a maximum of 1604.88 % when treated with GSNO and 821.63 % treated with SNAP, respectively ($p < 0.05$).

Tyrosine phosphorylation was significantly reduced in hepatocytes treated with GSNO at 25 and 50 ng/ml (17.59 - 99.47 %) and SNAP at 25 and 50 ng/ml (44.00 - 98.64 %) respectively ($p < 0.05$). Significant reductions were observed in myocytes treated with GSNO at 25 and 50 ng/ml (69.00 - 97.65 %) and SNAP at 25 and 50 ng/ml (57.90 - 94.41 %) respectively ($p < 0.05$).

In the presence of the NO scavenger carboxy-PTIO, no significant differences were observed in percentage phosphorylation of IR- β (adipose, liver and muscle) or IRS-1, serine and tyrosine phosphorylation (liver and muscle), when compared to their respective unstimulated and controls (100 and 1000 nM insulin), ($p > 0.05$).

Dexamethasone did not display significant reductions in IR- β and serine phosphorylation in hepatocytes and myocytes treated with GSNO and SNAP at 25 and 50 ng/ml, ($p > 0.05$), which was expected. Significant reductions were however observed in IRS-1 and tyrosine phosphorylation in hepatocytes and myocytes treated with GSNO and SNAP at 25 and 50 ng/ml ($p < 0.05$) which also was expected.

The study confirmed a mechanism by which NO induces insulin resistance as its presence caused a significant increase in serine phosphorylation in IRS-1.

The effects of NO were confirmed by the presence of the NO scavenger, carboxy-PTIO as opposed to some non-specific mechanism utilized by the drugs themselves. The results obtained showed that NO released from SNAP and GSNO exhibited dose dependency and tissue specificity. The study confirms the effect of NO on insulin-mediated signal transduction impairment in rats and this has serious implications for persons using NO-releasing drugs. Care should be taken when prescribing NO releasing drugs to persons who are especially obese as the effects of NO were demonstrated more in adipocytes than hepatocytes and skeletal myocytes.