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THE INFLUENCE OF ERYTHROCYTE ACETYLCHOLINESTERASE EFFECTORS IN THE BAND 3-DEPENDENT MOBILIZATION OF INTRACELLULAR NITRIC OXIDE STORES

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OBJECTIVE

The aim of this work was to assess the signal transduction pathway that leads to the nitric oxide translocation between intraglobular nitrosylated molecules and phosphorylated/dephosphorylated protein band 3 by tyrosine kinase and tyrosine phophatase proteins, correspondingly, in the presence of different acetylcholinesterase enzymatic complexes.

INTRODUCTION

- The machinery of release of nitric oxide (NO) bound particles by red blood cells is poorly settled hitherto, though it is purpose of numerous ongoing researches.
- Despite the fact that the role of the erythrocyte membrane-bound acetylcholinesterase (AChE) is not well-defined, the occurrence of more active (acetylcholine-AChE) *versus* less active (velnacrine-AChE) enzymatic complexes is already documented. Acetylcholine (ACh), its natural substrate, is capable of prompting an increase in nitric oxide (NO) concentration, as well as its oxidation metabolites (nitrites and nitrates, NOx), in red cell suspensions. The opposite is verified with velnacrine maleate (VM), an AChE inhibitor.
- A plausible hypothesis to explain this mechanism is based in the NO translocation among nitrosylated molecules and phosphorylated/dephosphorylated band 3 protein, by protein tyrosine-kinases (PTK: p72syk and p53/56lyn) and phosphotyrosine-phosphatases (PTP), respectively.

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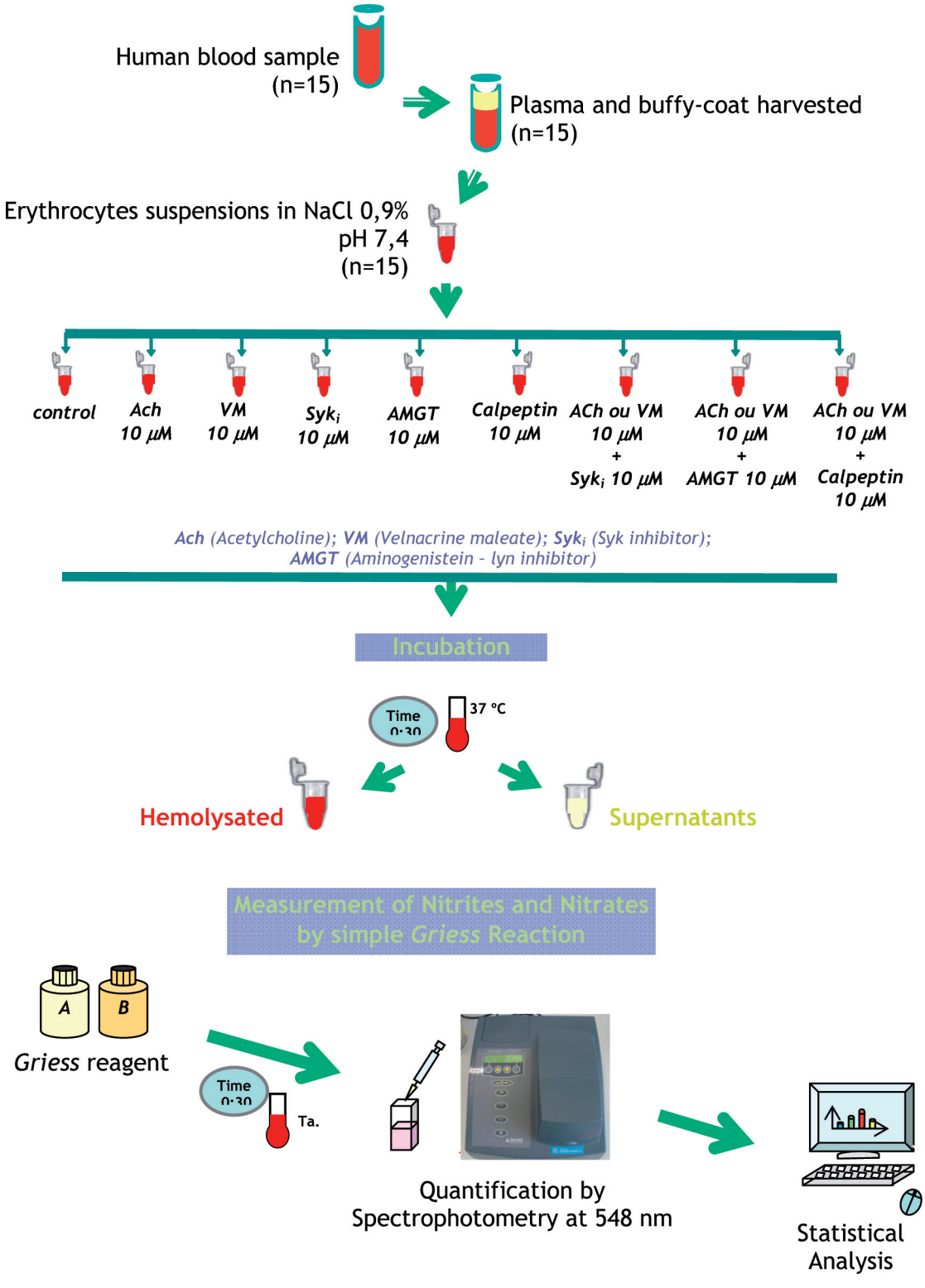
EXPERIMENTAL DESIGN



RESULTS

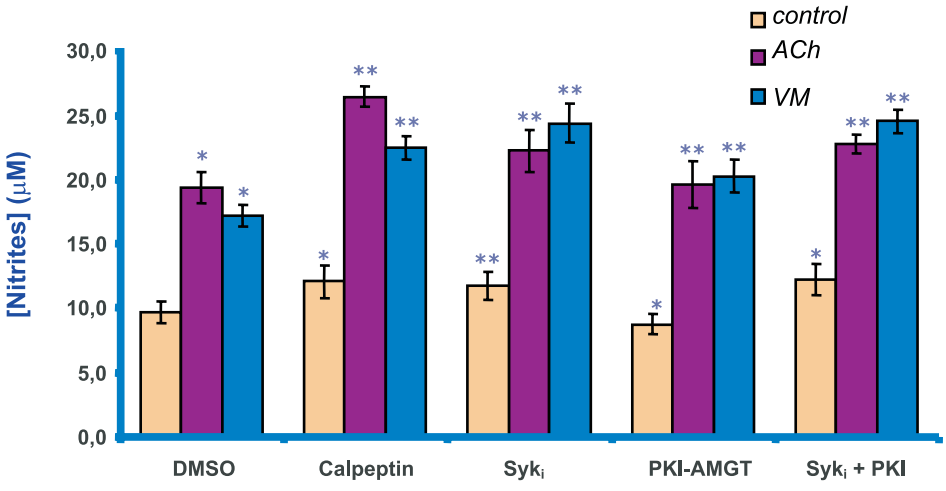


Figure 1. Changes on the *in vitro* nitrites concentration, after erythrocytes suspensions' incubation with ACh 10 mM, VM 10 mM, in the presence or absence of Syk inhibitor (Syk$_i$) 10 mM, AMGT 10 mM and calpeptin 10 mM.
Values are in mean ± SD, n = 15.
* $p<10^{-5}$; ** $p<10^{-9}$

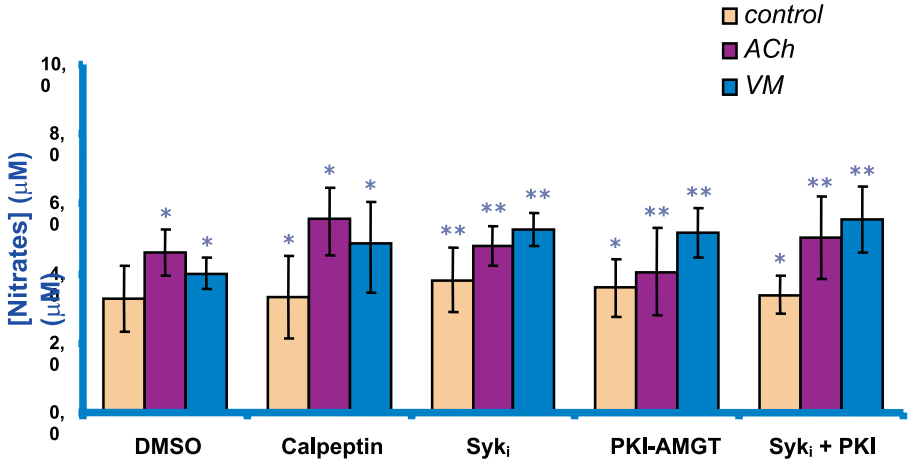
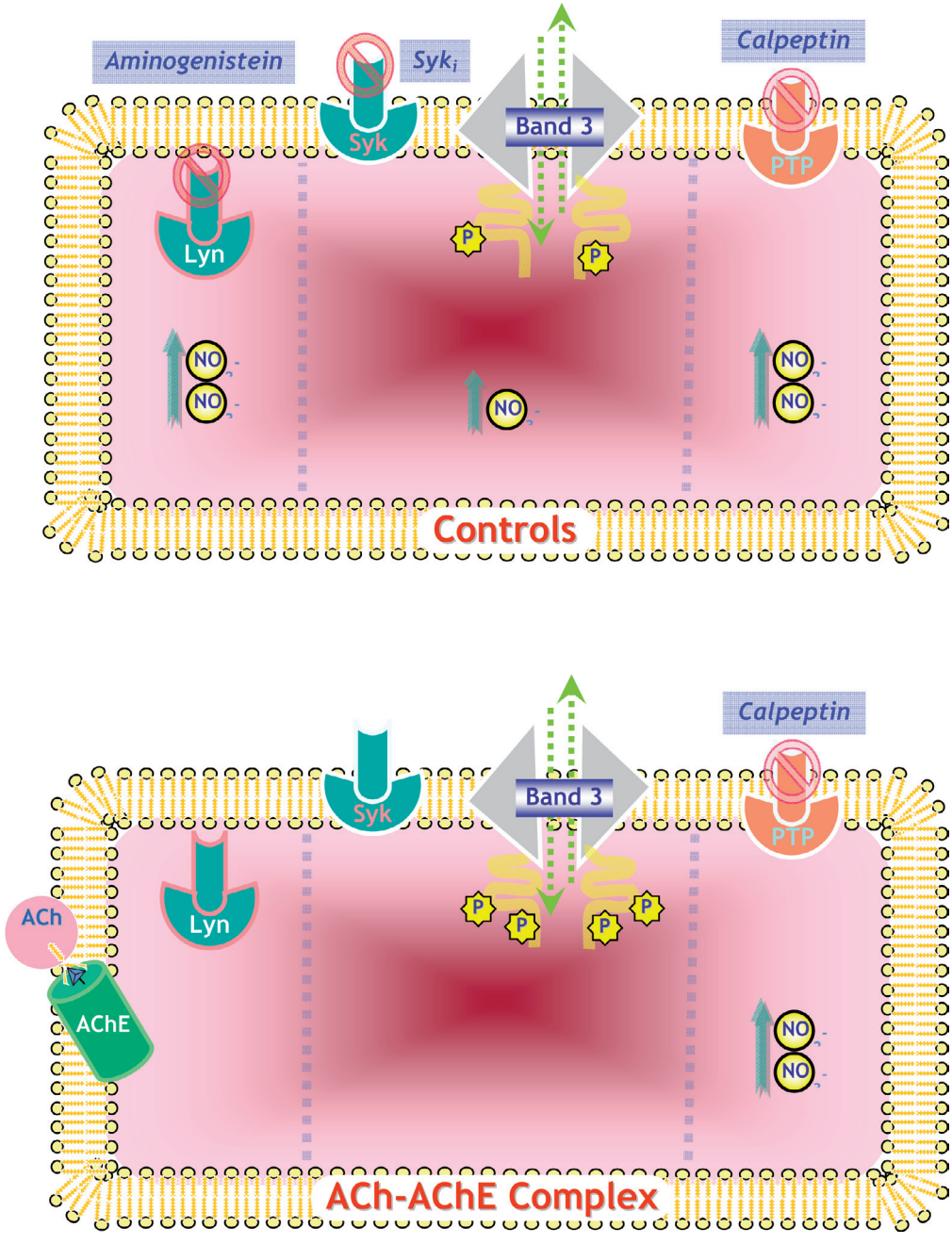
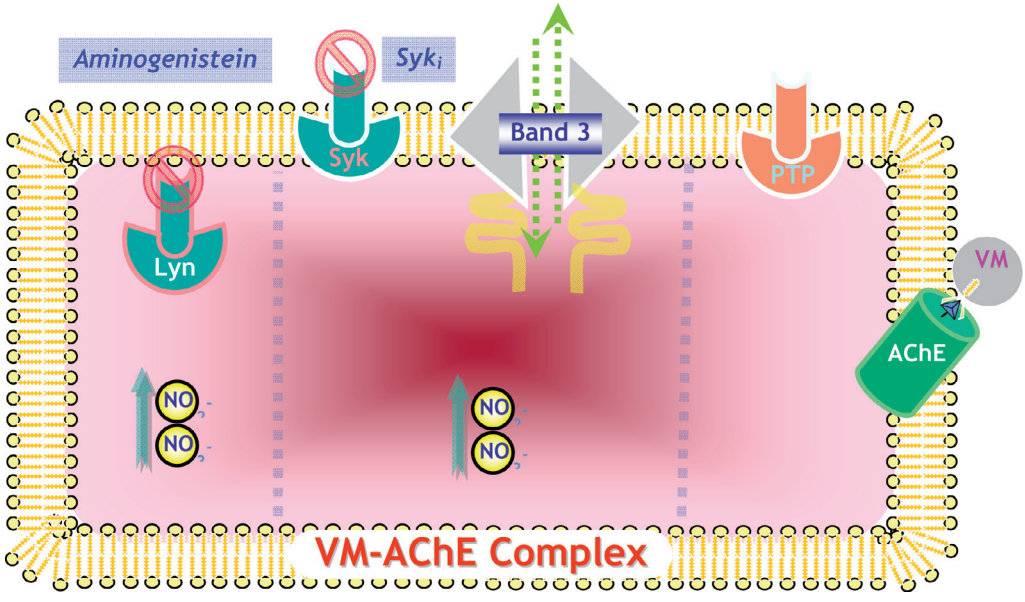


Figure 2. Changes on *in vitro* nitrates concentration, after erythrocytes suspensions' incubation with ACh 10 mM, VM 10 mM, and in the absence or presence of Syk inhibitor (Syk$_i$) 10 mM, AMGT 10 mM and calpeptin 10 mM.
Values are in mean ± SD, n = 15.
* p<0.001 ; ** p<0.0001


Aminogenistein
Syk
Band 3
Calpeptin
PTP
Lyn
P
NO
Controls
ACh
AChE
ACh-AChE Complex



- **Controls**

  – Both ACh and VM-stimulated erythrocytes significantly enhanced NO_x production;
  – Lyn and PTP enzymes inhibition increased NO^{2-}/NO^{3-} levels, while the presence of Sik_i increased only nitrates.

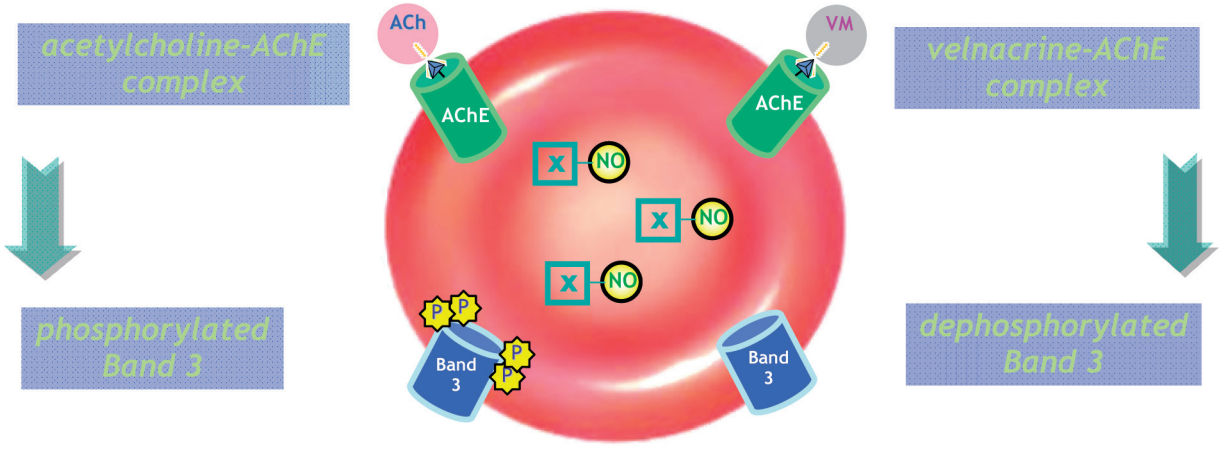
- **ACh-AChE complex**

  – Highest NO_x levels were seen in the presence of calpeptin, being band 3 totally phosphorylated.

- **ACh-AChE complex**

  – Highest NO_x levels were seen in the presence of tyrosine-kinase inhibitors, being band 3 partially or totally dephosphorylated.

CONCLUSIONS



- Both AChE and Band 3 modulators enhance intraglobular NO_x mobilization.
- Nitrites and nitrates levels depend on band 3 phosphorylated/dephosphorylated status.
- The less active AChE complex is associated with dephosphorylated band 3 protein.
- The more active AChE complex is associated with phosphorylated band 3 protein.

The study strongly suggests a band 3-dependent modulation of intracellular stockpile of nitric oxide mobilization.

The results could provide further insights regarding the dynamics of erythrocyte-dependent physiopathological events into the bloodstream.