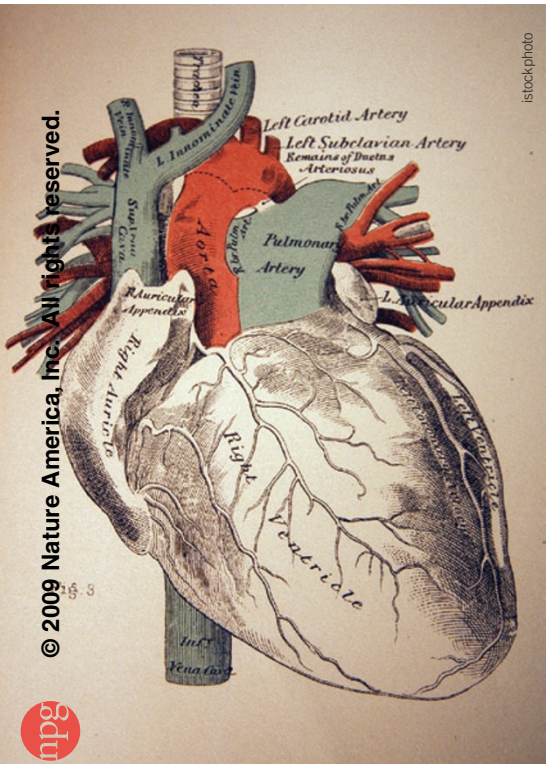
Clues emerge about benefits of briefly blocking blood flow

For people at risk of heart attack or stroke, protecting vital organs against grave damage presents a complex challenge. Danger lurks on both sides of the attack: when blood flow stops, heart and brain cells die of starvation; when blood flow returns, weakened cells suffer greater injury from reactive oxygen species, also known as free radicals.

Scientists have known since 1986 about a mechanism called ischemic preconditioning, or IPC, which describes a series of short interruptions in blood supply that builds cellular resistance to a subsequent and more severe loss of oxygen and nutrients (*Circulation* **74**, 1124–1136; 1986). The practice of IPC involves temporarily halting blood flow to prep tissues for longer periods without blood supply, such as during surgery. In one clinical trial, people undergoing open heart surgery who received IPC with a mechanical pump suffered less heart damage than those who did not receive the IPC treatment (*Cardiovasc. Surg.* **10**, 251–255; 2002). But exactly how IPC works is a complex mystery.



The beat goes on: LNO_2 might help when blood supply is interrupted

To explore the possible pathways involved in IPC, scientists tested the effects of an essential fatty acid compound, called nitrolinoleic acid, or LNO_2, on rat heart cells *in vitro*. They forced blood through rat hearts, induced IPC with a mechanical pump and then examined the mitochondria within cells. They found that LNO_2 forms naturally in mitochondria during temporary loss of blood flow (*Cardiovasc. Res.*, doi:10.1093/cvr/cvn323; 2008).

More importantly, the study showed that adding extra LNO_2 protects these cells against death during temporary blood loss and injury when blood flow returns. About 70% of control heart cells died compared to about 30% of experimental heart cells that received blood with extra LNO_2. Paul Brookes, senior author of the study, says that the amount of LNO_2 they added was not much more than the control levels they found, suggesting that a little LNO_2 has a potent effect.

How the cells use LNO_2 in these minievents to protect themselves is unclear. To investigate, Brookes and his team made a synthetic version of LNO_2 tagged with a marker and put it into rat heart cells by inducing IPC (*Cardiovasc. Res.*, doi:10.1093/cvr/cvn323; 2008). They used a technique called immunoprecipitation to track LNO_2 and found the marker on two proteins: adenine nucleotide translocase and uncoupling protein. According to Brookes, both proteins are involved in mitochondrial uncoupling—a process that diverts energy from food away from making ATP, the chemical used by cells as an energy currency, and toward generating heat.

Uncoupling proteins seem to play a part in the normal physiology of mammals through temperature regulation, such as during hibernation and regulation of insulin secretion in the pancreatic cells. And, crucially, these uncoupling proteins also might protect mitochondria against damage by free radicals (*Free Radic. Biol. Med.* **43**, 1351–1371; 2007).

Under stress

Scientists studying damage to human brains caused by stroke observe that mitochondrial uncoupling proteins are more abundant in damaged areas, suggesting that under stressful conditions involving loss of blood flow to the brain, neurons protect themselves by changing how they use energy (*Neuropathology* **27**, 442–447; 2007). In other *in vivo* and *in vitro* studies of rats, neurons producing extra uncoupling protein suffer less oxidative stress.

Elizabeth Murphy, a researcher at the pulmonary and vascular medicine branch of the US National Heart, Lung and Blood Institute in Bethesda, MD, who was not involved in these studies, speculates that LNO_2 may be an endogenous signaling molecule that leads to mitochondrial uncoupling, which ultimately prevents cellular damage and death. But she notes that there are other important cellular players involved in uncoupling, such as nitric oxide, protein kinase C and calcium-potassium channels—and LNO_2 probably comes in downstream.

For example, researchers investigated an alternate protective pathway in another study of rats and found that helium gas activates a calcium-sensitive potassium channel, which causes mitochondrial uncoupling and induces IPC in young rat heart cells (*Anesthesiology* **109**, 830–836; 2008).

"One of the major goals of this research is to find a compound that will activate these protective pathways by acting downstream. A drug that intervenes in just at the right spot to induce uncoupling and cause IPC will protect many different organs, such as the heart from heart attack, the brain from stroke, [or] the liver from ischemia," explains Murphy.

Researchers exploring the therapeutic potential of IPC and LNO_2 report mixed results. Adapting IPC from the bench to the clinic isn't straightforward, but reactive fatty acids such as LNO_2 show promise. Studies in rats show that the benefits of IPC are limited by diabetes, the use of beta-blockers and older age. "We need to figure out what it is about these groups that makes them resistant to preconditioning," says Brookes. It's been over 20 years since IPC was discovered, and scientists still haven't adapted it into routine clinical practice.

Signaling molecules such as LNO_2 may prove easier to adapt to the clinic than IPC. The results of a recent study show that several reactive fatty acids trigger a popular target of diabetes treatment, the peroxisome proliferator-activated receptor-gamma (PPAR-gamma) (*Nat. Struct. Mol. Biol.* **15**, 924–931; 2008). (The synthetic diabetes drug, rosaglitazone, also binds to PPAR-gamma and is commonly used as an insulin sensitizer to treat people with diabetes.)

But the future goal of protecting vital organs from damage during heart attack or stroke depends on of the success of even the smallest experiments. According to Brookes, "scientists have more work to do before drugs can be developed. We need to better at identify the signaling pathways that activate this natural protective mechanism in humans."

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