3 and β-catenin in endothelial cells, which is increased in response to NOS-3 agonists. Here we demonstrate that such agonists cause translocation of β-catenin into the nucleus. Confluent human umbilical vein endothelial cells (HUVEC) at passage 3 were exposed to the NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 100μM) or vehicle for 30 min, followed by histamine (100μM), thrombin (1U/ml), salbutamol (1μM), adenosine (100μM), ionomycin (5μM), spermine NONOate (10μM), or corresponding vehicle, all for 2 min at 37C. Nuclear lysates were immunoblotted for β-catenin, as well as the nuclear housekeeping protein lamin A/C. Bands were analysed by scanning densitometry, and band densities compared by repeated measures one-way ANOVA. $p<0.05$ (two-tailed) was taken as significant. All data are presented as mean ± SD. Nuclear extracts exhibited an increase in nuclear β-catenin relative to lamin A/C in response to adenosine (70±37.4%), histamine (153±105%), salbutamol (90±61%), thrombin (166±128%), ionomycin (135±52.7%) and spermine NONOate (97±93%) ($p<0.01$ for each, n=4-8). Pre-incubation with L-NAME abolished the nuclear translocation of β-catenin caused by Ca^{2+}-insensitive agonists (adenosine and salbutamol), but had no effect on that caused by Ca^{2+}-sensitive agonists (histamine and thrombin), the Ca^{2+} ionophore ionomycin or the NO donor spermine NONOate. We conclude that nuclear translocation of β-catenin occurs in response to NO, either from exogenous sources or generated by NOS-3 in HUVEC. On the other hand, NOS-3 agonists which act through increasing Ca^{2+} entry into the cell induce β-catenin nuclear translocation even when NOS-3 is inhibited, suggesting that such translocation can also be induced independently via Ca^{2+} entry.

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Specific Quantitative High-throughput Methods to Detect S-nitrosation

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The biotin switch assay for detection of protein S-nitrosation has been widely used in the field of nitric oxide and redox signaling. However, there are still challenges of the current assay. We found an ascorbate-dependent artifact that interferes with the interpretation of the biotin switch assay [1]. These years, we developed a series method to make the detection of S-nitrosation simple, specific, quantitative and high-throughput [2-6]. With these approaches, the S-nitrosated proteins can be directly visualized on-gel image[2], and the combined LC/MS performance was greatly improved [3]. We also developed ESNOQ (Endogenous SNO Quantification) method to detect endogenous S-nitrosation in cell system and ICAT switch method to quantify S-nitrosation in tissue samples [4,5]. Recently we found that there is experimental and theoretical interference of intermolecular disulfide bonds in S-nitrosated protein identification: proteins linked to S-nitrosated proteins by intermolecular disulfide bonds can be falsely detected as S-nitrosated targets. Then we developed irreversible biotinylation procedures (IBP) to prevent this interference [6]. Based on the above developed methods, we are continuing to study the network of S-nitrosation in diseases.

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A Modified Method for Accurate Nitrite Detection in Erythrocytes Using Reductive Chemi-luminescence

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Nitrite's importance in health, disease and therapeutics continues to grow. However, the levels of basal nitrite reported in whole blood versus that in plasma remain the subject of some debate.[1,2,3] Using a method that employs a nitrite preservation solution, consisting of potassium ferricyanide and n-ethylmaleimide, the levels of nitrite in the plasma were reported to be 121 ± 9 nM whereas those in red cells were reported to be 288 ± 47 nM.[2] We hypothesized that this result may be due to detection of erythrocytic iron nitrosyl hemoglobin (HbNO) that is recorded as nitrite. The ferricyanide is used to oxidize ferrous hemoglobin that can react with nitrite, forming methemoglobin which does not scavenge nitrite. However, it is possible that NO released from HbNO is oxidized to nitrite, probably via reductive nitrosylation, thereby increasing recorded nitrite levels in the red blood cell. To test this hypothesis, we carried out the nitrite preservation method on samples containing substantial HbNO using reductive chemiluminescence assays. When using the level of ferricyanide previously employed, we do detect slightly excess nitrite (22 ± 15 μM) presumably derived from HbNO (220 ± 20 μM), but this is probably not enough to account for excess nitrite detected in red cells. However, we find that using less ferricyanide can still oxidize the ferrous hemoglobin that is not bound to NO, but leaves HbNO intact and thereby does not lead to the artifact. Work is ongoing to see if the detection of HbNO-derived nitrite also occurs in whole preparations, and (if so) if modifying the method by using less ferricyanide this can be eliminated.

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Isolation, Characterization, and Activity of an Endothelilal Nitric Oxide Sythase in Human Red Blood Cells

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It has been shown that red blood cells (RBC) express a nitric oxide synthase (NOS), and release NO metabolites in a NOS-dependent fashion. The biochemical characteritics (isoform and activity) of this RBC-NOS have not been investigated so far. The aim of this study was to isolate and characterize the RBC-NOS for sequence, in vitro activity and ability to produce NO within RBC. We isolated a NOS protein under native conditions by immunoprecipitation with magnetic microbeads using anti human-NOS3 antibodies. The success of immunoprecipitation was verified by western blot. Electron spray ionization/mass spectrometric analysis revealed that the immunoprecipitated protein is an endothelilal nitric oxide synthase (NOS3 type 1). The isolated protein was active as measured by the conversion L-^{3}H-Arginine into L-^{3}H-Citrullin. The activity could be inhibited by adding the NOS inhibitors L-NAME or L-NIO, and was dependent on the presence of Ca^{2+}/Calmodulin in the reaction buffer. We also found a NOS-dependent intracellular NO production within