Online supplement to: Wenzel et al. Statin and eNOS coupling in diabetes mellitus

Extended research design and methods and supplementary results

The expression of the NADPH oxidase subunits nox1, nox2 and p67^{phox} in heart membrane fractions of STZ was increased in STZ as compared to Ctr and corrected by atorvastatin treatment (see Figure i).

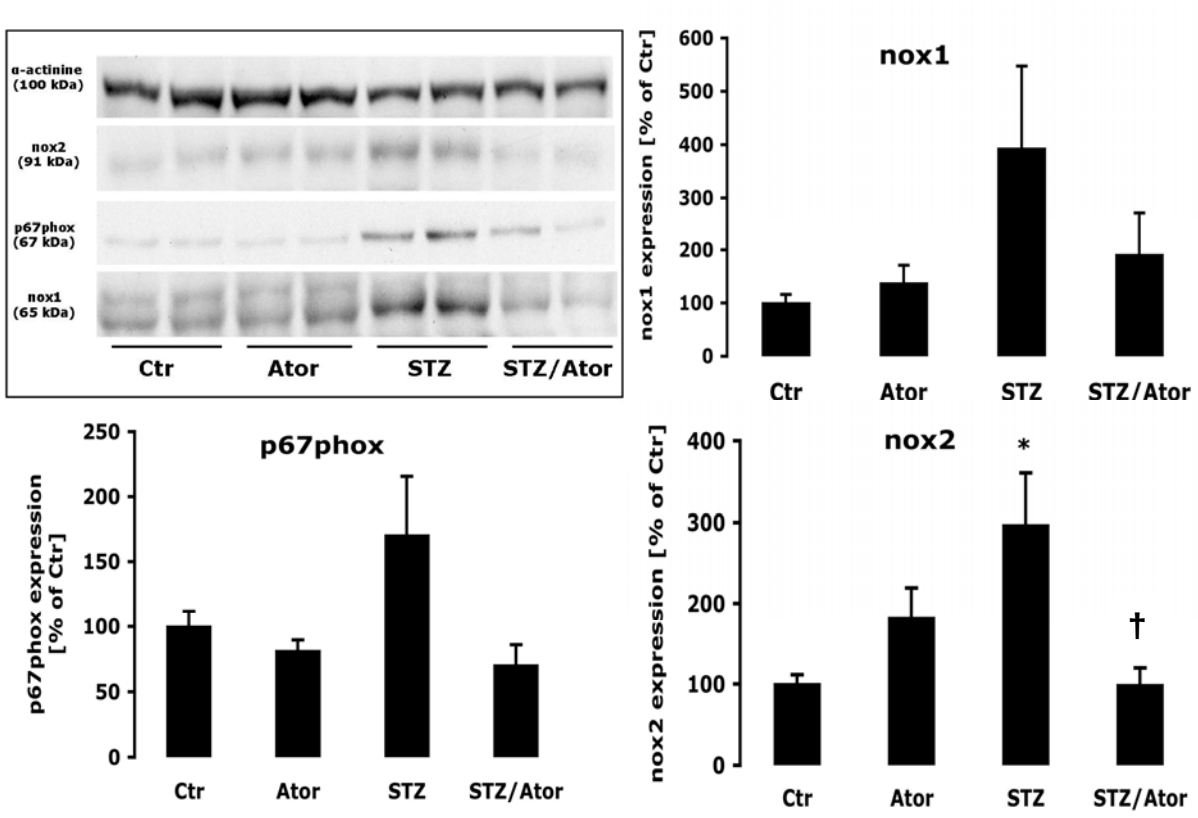

Figure i: Expression of NADPH oxidase subunits in heart membrane fractions Expression of nox1, nox2 and p67^{phox} was assessed by western blot in homogenates of heart membrane fractions. Original blots shown are representative for 6 to 8 independent experiments, data are mean ± SEM of 6-8 independent experiments. * p<0.05 vs Ctr, † p<0.05 vs STZ.

Determination of eNOS dimers and monomers: Dimers and monomers of eNOS were assayed using low-temperature SDS-PAGE as previously described [1]. Briefly, equal amount of aorta homogenate was added to fivefold Laemmli buffer (0.32 mol/l Tris-HCl, pH 6.8, 0.5 mol/l glycine, 10% SDS, 50% glycerol, and 0.03% bromophenol blue, 2.5% 2-mercaptoethanol), without boiling the sample mixture. Samples were then subjected to SDS-PAGE on 6% gels, using the Mini-Protean II

system from Bio-Rad Laboratories Inc. Gels and buffers were kept in an ice bath at 4°C. The gels were blotted onto nitrocellulose for Western blots. The nitrocellulose membranes were incubated with a monoclonal antibody against eNOS (1:1,000; Transduction Laboratories Inc.) in 5% fat-free milk for 2 hours at room temperature or overnight at 4°C. The blot was further incubated with a second horseradish peroxidase-conjugated antibody against mouse Ig G (1:7,500; Promega Corp., Madison, Wisconsin, USA) for 45 minutes at room temperature. The eNOS dimer and monomer were visualized using the ECL chemiluminescence kit (Amersham Pharmacia Biotech) according to the manufacturer's instructions.

Densitometric analysis of the recovered band revealed a descrease in the eNOS dimer fraction in STZ as compared to control (figure iii). In STZ/Ator, the eNOS dimer content was normalized.

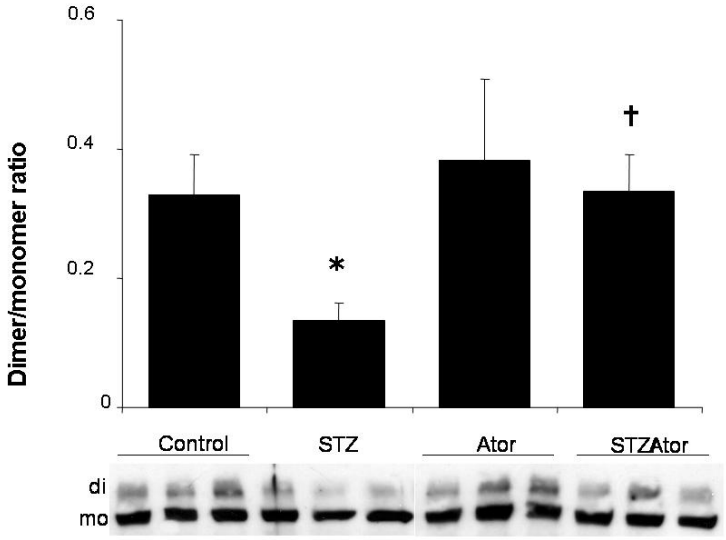

Figure ii: eNOS monomer and dimer. Levels of eNOS monomer and dimer were determined using Western blot after SDS-PAGE using a 4°C gel and non-reducing conditions. Data are mean ± SEM of 6-8 independent experiments * p< 0.05 vs Ctr; † p<0,05 vs STZ.

References

1 Zou, M. H., Shi, C. and Cohen, R. A., Oxidation of the zinc-thiolate complex and uncoupling of endothelial nitric oxide synthase by peroxynitrite, J Clin Invest, 2002, 109: 817-826.