

RESUMEN EN INGLÉS

The hydrogen peroxide (H_2O_2) is one of the most important reactive oxygen species (ROS). It is involved in multitude of intracellular process such as angiogenesis, apoptosis, cell survival, hyperplasia, atherosclerosis, cellular contraction and cellular relaxation. Some of these process, the roll of the H_2O_2 is well known however in other the effect of the H_2O_2 remains unknown. Throughout this thesis, analyzed the effect of the H_2O_2 in the contraction and relaxation process, in specify the *myo*-inositol 1,4,5-trisphosphate (IP_3) pathway and the cyclic guanilate monophosphate (cGMP) pathway respectively. As well, we studied the hydrogen peroxide in the hypertension mediated by L-NAME.

Stimulation of cell surface receptors results in the formation of the second messenger IP_3 via the activation of phospholipase C. IP_3 mobilizes intracellular calcium by binding to a family of receptors (IP_3Rs) that act as ligand-gated calcium channels. IP_3Rs are tetramers, and full-length sequences of at least three different isoforms have been identified by molecular cloning. Both homo- and heterotetramers are found in cells expressing more than one isoform. The acute regulation of IP_3Rs occurs primarily through feedback effects of cytosolic Ca^{2+} and/or by phosphorylation of the receptor. However, prolonged exposure of cells to agonists has also been shown to alter the expression of IP_3R protein levels. In the present study, we analyzed the effect of H_2O_2 in IP_3R level. Chronic stimulation of VSMC by H_2O_2 resulted in the down-regulation of both type I and type III *myo*-inositol 1,4,5-trisphosphate receptors (IP_3Rs). H_2O_2 -induced down-regulation of IP_3Rs could be detected within 4 h and resulted in an inhibition of IP_3 -induced Ca^{2+} release from permeabilized cells. The proteasomal inhibitor *MG132* completely prevented H_2O_2 -mediated down-regulation of IP_3Rs . However, the stimulation with H_2O_2 did not increase the amount of IP_3R immunoprecipitated by anti-ubiquitin antibodies. On the other hand, we analyzed the roll of the H_2O_2 in the ANGII- induced down regulation of IP_3Rs . The NAD(P)H inhibitors did not block the ANGII- induced down regulation in the long-term stimulation, however the catalase prevented the down-regualtion in the acute stimulation with ANGII. We conclude that H_2O_2 stimulated IP_3R degradation involves enhanced degradation by the proteasome pathway, but independent of the ubiquitination process; and the H_2O_2 generation is necessary in the ANGII- induced down regulation.

Cardiovascular disorders are characterized by impaired vasodilatory responses after acetylcholine administration. This abnormal response has been usually termed endothelial dysfunction, and is considered to be one of the main pathogenic mechanisms

responsible for the abnormal hemodynamic status of patients with hypertension, diabetes or atherosclerosis. Acetylcholine induces the synthesis of nitric oxide (NO) by endothelial cells, and a decreased synthesis of NO or an increased inactivation of this molecule has been proposed as the main cause of endothelial dysfunction. However, alternative mechanisms may also be proposed. NO induces cell relaxation by interacting with its intracellular receptor, soluble guanylate cyclase (sGC), which leads to an increased intracellular cyclic guanosine monophosphate (cGMP) concentration. Hence a reduction in sGC content or an abnormal response of the enzyme after its interaction with NO could also be involved in the previously mentioned vascular dysfunction. Some studies have shown a decreased sGC content in the vascular walls of animals with experimental hypertension or atherosclerosis. The sGC deficiency seems to be a more generalized phenomenon, since an attenuated glomerular cGMP production and renal vasodilation in streptozotocin-induced diabetic rats has also been demonstrated.

In the present study, we propose that the H₂O₂ might modulate the sGC content in contractile cells. For that purpose, we tested the effect of this compound on the sGC content of cultured vascular smooth muscle cells (VSMC). We observed an increased in the β 1 subunit levels of sGC, while the α 1 subunit remained without changes. This change in the β 1 subunit was accompanied with an increased in its activity, measuring like cGMP production and phosphorylation of VASP. By other hand, the H₂O₂ stimulation did not modify the β 1 subunit expression, analyzed by luciferasa activity. This suggests the possibility H₂O₂-mediated up regulation of the β 1 subunit is involved an increased of the mRNA stability, or a decreased of the degradation rate by the 26S proteasome.

Finally, we have examined the effect of endogenously produced H₂O₂ on BP using a transgenic mouse model that overexpresses catalase. We observed that the systolic blood pressure (SBP) of the transgenic mice (CAT) is similar to that of their wild-type littermates (WT). However, CAT mice showed a significantly reduced pressor response to L-NAME when compared to wild-type control mice. In addition, we observed that L-NAME significantly increased oxidative damage obtained from wild-type mice but did not alter from CAT mice. These results suggest that induction of H₂O₂ in the arterial wall is a mechanism by which L-NAME increase blood pressure.