EuroSciCon
NO news is good news – Detection, Measurements
and Quantification of Nitric Oxide: Tools for NO Research
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NO QUANTIFICATION IN ABNORMAL AND MANIPULATED RED BLOOD CELLS

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OBJECTIVE

The aim of this study was to access the *ex vivo* response of NO translocation in abnormal red blood cells after ACh 10 mM stimulation and to know if this action could be related to intraglobular nitrosylated molecules and phosphorylated/dephosphorylated protein band 3 by, tyrosine kinase and phosphatase proteins, respectively.

The Hypothesis of AChE / Band 3 Mediated Nitric Oxide Erythrocyte Mobilization

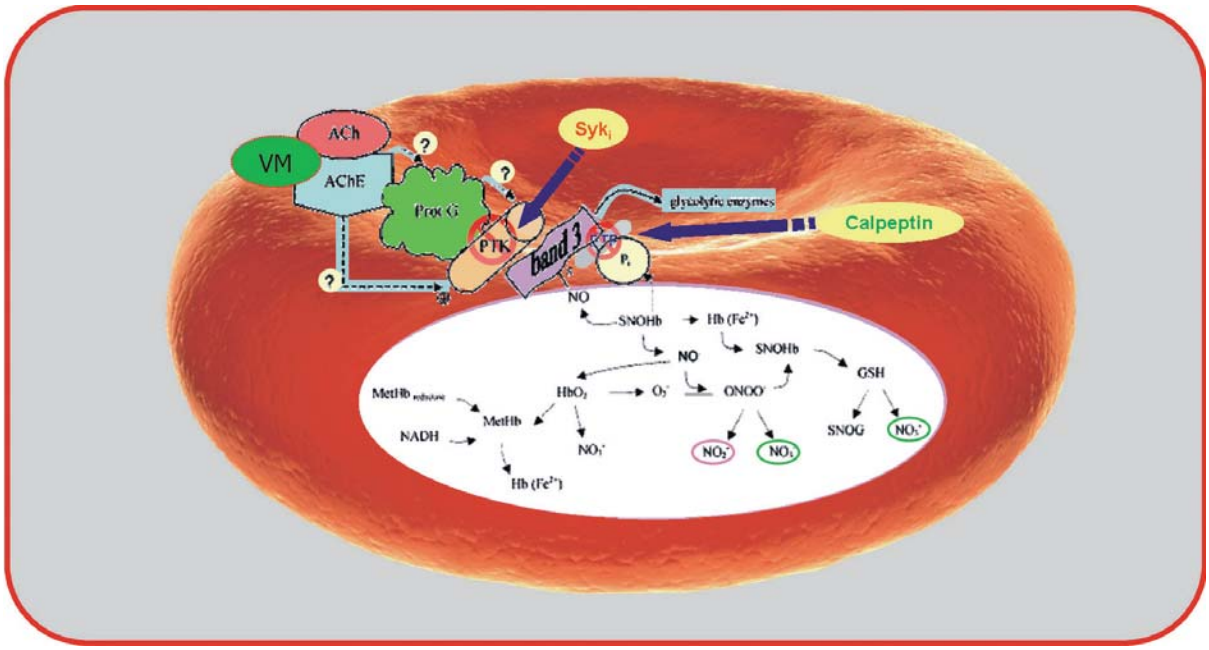

Figure 1. An hypothesis for acetylcholinesterase role on signal transduction mechanism in response to action of ACh or VM on NO production (and its metabolites) in human erythrocytes suspensions. Transnitrosilation process between phosphorylated / dephosphorylated band 3 and SNOHb could be associated to an unidentified mechanism mediated by formation of AChE-ACh (more active) or AChE-VM (less active) complexes, when PTK or PTP enzymatic activity is inhibited. This process could also be dependent of a G protein. **Abbreviations:** ACh (acetylcholine); VM (velnacrine maleate); AChE (acetylcholinesterase); Prot G (G protein); PTK (protein tyrosine kinase); PTP (protein tyrosine phosphatase); Pi (phosphate); NADH (nicotinamide adenine dinucleotide, reduced); Hb (hemoglobin); SNOHb (S-nitrosohemoglobin); NO (nitric oxide); O2.- (anion peroxide); ONOO- (peroxinitrite); NO3.- (nitrate); NO2.- (nitrite); GSH (glutatione reductase); SNOG (S-nitrosothiol); HbO2 (oxyhemoglobin); MetHb (methemoglobin); Calpeptin (PTP inhibitor) Syki (PTK inhibitor). (Carvalho F.A. *et al* (2004) *J. Appl. Toxicol.* 24: 419-427)

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EXPERIMENTAL DESIGN

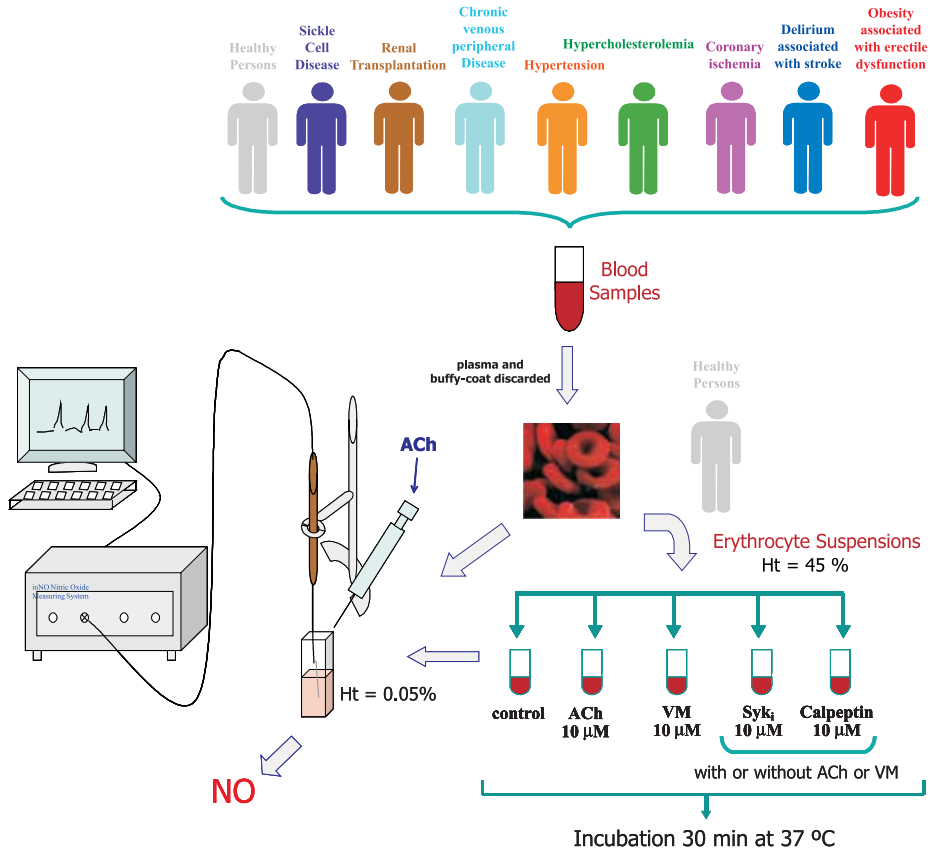

Figure 2. Venous blood samples were collected from healthy humans (n=27) and patients with sickle cell disease (n=9), renal transplantation (n=36), chronic venous peripheral disease (n=22), arterial hypertension (n=20), hypercholesterolemia (n=102), coronary ischemia (n=34), delirium associated with stroke (n=115) and obesity associated with erectile dysfunction (n=51). Erythrocyte suspensions from healthy humans were performed with addition of sodium chloride 0,9%, pH 7,4, to reconstitute the initial hematocrit (Ht, 45 %). Aliquots were incubated 30 minutes at 37ºC, with AChE effectors (either with ACh 10 mM or VM (acetylcholinesterase inhibitor, 10 mM)), in the presence and absence of p72syk inhibitor 10 mM and PTP inhibitor (calpeptin 10 mM). For amperometric NO quantification we used an amiNO-700 sensor (*Innovative Instruments Inc. FL, USA*). Erythrocyte suspensions (Ht= 0.05%) were incubated for 15 minutes (room temperature) and stimulated with ACh 10 mM for monitorized erythrocytic nitric oxide mobilization.

RESULTS

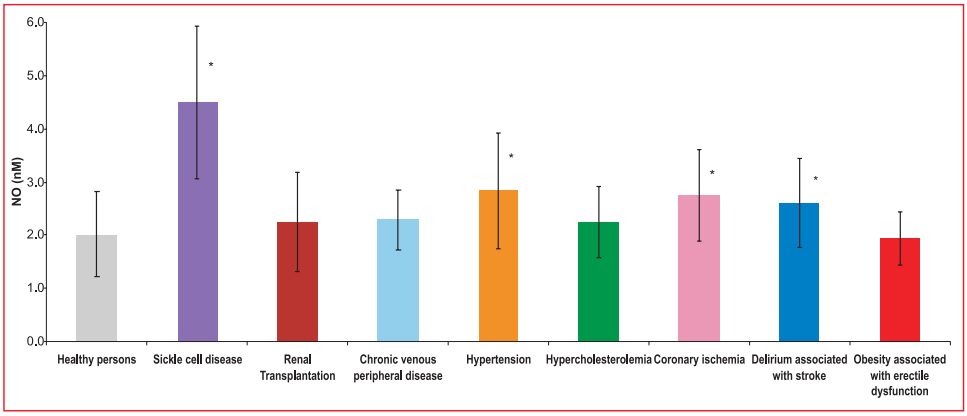

Figure 3. Changes on NO mobilization from erythrocytes suspensions incubated with ACh 10mM of control (healthy persons) and patients with sickle cell disease, renal transplantation, chronic venous peripheral disease, arterial hypertension, hypercholesterolemia, coronary ischemia, delirium associated with stroke and obesity associated with erectile dysfunction. Values are in mean ± SD.
* P < 0.001 for sickle cell disease, coronary ischemia and delirium;
* P = 0.005 for arterial hypertension

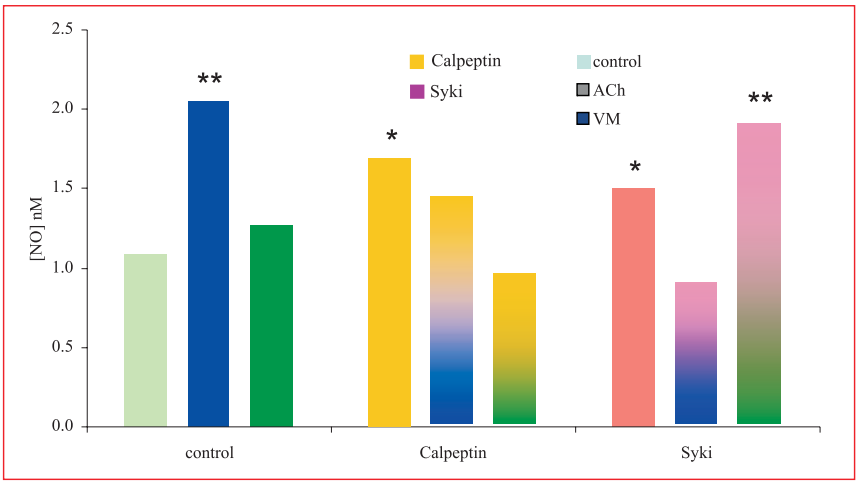

Figure 4. Changes of *in vitro* NO mobilization, after incubation of erythrocytes suspensions with ACh 10 mM, VM 10 mM, and in the absence or presence of Syk inhibitor (Syki) 10 mM, and calpeptin 10 mM.
Values are in mean, n = 4. Standard deviation values were not shown because is just about the same for every erythrocyte suspension.
* $P < 0.01$;
** $P < 0.001$

CONCLUSIONS

Abnormal Erythrocytes

- **In all pathologies studied -** Tendency to ⇑ NO mobilization
- **Highest NO mobilization –** Sickle cell disease
- **Significant NO increase:**
  - arterial hypertension
  - coronary ischemia
  - delirium associated with stroke

Manipulated Erythrocytes

- **ACh -** Significant ⇑ NO translocation
- **VM -** ⇑ NO not significant
- **Syk inhibitor and calpeptin -** Significant ⇑ NO translocation
  - with ACh - NO ⇓
  - with VM (only with syk_i) - NO significant ⇑

Human abnormal erythrocytes (microcirculatory diseases) have diverse physiological answers to ACh stimulation ⇒ Δ NO mobilization mechanisms

Manipulated erythrocytes - changes in band 3 protein phosphorylation/dephosphorylation - different NO translocation in presence of AChE substrate/inhibitor

⇓

Key-role of AChE effectors - associated band 3 phosphorylation status pathway in mobilization of erythrocyte NO stores