RESUMOS DOS TRABALHOS APRESENTADOS PELOS MEMBROS DA SPHM PRESENTES NA REUNIÃO CONJUNTA / *13TH INTERNATIONAL BIORHEOLOGY CONGRESS AND THE 6TH INTERNATIONAL CONFERENCE ON CLINICAL HEMORHEOLOGY, STATE COLLEGE, PENSILVÂNIA, 7-13 JULHO DE 2008*

ERYTHROCYTE NITRIC OXIDE MOBILIZATION

C. Saldanha

Nitric oxide (NO) released from endothelial cells and platelets is scavenged by erythrocytes via binding to the thiol groups of glutathione and formation of S-nitrosated hemoglobin (SNO-Hb). This process promotes the trans-nitrosation of NO to protein band 3, further allowing its escape from erythrocytes.

Acetylcholine is an endogenous compound with vasoactive properties that is present in circulating blood, and hence is part of the non-neuronal cholinergic system (NNCS). We have observed that acetylcholine is able to induce: (i) NO efflux and mobilization inside the erythrocyte; (ii) an increased of erythrocyte deformability and of NO_2^-, NO_3^- levels; (iii) a decrease of erythrocyte aggregation; (iv)only very minor alterations of basal peroxynitrite levels. We have determined that NO is strongly mobilized inside erythrocytes, with much less released to the extracellular medium due to the presence of dithiotreitol compared to the release induced by acetylcholine. Reduced glutathione concentration is not modified when dithiotreitol or acetylcholine are present. The trans-nitrosation process involving protein band 3 and SNO-Hb may be associated with the acetylcholinesterase-acetylcholine (AChE-ACh) complex, with participation by a G protein related to the degree of phosphorylation of band 3 as influenced by PTK and PTP. Modulation of protein band 3 phosphorylation states via PTK p53/56^{lyn} inhibitor and PTP inhibitor (calpeptin) does not affect erythrocyte deformability. However, erythrocyte aggregation increased when band 3 protein is phosphorylated and decreased when it is de-phosphorylated. Our results indicate that in presence of calpeptin and acetylcholine, protein band 3 is totally phosphorylated and NO levels are increased. Both active and less-active enzyme AChE complexes do not abolish the de-phosphorylation state of band 3 induced by protein tyrosine kinase inhibitors. We have also determined that the G protein sub-unit $G_{\alpha i1/2}$ is linked with protein band 3 at N and C-terminal sites, that the G_{β} sub-unit is associated with protein band 3 at the C-terminal domain, and that the G protein-protein band 3 associations are independent of the degree of protein band 3 phosphorylation.

Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Av. Prof Egas Moniz. 1649-028 Lisboa, Portugal