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Scientists map steps to block key enzyme action in heart failure

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Taking a cue from the way drugs like Viagra put the biological brakes on a key enzyme involved in heart failure, scientists at Johns Hopkins have mapped out a key chemical step involved in blocking the enzyme.

The Johns Hopkins team reports how the enzyme, phosphodiesterase 5, or PDE5A, slows down the breakdown of another, more vital compound in the body, cyclic guanosine monophosphate (cyclic GMP) which influences a variety of biological activities, including cell growth and muscle contraction. A buildup of cyclic GMP limits stress and overgrowth of heart tissue.

PDE5A is the same enzyme that earlier Johns Hopkins work in mice showed is slowed down by the drug sildenafil (Viagra), leading to a reverse of tissue damage from heart enlargement, or hypertrophy, and potentially heart failure. What the scientists are seeing more broadly in this new work is most likely the same braking mechanism, but through a natural chemical reaction in the cell instead of through a synthetic chemical.

In the latest study, to be presented Nov. 11 at the American Heart Association's annual Scientific Sessions in New Orleans, the Johns Hopkins team of protein biochemists confirmed precisely where a sulfur- and nitrogen-containing molecule, or S-nitrosyl group, chemically alters the enzyme's amino-acid building blocks. And they showed that so-called S-nitrosylation of amino acid cysteine 181 results in a 25 percent decrease in PDE5A activity, pinpointing how the enzyme's action is

suppressed.

"Knowing the molecular make-up and activity of a protein is critical to understanding heart failure because these problem-specific biochemical reactions are magnified in the disease," says senior study investigator Jennifer Van Eyk, Ph.D., a professor at the Johns Hopkins University School of Medicine and its Heart and Vascular Institute.

"Targeted drug therapies can now be developed and tested to work specifically on cysteine 181, to block the PDE5A enzyme, lower the breakdown of cyclic GMP, and potentially stall progression of heart failure and hypertrophy," adds Van Eyk, director of the Johns Hopkins NHLBI Proteomics Group and the Proteomics Center at Johns Hopkins Bayview Medical Center.

Van Eyk says that previous research by co-investigator Hunter Champion, M.D., Ph.D., had shown that other chemical pathways in cyclic GMP were controlled by the placement of the sulfur-nitrosyl combo, prompting her team to investigate if PDE5A was similarly influenced.

"This was the first solid evidence of S-nitrosylation in PDE5A," says Murray, who, after having confirmed what was happening to the enyzme, proceeded to map out just where on the enzyme S-nitrosylation was taking place.

As cysteine is the only amino acid to which an S-nitrosyl group can attach, Murray's next step was to narrow the search from among the 20-plus possible cysteine locations found in PDE5A.

In a separate experiment, Murray then determined which one of the two cysteines, or both, was being altered. Enzyme pools specific to each cysteine were created. One pool had cysteine 181 blocked, leaving only

cysteine 210 available for possible chemical modification, while the other pool had cysteine 210 blocked, leaving open cysteine 181.

Separate chemical attempts at S-nitrosylation of each pool gradually showed that if cysteine 181 was unavailable to react, then enzymatic activity proceeded at the same rate as in a control group with all cysteines free to react.

Researchers say their next steps are to investigate how PDE5A affects where cyclic GMP is broken down or made, with the goal of determining if the enzyme also controls the known cell pooling of the compound. Future studies will also examine other potential effects of PDE5A production, and how alterations to its structure affect its function.

Study co-investigator and cardiologist David Kass, M.D., in whose lab in 2005 much of the original work on sildenafil and PDE5A was performed, also plans to develop a mouse model with blocked cysteine 181 to see what happens when these mice develop heart failure.

Source: Johns Hopkins Medical Institutions