Drugs from toxins

Within their lifetimes, many people will suffer chronic pain due to injury or disease. One of the more potent, commonly prescribed analgesics is morphine, but it suffers from several side effects—not the least of which is addiction. Pain transmission and perception involve peripheral sensory neurons and their associated peptides communicating with central pathways. Injury or a painful stimulus causes the release of these peptides, which elicit action potentials in the pain pathways through both voltage-gated ion channels (VGICs) and ligand-gated ion channels (LGICs).

Because of the prevalence of pain, the search for new analgesics has been intense. Although some scientists have gone the route of medicinal and combinatorial chemistry, others have looked to nature to provide the answers, involving themselves in bioprospecting.

One group of naturally occurring analgesic compounds has been derived from the toxins of sea snails of *Conus* spp.— the conotoxins. The ω-conotoxins (ω-CTXs) such as ω-CTX MVIIA (marketed as Prialt by Elan Pharmaceuticals) are effective analgesics, but as N-type VGIC calcium channel blockers, they induce several side effects, including hypotension, nausea, and agitation.

Sensory nerves, however, also express nicotinic acetylcholine LGIC receptors, which are targets for another group of conotoxins. These α-CTXs are the research focus of Bruce Livett and colleagues at the National Aging Research Institute (Parkland, Australia) and the University of Melbourne.

At the recent Venoms to Drugs 2002 Conference in Heron Island, Australia, Livett's group presented their findings on an analgesic α-CTX that they have dubbed ACV1. A 16-residue polypeptide, ACV1 binds to the nicotinic receptor and inhibits the nicotine-evoked release of catecholamines from neuroendocrine cells in vitro. In rats, ACV1 is an effective analgesic and is more potent and longer-lasting than ω-CTX MVIIA. It also showed no adverse side effects in rats during the 12-week study. And unlike the N-type calcium channel blockers, ACV1 does not have to be infused into the spine but can be administered intramuscularly or subcutaneously. Furthermore, there are indications that ACV1 accelerates the rate of recovery from nerve injury.

So next time that you see signs at the beach warning of venomous animals, you might just begin to think of the pharmaceutical potential of the danger under your feet.

—RANDALL C. WILLIS

Babes and blood pressure

Pureed peaches, green beans, and applesauce might top your infant's list of favorite foods. But if he or she shows an early preference for salt and has a hypertensive grandparent, chances are good that your baby has higher blood pressure than peers without these characteristics, according to a recent study.

Stephen H. Zinner and colleagues at Mount Auburn Hospital in Cambridge, MA, tested 283 infants to determine whether their preference for salt or sugar was associated with a difference in blood pressure (*Hypertension* **2002,** *40* (3), 280–285). Because blood pressure is not routinely measured in healthy newborns, the researchers did not compare blood pressure measurements at birth to normal or set values, as would be done in adults. Instead, they grouped the newborns on the basis of their sucking response to water, water and sugar, or water and salt. A preferential response was measured within three days of birth by a relative increase in sucking activity, using a recording device connected to a specially designed nipple that delivers microdrops of fluid at a time. Increased sucking indicated a preferential response, and decreased sucking indicated an aversive response.

At a follow-up blood pressure measurement at one month of age, those babies who showed a preference for salt averaged 3.1 mm Hg higher diastolic blood pressures and 3.3 mm Hg higher systolic blood pressures than babies with a neutral or aversive response. If the salt-preferring newborns also had a grandparent being treated for high blood pressure, their average diastolic pressure was 5.0 mm Hg higher, and their average systolic pressure was 6.7 mm Hg higher than babies with an aversive or neutral response. A preference for sugar had no relationship to increased blood pressure in the infants.

Although the researchers cautioned that it would be premature to identify individuals at risk for adult hypertension through such neonatal testing, previous studies have linked salt intake to high blood pressure in some adults, especially those who are considered salt-sensitive. Salt-sensitive individuals suffer sharp increases in blood pressure at low intakes of salt. Both hypertension and salt sensitivity are known genetic conditions.

—CHRISTEN L. BROWNLEE

PHOTOS: PHOTODISC AND EYEWIRE

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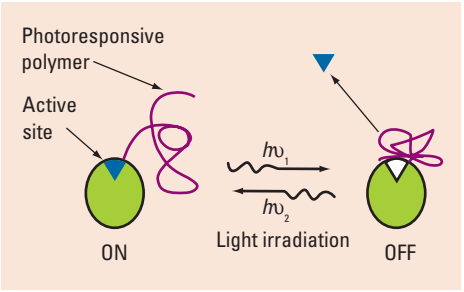
Photoswitchable proteins

The design of biomedical tools like diagnostic assays, affinity separations, and drug delivery systems requires not only an understanding of molecular recognition processes but also the ability to control them—to turn them on and off—as needed. A typical means of control is to change solution conditions such as temperature or pH. Such solution changes, however, are often damaging to biomolecules.

Light activation represents a milder alternative, especially appealing because its external reversibility makes it amenable to automation. In this direction, Allan Hoffman and colleagues at the University of Washington (Seattle) have recently prepared photoresponsive molecular switches that can physically open and close an active binding site of a protein based on the wavelength of light shone upon them (*Bioconjugate Chem.* **2002,** in press).

The "photoswitches" studied by Hoffman's group were two different *N*,*N*-dimethylacrylamide copolymers, denoted DMAA and DMAAm, respectively. The researchers had previously shown that isomerization of the diazo chromophores of these polymers, triggered by photoexcitation, causes a shift in polymer structure between a water-soluble extended random coil and an insoluble collapsed entity. For DMAA, the soluble coil is present under UV light, whereas the collapsed structure forms in visible light. The exact opposite response is observed for DMAAm—the insoluble species arises at UV wavelengths and the soluble coil is present in the visible spectrum.

The scientists were able to accomplish site-specific conjugation between the polymers and streptavidin (SA) mutants at a position adjacent to where SA–biotin binding takes place. Subsequently, biotin-binding assays amid UV–vis cyclic irradiation clearly indicated light-regulated switching. The SA–DMAA conjugate exhibited strong binding—approaching that of free SA—under UV light, whereas visible frequencies caused significant blocking of biotin as well as the release of already bound molecules. The SA–DMAAm entity demonstrated the exact same phenomena in reverse. The researchers offer the explanation that the loosely wound coil leaves a mostly open path for biotin to slip into the active site, whereas the insoluble collapsed polymer sterically impedes biotin from making or maintaining contact with the protein (see figure)—providing a general approach to the design of photoswitchable proteins.



Polymer patrol. Regulating an active site of a protein with a photoresponsive polymer.

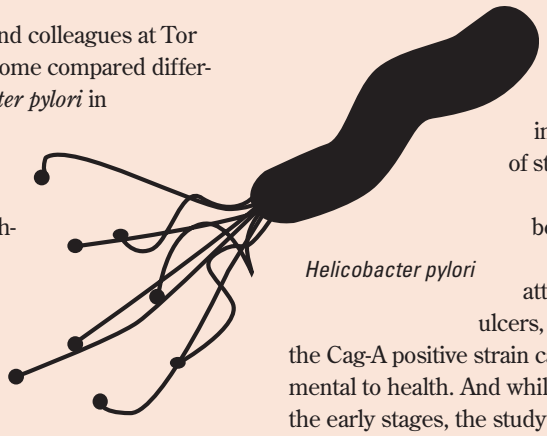
—DAVID FILMORE

Bacterial stroke strain

This year in America, about 600,000 people will have a stroke, and almost 160,000 of those will die from sustained injuries, according to the American Stroke Association. Although researchers are aware of many risk factors for stroke, such as a history of high blood pressure, diabetes, or excessive alcohol intake, a recent study suggests that the prevalence of a particular bacterial strain in the bloodstream could signal an increased chance of certain kinds of strokes.

Antonio Pietroiusti and colleagues at Tor Vergata University in Rome compared different strains of *Helicobacter pylori* in the bloodstream of three groups of test subjects: 138 patients who had suffered an atherosclerotic stroke (group A), 61 patients who suffered a cardioembolic stroke (group B), and 151 healthy patients (group C) (*Circulation* **2002,** *106,* 580–584). While groups A and C had similar amounts of *H. pylori* infection, a certain strain of *H. pylori* known as Cag-A positive was found in 42.8% of the atherosclerotic group, compared to only 19.7% in the cardioembolic group and 17.9% of the control group. An atherosclerotic stroke is initiated by a buildup of cholesterol and other lipid material on the inner lining of an artery, which causes the artery wall to harden and fill up with deposits. Clots initiated by these deposits prevent blood flow to the brain, which often leads to a stroke. Cardioembolic strokes occur when a clot travels to the brain, causing a cerebral artery to be suddenly obstructed.

Cag-A positive *H. pylori* possess a gene known as cytotoxin-associated gene A. These bacteria produce a toxin that attacks cells, which can lead to tissue inflammation and lesions. According to the researchers, when this strain of bacteria circulates in arteries leading to the brain, it can damage artery walls and cause lesions. Ultimately, this narrows the blood vessels and further increases the risk of stroke.



Helicobacter pylori

While *H. pylori* has been linked in the past with heart attack and stomach ulcers, Pietroiusti says that the Cag-A positive strain can be especially detrimental to health. And while this research is in the early stages, the study's conclusions indicate that the association between *H. pylori* and stroke might be due to a higher prevalence of these virulent *H. pylori* strains in patients with the large-vessel, or atherosclerotic stroke.

"Cag-A positive strains are more virulent than the other *H. pylori* strains because they are able to increase the expression of the interleukin-1 β gene and consequently the production of this cytokine, which has strong proinflammatory properties," he said.

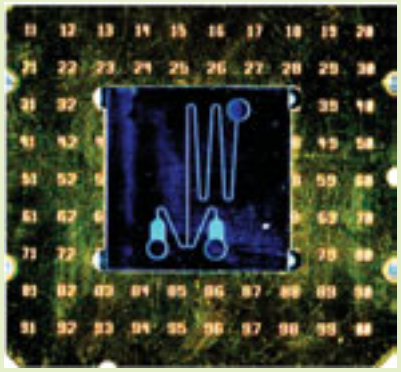
—JULIE L. McDOWELL

Microfluidics and MALDI-TOF

Recent advances in microfluidic technology—the lab-on-a-chip—have added impetus to the development of micrometer-scale reactors. But on-line integration of microfluidic chambers with analytical instruments has been difficult because standard spectroscopic and electrochemical detection methods do not always provide sufficient sensitivity.

Mass spectrometry (MS), however, does offer adequate sensitivity for integration with microreactors. And electrospray ionization (ESI)-MS has typically been the technique used for such purposes because it allows for simple interfacing between the reactor and detector and is run at atmospheric pressures. But ESI-MS suffers from sample preparation problems—mainly because it requires the removal of ionic species from the original sample buffer—that are best addressed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS. David Reinhoudt and his colleagues at the University of Twente (The Netherlands) have developed an on-line microreactor-MALDI-TOF MS system that could potentially follow chemical and biochemical reactions in real time (*Anal. Chem.* **2002,** *74*(16), 3972–3976).

The microreactor is composed of two 5-µL inlet reservoirs and one 5-µL outlet port separated by an 80-mm-long microchannel. After the sample is loaded into the inlets, the microreactor is placed in a MALDI-TOF sample plate. Under vacuum, the two inlet samples are drawn into the microchannel, they react, and the products are analyzed in the outlet port.



Lab-on-a-chip-on-a-sample-plate. By combining a microfluidic reactor with MALDI-TOF MS, researchers hope to follow small-scale reactions in real time. (Reprinted from *Anal. Chem.* **2002,** *74*(16), 3972–3976.)

The researchers used their chip-MS system to sequence a 41-base oligonucleotide. The oligonucleotide was digested with snake venom phosphodiesterase, which hydrolyzes in the 3′-to-5′ direction. As MS determines the masses of the resulting truncated oligonucleotides, the sequence can be deduced from the mass difference between adjacent pairs of peaks in the spectra. For example, the loss of an adenosine will result in two peaks separated by 313.2 mass units. The researchers similarly followed the sequencing reaction of a small hormone polypeptide as it was digested by a protease that sequentially hydrolyzes the C-terminal residues of a peptide.

By performing the protein digestions and product analyses directly on the chips, the researchers were able to minimize sample handling, sample loss, and method development time. They propose that a chip designed with multiple outlet ports will allow quasi-continuous monitoring of the reaction products.

—RANDALL C. WILLIS

Eye contact that delivers

Almost everyone, at one time or another, has used eyedrops to deliver a drug. But even though improvements in excipients have increased the efficacy of these delivery systems, the problems of blinking and tear production are still serious impediments to their use. This has led researchers to look for ways to use soft contact lenses, loaded with a drug, as a delivery vehicle. But the challenge of making lenses that absorb and release therapeutically relevant doses remains.

Carmen Alvarez-Lorenzo and colleagues at the Universidad de Santiago de Compostela (Spain) and Menicon Corp. (Kasugai, Japan), a contact lens developer and distributor, have answered this challenge by testing a variety of lens polymers with timolol, a drug for glaucoma (*J. Pharm. Sci.* **2002,** *91,* 2182–2192).

The researchers combined poly(hydroxyethyl methacrylate) [poly(HEMA)] hydrogel with small amounts of either methyl methacrylate (MMA) or methacrylic acid (MAA) and examined the effects of copolymerization with or without timolol on the resulting lenses. By adding timolol during polymerization, the researchers hoped to molecularly imprint the drug on the copolymer and thereby increase subsequent drug absorption.

The lenses were visually identical in the presence or absence of the drug, showing high optical clarity both before and after oiling (the usual cleaning and sterilizing treatment).

Similarly, any elasticity or viscosity differences were slight, although there was some indication that copolymerization with timolol altered the inter- and intrachain interactions between poly(HEMA) and MAA or MMA, which suggested that molecular imprinting did occur. The drug, however, had no effect on hydrogel swelling.

The rate of drug release decreased as the MAA and MMA concentrations increased, but timolol release was higher in the presence of low concentrations of MAA than in its absence. Likewise, hydrogels containing low concentrations of MAA showed maximum absorption of timolol. Imprinting, however, had no effect on absorption, and loading in all cases reached its maximum by 8 h.

The researchers see their efforts as providing a first step in the development of a reusable drug delivery system based on soft contact lenses that can be reloaded overnight.

—RANDALL C. WILLIS

Honey! I lowered my cholesterol

Most people know honey only as a sticky sweetener, and some may even use it as a quick, natural energy aid. Few, however, probably look to it specifically for heart health. But according to a recent study presented at the 224th American Chemical Society national meeting held in Boston in August, honey contains about the same level of antioxidants as spinach, apples, bananas, oranges, and strawberries.

Nicki Engeseth and colleagues of the University of Illinois at Urbana-Champaign conducted a five-week study, funded by the National Honey Board, to test honey's effects in 25 men aged 18–68. Subjects drank a mixture of 4 tablespoons of honey in 16 ounces of water daily while continuing their regular diets for the duration of the study. Engeseth found that the mixture improved the antioxidant levels in their blood—thereby showing the potential to protect against high cholesterol and heart disease, all known effects of antioxidants. An earlier study by Engeseth's laboratory confirmed that the darker the honey, the better at lifting antioxidant levels in the blood.

Engeseth pointed out that to get the same amount of antioxidants from honey as from fruits and vegetables, you would have to eat the equivalent amount of honey per weight, and of course that would be excessive.

"People could incorporate more honey in places where they might be using some sort of sweetening agent, like sugar, and this might contribute a significant amount of dietary phenolics," Engeseth said.

Currently, Engeseth's research group is conducting a study with rabbits to determine if honey has an inhibitory effect on hardening of the arteries, or atherosclerosis, a leading cause of death in the United States. The group is also collaborating with scientists at the University of Illinois at Chicago to evaluate honey's ability to inhibit oral pathogenic bacteria, such as *Streptococcus mutans*, which leads to tooth decay. "Some types of honey seem to be protective against these bacteria," Engeseth said in the press release. "Sage honey and Tupelo honey are two of the tested honeys to show the most inhibitory effects."

—FELICIA M. WILLIS

Arthritis: Monoclonal medicine

The observation that tumor necrosis factor alpha (TNF-α) is typically elevated in the blood serum and sinovial fluid of arthritis sufferers has made the molecule a site of intense study of the disease. To date, researchers have observed that TNF receptor or anti-TNF antibodies can prevent collagen-induced arthritis in mice; a mouse line engineered to overexpress human TNF-α develops chronic multijoint arthritis similar to the human disease; and onset of the disease can be prevented by treatment of these transgenic mice with anti-TNF-α antibodies.

In a recent study, researchers at Centocor, Inc. (Malvern, PA), a biotechnology company that currently markets anti-TNF-α antibody under the trade name Remicade, and Wayne State University Medical School (Detroit) showed that these same polyarthritic transgenic mice could have their existing joint damage healed by the use of anti-TNF monoclonals (*Arthritis Research* **2002,** *4* (5), R7). Arthritis-afflicted juvenile (7–8-week-old) and aged (27–28-week-old) mice were tested for their response to anti-TNS monoclonals. A healthy aged population of this mouse line was produced by continuous use of anti-TNF antibody from 4 weeks until 24 weeks, when treatment was stopped and arthritis was allowed to develop in these older animals, mimicking adult onset in humans. Then saline versus anti-TNF antibody treatment was compared to determine if the antibodies were therapeutic and not just preventive.

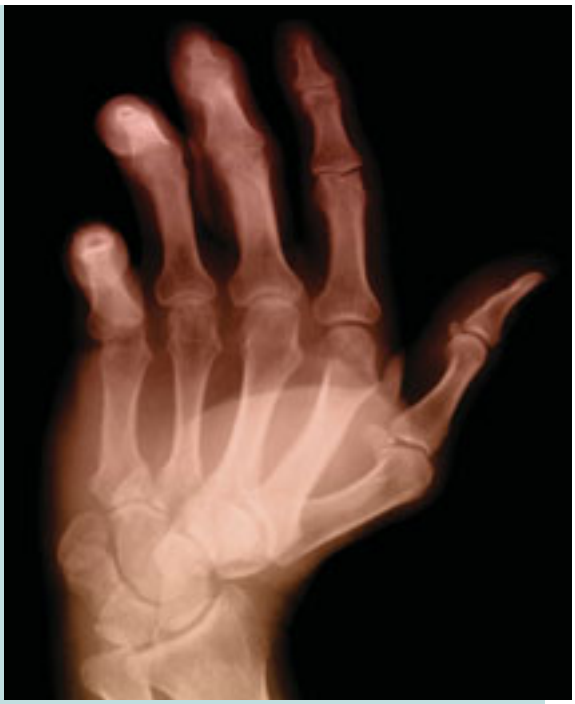


PHOTO: PHOTODISC

For both the juvenile and the aged populations, dramatic reversal of arthritic symptoms was seen with the therapeutic use of the antibodies. By 6 weeks of treatment, all histopathological evidence of bone or cartilage erosion and cellular proliferation or infiltration at the joints had disappeared. Extended treatment for 16 weeks showed maintenance of normal joint architecture. Although these results are encouraging, they are based on studies of animals with a unimolecular cause of their symptoms. Whether the results can be extrapolated to human rheumatoid arthritis, which might have multiple etiologies, remains to be seen.

—MARK S. LESNEY