Innovations

The snail companies
Neurex Corporation & Cognetix Inc.

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The fish-eating cone snails do not mess around; they don't have that luxury. Their prey — often larger than the snails themselves, and considerably more mobile — cannot be allowed to wriggle free or attract the attention of other predators. So the cone snails hit fish with a cocktail of peptide toxins that have a succession of nasty effects. Some toxins open ion channels to immobilize the fish with a quick tetanic shock; others block channels at the neuromuscular junction to more permanently shut down any muscle action; still others block calcium channels to shut off all communication between nerves. The end result of this chemical carnage is not surprising. The fish becomes an easy meal.

It is from this poisonous mixture that Neurex Corporation (Menlo Park, California) and Cognetix Inc. (Salt Lake City, Utah) think they can extract human pharmaceuticals.

Simple beginnings

Baldomero Olivera was trained in the well-funded laboratories of the California Institute of Technology and Stanford University. But when he returned to his home country, to take a position at the University of the Philippines, money and equipment became a problem. Molecular biology was out of the question.

He turned to the genus *Conus*, the cone snails that shuffled around local coral reefs. He knew about them from his youth, and was familiar with the reports that one species, *Conus geographus*, had killed humans. It seemed a reasonable proposition to study *Conus geographus* to find what made it lethal. "We thought the venoms would be relatively simple," he says. "We just thought we would categorize a couple of toxins."

The analysis soon became far more complex. The toxin had many different molecules in it, visible as separate chromatographic peaks. But was the complexity a red herring? "There were a lot of peaks, but a lot of them were inactive," says Olivera. "We weren't sure what to make of these inactive peaks."

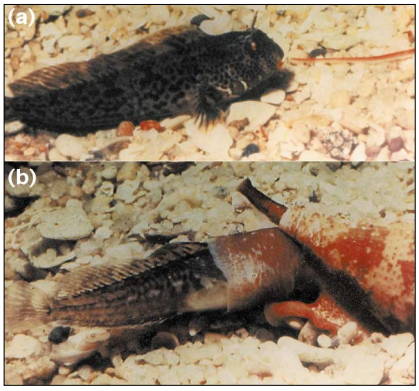
The toxins, dubbed conotoxins, were small peptides of 10–30 amino acids. They paralyzed fish, so Olivera was testing them by looking for paralysis in mice after intravenous injection. When so many toxins tested negative he went back to studying DNA replication, eventually at the University of Utah (Salt Lake City, Utah). Everything changed when graduate student Craig Clark decided to inject the individual peptide toxins into the central nervous system of the test mice. Previously inactive toxins now caused the mice to jump, sleep, scratch, drag their hind legs, swing their heads or shake. Olivera had hit a neurological gold mine.

Lucky for some

Unfortunately the University of Utah was not ready for Olivera's gold mine. They patented some of the earliest conotoxins, but not the initial ω-conotoxins. "I did make some moves to get them patented but the University of Utah tech transfer office was in transition and disarray," says Olivera. "We needed to get our stuff out and published, because we needed to renew our grants, so the ω-conotoxins became part of the public domain."

George Miljanich of the University of Southern California wanted ω-conotoxins for his work on neurotransmitter release. Unlike existing calcium channel blockers, such as the cardiac drugs nifedipine and nitrendipine, the ω-conotoxins bound to the neuronal (N-type) not cardiac (L-type) calcium channels, thus preventing the calcium influx needed for neurotransmitter release.

Figure 1



The diner becomes dinner. (a) The fish-eating *Conus* snails use a proboscis that looks like a tasty worm to attract a fish. (b) *Conus* injects poison using a chitin harpoon launched from the proboscis, and then emerges from the gravel to engulf the paralyzed fish.

In 1988, Miljanich was recruited by a start-up called Neurex. The year-old company wanted to find drugs specific to subtypes of neurological ion channels and receptors. But Neurex had a long hard road ahead of it. It was hoping to identify receptors, clone them, and then find subtype-specific inhibitors. When Miljanich brought the ω-conotoxins he skipped straight to the last step, and in the process he probably saved the company.

Things moved slowly at first. "The concept of peptides that could become drugs was met with some degree of skepticism," says Miljanich. "They were more keen on a small-molecule approach."

Fortunately the conotoxins already showed some properties more usually associated with small molecules than with peptides. All this is thanks to the snail's hunting strategy. The snails use small peptides because they diffuse rapidly through a poisoned fish, but for Neurex that meant a simple solid-phase synthesis. And the snail had evolved rigid molecules that avoid dilution by binding only one type of receptor very tightly, so Neurex now had potent, specific drugs that were resistant to degradation.

Intrathecal snails for pain relief

Based on binding affinities, Neurex's lead ω-conotoxin became the *Conus magus* toxin variously known as MVIIA, SNX-111, or ziconotide. The drug now had to be matched with a disease. "Any disease that could be improved by controlling synaptic release was fair game for treating with conotoxins," says Miljanich. The pattern of ziconotide binding to a specific part of the spinal cord suggested that the drug might be useful for pain. Ziconotide would not shut down the entire nervous system because P- and Q-type calcium channels were regulating synaptic release in other neurons.

Animal models of pain gave promising results. With an assay in hand, Neurex tried to improve ziconotide. "We tried to do better than what fifty million years of evolution had given us, but SNX-111 proved to be the superior compound," says Miljanich. "After hundreds of analogs, we went back to the original compound."

In animals and then humans, ziconotide was proving to have several advantages over opiates such as morphine. In some patients ziconotide caused mental fogginess (as is seen with morphine), but it did not cause constipation or respiratory suppression, and unlike the opiates it was effective against neuropathic pain, which is the result of nerve rather than tissue injury. And there was no tolerance. "We never saw any signs of tolerance in animals or in patients," says Miljanich. "The dose we give in the first week is equally effective a year later."

The reason is simple. "Ziconotide is an antagonist whereas morphine is an agonist of its receptor," says Miljanich. "To subvert the actions of an agonist, the cell just has to inactivate or to stop producing the receptor — then you've got tolerance. To overcome an antagonist the cell would have to increase the amount of receptor and there's a limit to that. There's only so many calcium channels that a cell can jam into a membrane."

What exactly is being blocked is unclear, although Miljanich guesses that neurotransmitters such as glutamate and substance P are no longer released. Ziconotide's method of action is clearer for its second application: blocking cell death in the brain after head trauma and stroke. It prevents the calcium influxes that both directly lead to apoptotic cell death, and that lead to excess glutamate release and so excitotoxic cell death.

But clinical trials with head trauma victims showed that ziconotide was not without its problems. The trials were suspended when intravenous ziconotide depressed blood pressure: the drug had inhibited norepinephrine release by sympathetic nerves to the smooth muscles that maintain blood pressure. This problem is now managed in hospitals with fluids and counteractive drugs, and intravenous ziconotide is now in phase III trials.

The solution for pain patients is to deliver ziconotide directly to the spine so that it never circulates through the rest of the body. Neurex has teamed up with Medtronic, Inc. (Minneapolis, Minnesota) to produce a pump system that delivers a constant flow to the spinal cord. The pump is the size of a hockey puck, and is implanted in the chest and refilled by syringe. Phase III trials for pain were completed earlier this year, and Neurex expects to file a new drug application in the next six months.

Olivera finally gets some action

The University of Utah had lost the ω-conotoxins, but now they were paying more attention to Olivera. Unfortunately he just wouldn't stop producing more, novel conotoxins. "We were beginning to eat up a significant portion of the University budget for patents," he says.

The solution was Cognetix. Formed in 1993, the company now has or has applied for patents covering over one hundred conotoxins. Cognetix isolates toxins based on their effects on ion channel activity, using both electrophysiology and fluorescent dyes that respond to differences in intracellular ion concentration. Toxin targets include nicotinic acetylcholine receptors (nAChR), calcium channels, *N*-methyl-D-aspartate receptors (NMDA-R), sodium channels, potassium channels and, recently, a serotonin receptor.

The main disease targets, all preclinical, include Parkinson's disease (using NMDA-R antagonists to indirectly increase dopamine release), urinary dysfunction in spinal-cord patients (using nAChR or NMDA-R antagonists to open the urinary sphincter), epilepsy (using NMDA-R antagonists to prevent over-excitation) and pain (various approaches). Cognetix hopes to start clinical trials for intractable epilepsy in 1999. The possible market includes the many epileptics (up to one third) who do not respond to existing drugs.

Both Neurex and Cognetix are trying to replace conotoxins with orally available small molecules. Olivera sees this as a challenge that may take a new approach. "If one simply screens for competitors of toxin binding it may be difficult to obtain the same discrimination between receptor subtypes," he says.

If the companies cannot solve the rules of conotoxin binding and apply them to small molecules, there is always luck. It has already struck once in this story. Olivera focused on fish-eating rather than worm-eating snails because he hoped that toxins designed to hit certain vertebrates (fish) would also work on mice and humans. But ziconotide doesn't kill humans precisely because it doesn't fit this pattern. Mammals have evolved calcium channels at the neuromuscular junction that, unlike those in fish, are insensitive to ziconotide. As Miljanich says, "lucky for pain sufferers, and for Neurex."

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