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EFFECTS OF ACETYLCHOLINE STIMULATION ON ERYTHROCYTES.
NITRIC OXIDE MOBILIZATION THROUGH PHOSPHORYLATION / DEPHOSPHORYLATION OF BAND 3 PROTEIN

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OBJECTIVE

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

THE HYPOTHESIS OF AChE / BAND 3 MEDIATED NITRIC OXIDE ERYTHROCYTE MOBILIZATION

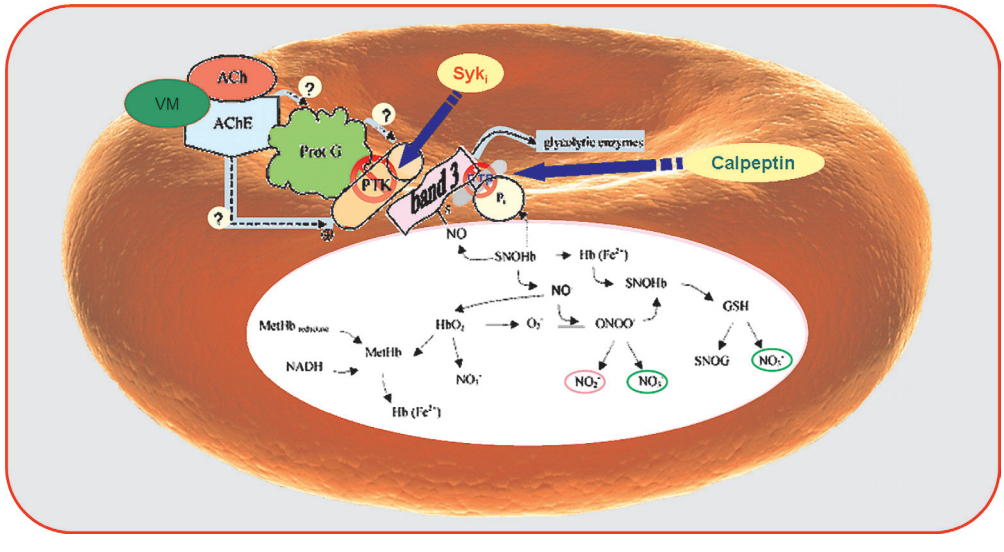
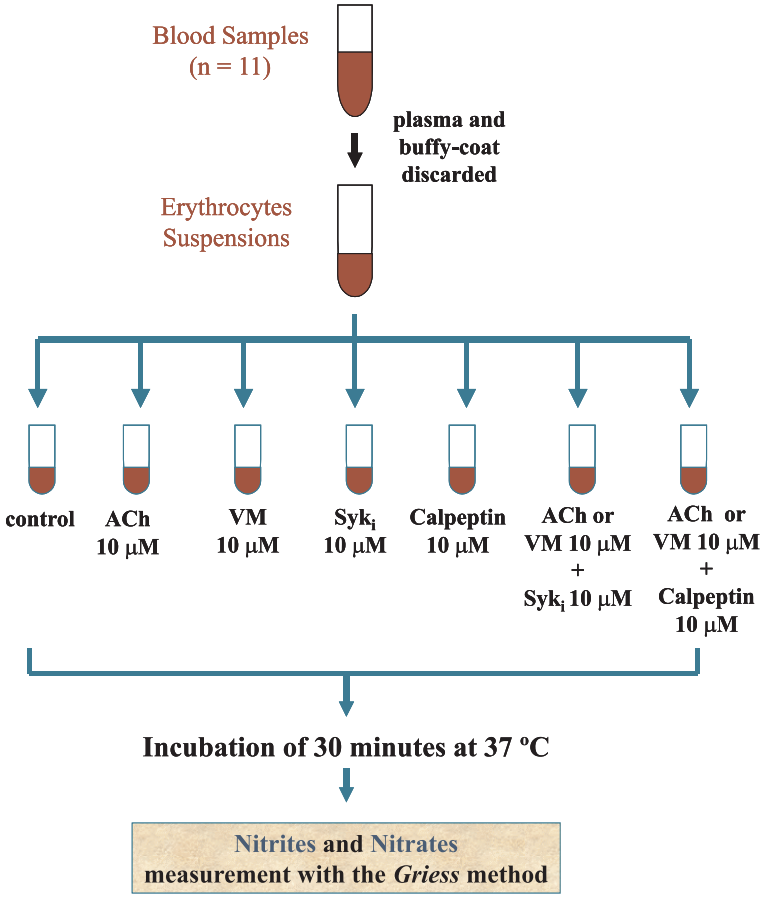


Figure 1: An hypothesis for the possible acetylcholinesterase role on the signal transduction mechanism in response to the action of acetylcholine or velnacrine maleate on nitrites and nitrates production of human erythrocytes suspensions. The transnitrosilation process between phosphorylated / dephosphorylated band 3 and SNOHb could be associated to an unidentified mechanism mediated by the 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 on a protein G.
Abbreviations: ACh (acetylcholine); VM (velnacrine maleate); AChE (acetylcholinesterase); Prot G (protein G); 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)]**

EXPERIMENTAL DESIGN



RESULTS

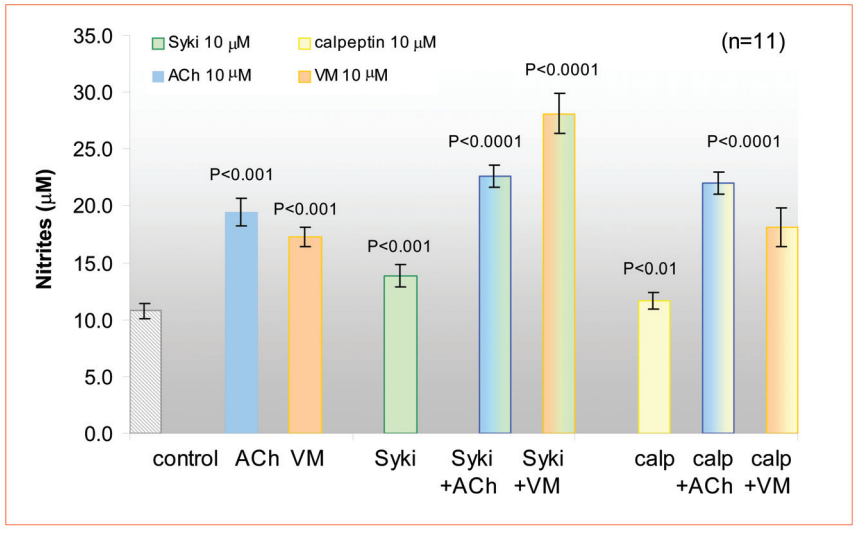


Figure 2: Changes on *in vitro* nitrites erythrocytes concentration when the erythrocytes suspensions were incubated with ACh 10 mM, VM 10 mM, and in the absence or presence of Syk inhibitor (Syki) 10 mM and calpeptin 10 mM. Values are in mean ± SD, n = 11.

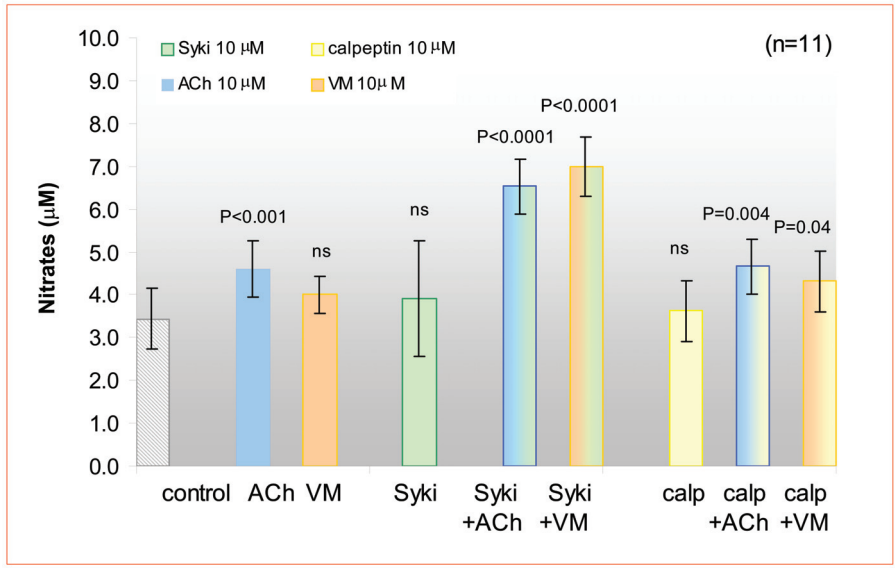


Figure 3: Changes on *in vitro* nitrates erythrocytes concentration when the erythrocytes suspensions were incubated with ACh 10 mM, VM 10 mM, and in the absence or presence of Syk inhibitor (Syki) 10 mM and calpeptin 10 mM. Values are in mean ± SD, n = 11.

CONCLUSIONS

- **ACh** increased the erythrocytes NOx production;
- **VM** significantly decreased the NOx production when erythrocytes were stimulated with ACh;
- **Calpeptin:** ⇑ the NOx values in the presence of AChE-ACh complex
  ⇑ the NOx values in the presence of AChE-VM complex
- **Syk inhibitor:** ⇑ the NOx values in the presence of the AChE-ACh complex
  ⇑ ⇑ the NOx values in the presence of AChE-VM complex

The less active AChE (in presence of VM) could be associated with dephosphorylated protein band 3 (in the presence of PTK inhibitors)

The more active AChE (in presence of ACh) could be related with phosphorylated protein band 3 (in the presence of PTP inhibitors)