Kuster GM, Pimentel DR, Adachi T, Ido Y, Brenner DA, Cohen RA, Liao R, Siwik DA, Colucci WS. α-adrenergic receptor-stimulated hypertrophy in adult rat ventricular myocytes is mediated via thioredoxin-1-sensitive oxidative modification of thiols on Ras. *Circulation*, in press. Epub ahead of print Feb. 22, 2005.

Lay summary:

Background: **Enlargement (or hypertrophy) of the heart muscle is a disease process underlying heart failure. Although reactive oxygen species (ROS) are known to be involved in this process, relatively little is known about precisely how ROS -- highly reactive molecules that arise from incomplete chemical reaction of oxygen -- activate the pathways that direct cardiac myocytes to enlarge. ROS can cause tissue damage but also are involved in the regulation of cell growth, consequently they are important for the study of biochemical processes in normal and diseased cells. Stimulation of the α-adrenergic receptor causes hypertrophy in adult rat cardiac myocytes in culture, thus providing a useful model to study the cellular mechanisms of cardiac hypertrophy. A small protein, Ras, that contains sulfur molecules (thiols), is known to be activated following α-adrenergic receptor stimulation and to mediate molecular signaling events leading to hypertrophy. Ras was studied to understand the precise mechanisms of ROS interactions with its thiols.**

Findings: **In this research, the authors found that α-adrenergic receptor stimulation produces ROS that modify thiols of Ras. In chemical terms, this is known as an "oxidative modification" of Ras thiols. Furthermore, they found that the chemical reaction of ROS with Ras thiols can be prevented if the endogenous level of thioredoxin-1, a protein capable of protecting protein thiols in the cell, is increased by viral gene transfer. It was further shown that preventing the oxidative modification of Ras thiols also inhibited α-adrenergic receptor-stimulated hypertrophy.**

***Importance for public health*: This is the first direct demonstration that an oxidative modification of a small signaling protein, Ras, mediates myocyte hypertrophy. These observations show how those ubiquitous ROS molecules that we get from incomplete cellular processes can interact with protein thiols in a damaging way, playing an important role in cardiac disease. Furthermore, these observations raise the possibility that by knowing the way in which the interaction takes place, new treatments may be developed to protect thiols from oxidative modification.**

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Abstract

Background: Alpha-adrenergic receptor (αAR)-stimulated hypertrophy in adult rat ventricular myocytes (ARVM) is mediated by reactive oxygen species (ROS)-dependent activation of the Ras-Raf-MEK1/2-ERK1/2 signaling pathway. Since Ras is known to have redox-sensitive cysteine residues, we tested the hypothesis that αAR-stimulated hypertrophic signaling is mediated via oxidative modification of Ras thiols. **Methods and Results:** The effect of αAR stimulation on the number of free thiols on Ras was measured using biotinylated iodoacetamide (BIAM) labeling. αAR stimulation caused a 49 % decrease in BIAM-labeled Ras that was reversed by dithiothreitol (DTT; 10 mM), indicating a decrease

in the availability of free thiols on Ras due to an oxidative post-translational modification. This effect was abolished by adenoviral overexpression of TRX1 and potentiated by the TRX reductase inhibitor azelaic acid. Likewise, αAR-stimulated Ras activation was abolished by TRX1 overexpression and potentiated by azelaic acid. TRX1 overexpression inhibited the αAR-stimulated phosphorylation of MEK1/2, ERK1/2 and p90RSK; and prevented cellular hypertrophy, sarcomere reorganization and protein synthesis (vs. βgal). Azelaic acid potentiated αAR-stimulated protein synthesis. While TRX1 can directly reduce thiols, it can also scavenge ROS by increasing peroxidase activity. To examine this possibility, peroxidase activity was increased by transfection with catalase (CAT), and intracellular ROS were measured using dichlorofluorescein diacetate (DCF). While CAT increased peroxidase activity ≈20-fold, TRX1 had no effect. Likewise, the αAR-stimulated increase in DCF fluorescence was abolished with CAT, but retained with TRX1. **Conclusion:** αAR-stimulated hypertrophic signaling in ARVM is mediated via a TRX1-sensitive post-translational oxidative modification of thiols on Ras.