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Boston University
Exploring the factors that trigger vasospasm in the coronary arteries

The elusive issue of the causes and character of coronary-artery vasospasm is the principal research interest of Richard A. Cohen, M.D., and his associates.

Vasospasm plays an important role in the functioning of blood vessels throughout the body, says Dr. Cohen. It has been identified as a cause of angina pectoris, migraine headaches and Raynaud's phenomenon, among other conditions.

A key feature of the coronary arteries, notes the investigator, a member of the Peripheral Vascular Section, is that they react in the opposite way from other arteries to stimulation through the sympathetic nervous system's adrenergic pathways. When norepinephrine is released by the nerves in coronary-artery walls, the result is vasodilation, not vasoconstriction.

The apparent reason is that the neurotransmitter is acting primarily on the vasodilatory beta adrenergic receptors in the smooth muscle, not the vasoconstricting alpha receptors. "That's why propanalol, which blocks the beta adrenergic receptors, can cause problems in some patients with angina," says Dr. Cohen, who is also an assistant professor of medicine at BUSM.

A complex phenomenon

Though there's obviously much to be gained from shedding light on the mechanisms underlying vasospasm, they do not yield readily to efforts at unraveling them. For one thing, a range of factors are involved in producing constriction in the coronary arteries. Cholesterol, for example, can magnify the effects of important vasoconstrictive stimuli. For another, platelets, key players in arterial regulation, are subject to an elaborate feedback system. "Platelets release several mediators involved in vasospasm," explains Dr. Cohen, "and many of the released substances in turn accelerate the activation of the platelets."

Yet while the regulatory machinery of vasospasm is complex, that doesn't mean clinically useful findings lie decades off. On the contrary, Dr. Cohen suggests that if the key influences on vasospasm are identified, clinical applications may soon follow.

One starting point for any exploration of vasospasm is the arterial endothelium, recently identified as a major regulator of vasoconstriction. Studies of the endothelium, says Dr. Cohen, demonstrate that it harbors a diverse range of regulatory tools: hormone receptors, ion-exchange pumps, and both synthesizing and degrading enzymes.

One of the endothelium's abilities is that, in response to certain stimuli, it can trigger a relaxation of the blood vessel. "This effect was first discovered with acetylcholine," says Dr. Cohen. "When it's released and comes in contact with the endothelium in vitro, the result is a strong relaxation effect. It's clear that the endothelium is releasing some sort of mediator."

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Studying the impact of platelets

Dr. Cohen's group is particularly interested in the interactions between the endothelium and platelets, and in the impact of platelet-generated mediators on other parts of the vessel. They have done extensive in vitro studies in which rings of animal arteries in various states are exposed to platelets or individual mediators.

"When we cause platelets to aggregate in one of these rings, we see two contradictory types of responses," explains Dr. Cohen. "The response mediated by the endothelium is a relaxation, while the vessel's direct response to the platelets is a contraction."

The contraction effect is present even when the endothelium is intact, and studies by Dr. Cohen's group have helped to show why. The group has found that platelet products—among them thromboxane and serotonin—can enter the endothelium, and can thereby influence the vessel's functioning in various ways.

Serotonin, for example, can link up directly with receptors on the smooth muscle. It also can act on receptors near the endings of the adrenergic nerves that control the arterial blood flow. In both instances, he adds, the mediator promotes constriction.

"We don't know exactly what serotonin does when it acts on the nerve endings," says Dr. Cohen. "However, we do know that it inhibits the release of norepinephrine, and that leads to a greater contraction of the vessel."

When constriction predominates

The ability of platelet-generated mediators to produce such effects probably has little relevance to the functioning of normal vessels. Platelets don't generally collect on normal tissue. Even when they do—as, for example, when there's endothelial damage upstream—their net impact is strongly weighted toward relaxation because of their stimulating effect on the endothelium.

If the endothelium is damaged, however, it's another matter. "We find that when we remove the endothelium from an artery, and expose it to platelets, the result is a strong constriction," says Dr. Cohen. He says the finding is convincing evidence that the direct effects of the platelet mediators on the smooth muscle and the nerves are dominating the vessel's response to the mediators.

Other experimental evidence suggests that it's not necessary for the endothelium to be eliminated altogether to produce vasospasm. Endothelial damage like that done by atherosclerosis appears to be enough to tip the balance strongly toward constriction.

Besides looking at the impact of platelets on coronary arteries, Dr. Cohen's group has also explored which platelet products are most influential in vasospasm.

Serotonin's central role

"In studies I began when I was at the Mayo Clinic, I found that serotonin was clearly responsible for the most direct effects," says Dr. Cohen. "That surprised some people, because thromboxane has been considered the most potent vasoconstrictor in platelets."

The group also has discovered that serotonin probably has chronic effects on blood vessels as well as acute ones. "When we put a balloon catheter into an animal's artery and remove the endothelium, we get platelets aggregating in there," explains Dr. Cohen. "When we go back 24 hours later and measure the serotonin in the sympathetic nerves innervating those vessels, we find very high levels. This adversely affects the functioning of those nerves."

This finding points to long-term buildups of serotonin in the nerves as major contributors to vasospasm. Dr. Cohen believes there's solid indirect evidence for such an effect.

"If there's chronic platelet aggregation," he explains, "and the serotonin becomes incorporated into the nerve ending, it's likely to remain there for a long time, because once it's in there it's protected from metabolic degradation."

Implications for treatment

What is the potential significance of such findings for patients with cardiovascular disease? "If you establish that serotonin plays a major role in vasospasm, that probably isn't going to mean much for the angina patient at the time he's experiencing symptoms," says Dr. Cohen. "There are an awful lot of medications, like the calcium channel blockers and nitroglycerin, that can reduce arterial constriction."

On the other hand, he says, the findings could speed the search for preventive therapies. If important mediators of vasospasm are collecting in the nerves, for example, it might be possible to reverse the accumulation, or at least to moderate the mediators' effects.

In fact, he notes, Jay D. Coffman, M.D., chief of the Peripheral Vascular Section, is U.S. coordinator for an international study aimed at finding out whether administering ketanserin, a serotonin antagonist, helps to extend the lives of patients suffering from atherosclerosis and its complications.

"As far as I know, this is the first time that a serotonin blocker has been tested in this way," says Dr. Cohen. "It should tell us a lot about the clinical significance of what we've been doing."

Suggested further reading

Complement's crucial role in bringing on a common disease of the kidney

As many as 30 to 40 percent of adults with nephrotic syndrome have membranous nephropathy, a condition that is a common cause of kidney failure.

In membranous nephropathy, the glomerulus, which is the kidney's filtering mechanism, becomes damaged. The result is that the kidney loses some of its selective filtering capability, allowing large-sized protein molecules, especially albumin, to be excreted rather than retained in the blood. The reduction in serum albumin, in turn, leads to the formation of edema and the nephrotic syndrome.

David J. Salant, M.D., a member of the Evans Renal Section, says that membranous nephropathy may be a complication of cancer, hepatitis and lupus, and is a side effect of various medications, including gold, penicillamine and captopril. Usually, though, it's a primary condition.

"Membranous nephropathy is most prevalent among the middle-aged, with about a three-to-two preponderance of men over women," says Dr. Salant, who also is an associate professor of medicine at BUSM.

There are palliative treatments for nephrotic syndrome, and occasionally it goes into remission. Generally, though, the syndrome will either settle down into a chronic state or produce kidney failure.

There's no doubt that the membranous nephropathy form of the syndrome, when it's a primary condition, is immunologic in origin, says Dr. Salant. Biopsies show large immunoglobulin buildups at the site of the glomerular damage. Not so clear, however, are the specific immune-system events that trigger the condition.

Doubts about the prevailing view

Until about a decade ago, the accepted theory was that membranous nephropathy results when immune complexes circulating in the bloodstream become trapped in the kidney. But that theory didn't square with findings in experiments with rats.

"One couldn't reproduce membranous nephropathy by introducing immune complexes into rats," explains Dr. Salant, "so certain people began to wonder whether the entrapment theory was correct."

Among the skeptics was William Couser, M.D., an Evans investigator whose lab Dr. Salant joined in 1977. [Dr. Couser left in 1982 to become chief of nephrology at the University of Washington School of Medicine in Seattle.]

The Evans group determined that immune complexes in the rat model in fact form in situ, with the triggering mechanism being the binding of an antibody to a glomerular antigen. These findings matched those of Dutch investigators who were exploring the origins of membranous nephropathy at about the same time. (Subsequent studies by other groups have shown that the antigen involved is a component of glomerular epithelial cells.)

Early studies of complement

Dr. Salant and his colleagues also were interested in the role of complement, the nine-member family of immune-system proteins best known for helping to combat such organisms as pneumococcus and gonococcus.

"It has been recognized for several years that you find complement components in the diseased tissue from patients with membranous nephropathy," says Dr. Salant.

At the time the group was beginning its studies, complement's only known role was to draw cells like neutrophils to a target, and permit them to carry out their destructive mission once on the scene. Other findings, however, suggested that complement was almost certainly not playing such a role in membranous nephropathy.

"If you look under light microscopy at tissue affected by membranous nephropathy, you won't see neutrophils or any other inflammatory cells," notes the investigator.

"Since it was thought that the only role of complement in tissue injury is through its influence on inflammatory cells, the absence of such cells implies that complement is not involved."

But he and Dr. Couser, says Dr. Salant, suspected that complement might somehow be directly injuring the glomerulus, without having to call on neutrophils. To test their theory, they devised experiments involving the rat model of membranous nephropathy.

Initial tests of a theory

The condition is generated by injecting antibodies that have an affinity for an antigen located on the walls of glomerular capillaries. "What we did first was to deplete some
Photomicrograph shows glomerular capillary from rat kidney affected by early membranous nephropathy. Lower area (CL) is capillary lumen, area above (US) is urinary space. Dark spots denoted by arrows along epithelial cell (Ep) borders are immune complexes.

animals of complement," says Dr. Salant. "Then we injected the antibody into those animals and a group of controls. The group with depleted complement did not develop albumin in their urine, but the controls did."

Though the first sign that complement might indeed be directly involved in damaging the glomerulus, the experiments did not prove complement was playing such a role. "The idea was that perhaps inflammatory cells were involved, and we simply missed seeing them," says the investigator. "Maybe they just came through, did their 'dirty work,' and passed on."

To check that possibility, the group performed new experiments. They first reduced the levels of neutrophils and macrophages in rats, and then induced membranous nephropathy. This time, both sets of animals developed proteinuria.

Although this was convincing evidence that complement does its damage directly, it did not explain how that comes about. The investigators suspected that a compound made up of five of the complement proteins might be to blame. This compound, now called the membrane attack complex (MAC), had been found by other groups in the mid-'70s. Although subsequently revealed to be responsible for the lysis of red blood cells and the destruction of bacteria, the complex's potential for damaging kidneys had not been recognized by the time the Evans investigators began their studies of it.

**Probing how the damage is done**

To explore whether the complex was in fact injuring glomerular membranes, therefore, the investigators performed a series of experiments, at the start using rabbits that were deficient in one of the MAC proteins.

Those experiments, and a subsequent series in which plasma from a patient deficient in one of the MAC proteins was circulated through an in vitro rat kidney, helped pinpoint the role of the membrane attack complex. "We proved conclusively that the full MAC is needed to produce albumin leakage in experimental membranous nephropathy," says Dr. Salant.

From microscopic studies of affected kidneys, the group also was able to establish that glomerular epithelial cells are the main targets of the attack complex. So now they knew not only that complement was injuring the glomerular capillaries, but also how it does so: The buildup of complement, triggered by antigen-antibody linkages on the capillary walls, leads to formation of the attack complex. The complex, in turn, attaches to the walls, creates holes in them, and by a process still not fully understood, alters their ability to carry out normal filtering functions.

Identifying the attack complex as the chief culprit in membranous nephropathy has raised the question of whether it may be involved in causing other conditions as well.

**Other roles for the complex?**

"The complex has been found in the skin of patients with lupus and bullous pemphigoid, and in inflamed muscle tissue from patients with dermatomyositis," notes Dr. Salant. "It also has been identified in nerve tissue from myasthenia gravis patients. In addition, there's a study in which MAC is reported in kidney samples from patients with a variety of different conditions, including hypertension and diabetes."

The simple fact that the complex is present, he adds, doesn't imply that it's playing a pathological role. Moreover, finding MAC does not rule out the possibility that complement is exerting its effects through its traditional, indirect mode of action.

"In point of fact," says Dr. Salant, "there's a kidney condition that is caused by inflammatory cells, and we've found that we can distinguish it from membranous nephropathy simply by determining the specific site within the capillary wall at which the immune deposits form."

**Thinking about potential applications**

At this point, Dr. Salant is trying to pin down the specifics of the attack complex's effects on the kidney. Using glomerular epithelial cell cultures, he hopes to pinpoint where and how it does its damage. He also hopes to explore whether the complex operates the same way in human kidneys as in animal kidneys.

Though the research has not yet reached the point of clinical usefulness, the investigator says he has given some thought to potential applications.

From a therapeutic standpoint, says Dr. Salant, one significant recent finding is that

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Richard Eglow, M.D., has been named chief resident of the Evans for the 1986-1987 academic year. A native of Syosset, N.Y., he received his medical degree from the Chicago Medical School.

Dr. Eglow's main research interest is the chemical makeup of the clostridium difficile toxin B. He's currently working with investigators from the Section of Gastroenterology in an effort to sequence the toxin, which causes a virulent form of diarrhea in some patients on antibiotic regimens.

"When patients are put on antibiotics, and the drugs kill the other bacteria, it permits C. difficile to secrete its toxins within the lumen of the colon," explains Dr. Eglow.

The toxin, one of two produced by C. difficile, is difficult to isolate because it is very unstable, he adds. If it can be sequenced, however, the investigators will then be able to compare it with other toxins, such as the cholera toxin, in an effort to better understand its mode of action in the colon.

Dr. Eglow, who came to the Evans after graduating from medical school, says he expects to become a fellow in the Section of Gastroenterology following completion of his service as chief resident.

The chief resident also is an ardent Yankee fan—a passion that stems in part, he says, from the fact that Joe Dimaggio is a close family friend.

Yet despite the fact that he has a large Yankee poster and two pictures of Dimaggio in his office, Dr. Eglow doesn't begrudge Red Sox fans their team's success this past season. "They waited a long time for it," he says graciously.

A total of 17 interns have joined the Evans this year. They are:

Lee Albert, M.D., Johns Hopkins School of Medicine
Jeffrey Altholz, M.D., Einstein College of Medicine
David Cohen, M.D., Jefferson University Medical College
Susan DeCoste, M.D., University of Connecticut School of Medicine
Mary Delaney, M.D., Boston University School of Medicine
Katherine Duffy, M.D., University of Maryland School of Medicine
Richard Fogel, M.D., Brown University School of Medicine
Michael Goldaber, M.D., Albany Medical College
Dale Janik, M.D., University of Pennsylvania School of Medicine
Michael Kraut, M.D., Einstein College of Medicine
Michael Lev, M.D., Brown University School of Medicine
Scott Mackler, M.D., University of Pennsylvania School of Medicine
Max Rosen, M.D., Tufts University School of Medicine
Harry Shapiro, M.D., Boston University School of Medicine

Dr. Eglow (foreground) checking patient's chart in UH's Medical Intensive Care Unit with resident Robert M. Weiss, M.D., and Nadine Barry, R.N., staff nurse. MICU is located on seventh floor of Evans Building.

Alice Sheridan, M.D., New York Medical College
DuWayne Willett, M.D., University of Wisconsin School of Medicine
John Wilson, M.D., Pennsylvania State University School of Medicine.
This past year's Evans graduates, and their current pursuits, are:

Richard Eglow, M.D., The University Hospital (chief resident)
Steven Feske, M.D., primary-care practice, Boston area
Howard Fogel, M.D., Massachusetts General Hospital (endocrinology)
Stephan Gaede, M.D., Boston Veterans Administration Medical Center
Kamran Ghalili, M.D., The University Hospital (cardiology)
Bruce Kriegel, M.D., primary-care practice, Boston area
Vanessa Lucarellla, M.D., University of Pennsylvania (cardiology)
Judith Melin, M.D., Lahey Clinic (general internal medicine)
Chester Mohr, M.D., New England Medical Center (pulmonary medicine)
Debra Nichols, M.D., Carney Hospital, Boston (chief resident)
Daniel O'Dea, M.D., Valhalla Westchester County Medical Center (cardiology)
Jerome Siegel, M.D., Bedford (Mass.) Veterans Administration Medical Center (geriatrics)
Zachary Spigelman, M.D., The University Hospital (oncology)
David Strumpf, M.D., Rhode Island Hospital, Providence (pulmonary medicine).
Linda Lesky, M.D., has been named a member of the Evans Section of General Medicine. A graduate of the University of Chicago School of Medicine, Dr. Lesky did her residency at Michael Reese Hospital in Chicago. She also served as medical director of that hospital’s internal medicine clinic and as director of a preventive medicine facility in Chicago. In addition to her Evans appointment, Dr. Lesky has been named to the general medical staff of The University Hospital and will head the new Women’s Health Group of UH’s Evans Medical Group. She has also been appointed an assistant professor of medicine at Boston University School of Medicine.

David M. Center, M.D., has been appointed chief of the Evans Pulmonary Section. Dr. Center succeeds Jerome S. Brody, M.D., who will continue to be active in the Section. Dr. Brody heads Boston University School of Medicine’s Pulmonary Center.

Complement...
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thromboxane and other arachidonic-acid products play a supporting role to complement in damaging the kidney. Complement, he notes, triggers the output of these products, and they in turn appear to aggravate the damage.

"By the time we see membranous nephropathy, the disease usually is established," he notes. "But we have effective blockers for these arachidonic-acid products, so we can think about inhibiting some of their effects, and perhaps reducing the amount of damage sustained by the kidney." □

Suggested further reading

Gordon L. Snider, M.D., is serving as president of the American Thoracic Society, the medical arm of the American Lung Association. Dr. Snider, a member of the Evans Pulmonary Section, was recently named chief of medicine at the Boston Veterans Administration Medical Center.

Joseph Stokes III, M.D., a member of the Section of Preventive Medicine and Epidemiology, has been named editor of the American Journal of Preventive Medicine. The appointment took effect July 1, and the first issue prepared under Dr. Stokes' direction will appear in January 1987. Dr. Stokes is a principal investigator for the Boston University-Framingham Heart Study.

William E. Huckabee, M.D. 1926–1986
William E. Huckabee, M.D., a member of the Evans from 1950 to 1971, died in July of this year.

Dr. Huckabee, who headed the Evans Section of Cardiac Metabolism, carried out landmark investigations that brought him international renown. His major research finding, published in 1958, was that the rate of cellular respiration is reflected in the ratio of lactate to pyruvate in the human body, with a disproportionate increase in lactate indicating cellular dysfunction. In later papers, he described and defined the clinical problem of lactate acidosis, an important concept that is still under investigation.

Dr. Huckabee was a professor of medicine at Boston University School of Medicine. He served as president of the American Federation of Clinical Research from 1965 to 1967. He was also associate editor of the Journal of Clinical Investigation.

Born in 1926 in Brownwood, Texas, Dr. Huckabee received his undergraduate education at Southern Methodist University in Dallas, and earned his medical degree at the Southwestern Medical College of the University of Texas, also in Dallas. He interned at Massachusetts Memorial Hospitals (now The University Hospital) and was a cardiology fellow under the late Robert W. Wilkins, M.D., former director of the Evans.