

Adachi T, Weisbrod RM, Pimentel D, Ying J, Sharov VS, Schoneich C, Cohen RA. S-Glutathiolation by peroxynitrite activates SERCA during arterial relaxation by nitric oxide. A mechanism targeted by oxidants in vascular disease. *Nat Med* 2004; 10: 1200-1207.

Lay summary: This paper describes a new mechanism by which nitric oxide causes vasodilation and identifies how vascular disease interferes with that mechanism. Nitric oxide reacts with low levels of cellular oxidants to produce a more reactive chemical, peroxynitrite. In turn, peroxynitrite reacts with cysteine amino acid groups on the protein, SERCA¹, and a cellular antioxidant, glutathione. The glutathiolation of SERCA stimulates its activity which lowers cell calcium levels and results in relaxation. This is a surprising finding because previously, peroxynitrite was thought to be a harmful oxidant associated with diseases like atherosclerosis and Alzheimer's disease. The authors showed that glutathiolation of SERCA appeared in vascular smooth muscle during normal endothelium-mediated relaxation. They identified one cysteine which normally was most reactive with peroxynitrite and glutathione, and showed that if this cysteine was eliminated from SERCA, nitric oxide no longer increased SERCA's activity. Diseased arteries do not vasodilate normally and nitric oxide does not stimulate SERCA as well. The authors found that the important cysteine was irreversibly oxidized. This is evidence that high levels of oxidants produced in diseased vascular cells adversely affect the SERCA protein function by oxidizing cysteines that normally are involved in its physiological regulation by lower levels of oxidants. The research was supported by NIH and NHLBI grants and contracts including the Boston University- and NHLBI-funded Cardiovascular Proteomics Center, part of the NHLBI Proteomics Initiative of the National Heart, Lung, and Blood Institute, National Institutes of Health, under Contract No. N01-HV-28179. As a focus of the Center, oxidative modifications² of proteins are being studied to better understand normal protein function, as well as mechanisms of, and markers for, disease.

Abstract

Nitric oxide (NO) physiologically stimulates the sarco/endoplasmic reticulum calcium (Ca²⁺) ATPase (SERCA) to decrease intracellular Ca²⁺ concentration and relax cardiac, skeletal and vascular smooth muscle. Here, we show that NO-derived peroxynitrite (ONOO⁻) directly increases SERCA activity by S-glutathiolation and that this modification of SERCA is blocked by irreversible oxidation of the relevant cysteine thiols during atherosclerosis. Purified SERCA was S-glutathiolated by ONOO⁻ and the increase in Ca²⁺-uptake activity of SERCA reconstituted in phospholipid vesicles required the presence of glutathione. Mutation of the SERCA-reactive Cys674 to serine abolished these effects. Because superoxide scavengers decreased S-glutathiolation of

¹ SERCA, sarco/endoplasmic reticulum calcium (Ca²⁺) ATPase, a protein that pumps calcium into cell storage

² Oxidative modification, a chemical alteration of a protein that happens during oxidant stress, a situation in which cellular oxygen balance is awry either from production of too many oxygen radicals or from not enough antioxidant mechanisms taking place

SERCA and arterial relaxation by NO, ONOO⁻ is implicated as the intracellular mediator. NO-dependent relaxation as well as S-glutathiolation and activation of SERCA were decreased by atherosclerosis and Cys674 was found to be oxidized to sulfonic acid. Thus, irreversible oxidation of key thiol(s) in disease impairs NO-induced relaxation by preventing reversible S-glutathiolation and activation of SERCA by NO/ONOO⁻.