Unique Enzyme Targets Neuropeptide

At least one neuropeptide seems to be made by a specific enzyme, a finding that has basic research and clinical applications

"With all neurotransmitters and hormones, identifying the enzymes that make them is terrifically important," says Solomon H. Snyder, director of the Department of Neuroscience at Johns Hopkins University School of Medicine. "If there is a specific enzyme to make each of them, then you can in theory make a specific inhibitor and turn off one of them without affecting hundreds." In addition to being of clinical importance, such inhibitors can also enable researchers to learn exactly what each neurotransmitter and hormone does.

But, in the case of the neurohormones that Snyder studies, until recently it was not at all clear that such specific enzymes even exist. Snyder and his colleagues have just gotten evidence that they do. Snyder presented his results at a meeting held on 31 May and 1 June in recognition of Nobel laureate Julius Axelrod and sponsored by the National Institute of Mental Health.

Snyder is primarily interested in the neuropeptides—small protein fragments that seem to play major roles in the brain, controlling pain, for example, and also in other parts of the body, such as the gut, where they may aid in digestion and in appetite control, and in the kidney, where they may help regulate blood pressure. Researchers believe that there are massive numbers of these neuropeptides and that all are made in the same way. In every case, the cells that produce neuropeptides make a large precursor molecule that is then chopped apart in two steps. First, a trypsin-like enzyme makes a cut and then an enzyme that resembles carboxypeptidase B cuts it again (*Science*, 22 April 1983, p. 395). The process, says Snyder, is "very clever" and "very simple."

Yet it is the simplicity of the process that made it seem possible that there are no unique enzymes for the synthesis of these neuropeptides. "Peptidases are everywhere," Snyder says. "And they are more generalized than other enzymes. If you've seen one peptide bond you've seen them all." It would be possible for the brain to selectively make one neuropeptide or another by sequestering the neuropeptide-producing cells and using general rather than specific enzymes.

The work on finding a specific enzyme began a couple of years ago when Snyder and his graduate student Lloyd Fricker began looking for enkephalin convertase—the enzyme that acts like carboxypeptidase B and is used to make the enkephalins, which are sometimes referred to as the brain's own opiates. They knew that enkephalin and the hormone epinephrine are made in chromaffin granules in the adrenal gland and that, presumably, not much else is made there. They also knew that any peptidase they might find in the granules would synthesize enkephalin, a neuropeptide, and not epinephrine, which is not a peptide. So they set out to purify the granules and look for a carboxypeptidase.

But the task was not, at first sight, a simple one. "It is extremely cumbersome if you have to take as your substrate enkephalin with basic amino acids still attached at one end and then look for an enzyme that converts it to enkephalin. If you work as hard as hell, you can do ten samples in a day." Fricker, however, developed a trick that enables him to examine 100 samples in an hour.

What Fricker did was to make a product consisting of the fluorescent moiety dansyl attached to the amino acid chain phenylalanine-leucine-arginine. The carboxypeptidase he was looking for would cleave the chain between the leucine and the arginine. The original compound—uncleaved—is insoluble in chloroform because of the electric charge of the arginine. When the enzyme breaks off the arginine, the remaining compound is soluble in chloroform and, because of the dansyl, it fluoresces. Thus the assay consisted of looking for an enzyme that would make the original insoluble fluorescent compound soluble.

By using this simple assay, Fricker found a new enzyme in the chromaffin granules, an enzyme that was distinct from any other carboxypeptidase ever before found. Since it was localized in the granules, it was possibly selective for the synthesis of enkephalins. Other carboxypeptidases are everywhere in the brain. But this presumptive evidence that the enzyme was selective was not, of course, proof.

To get proof, Fricker set out to find a compound that would selectively inhibit the new carboxypeptidase. He went to the library and started looking up compounds that were known to block carboxypeptidases and one of the ones he found was GEMSA. When he tried it out, he found that it was tremendously effective—it inhibited the new carboxypeptidase in nanomolar concentrations, whereas 1000 times more of the compound was required to inhibit other carboxypeptidases. "It was just spectacular," Snyder says.

The potency of GEMSA, in fact, was so great that it was in the range of potencies of the drugs that are used to study receptor binding. So Snyder and his colleagues realized that they could measure the distribution of this new enzyme with the same techniques that they use to study the distribution of hormone receptors in the brain.

Working with Stephen Strittmatter and David Lynch of Johns Hopkins, Snyder added tritium-labeled GEMSA to thin slices of brain. The group could then do autoradiography, using the radioactively labeled GEMSA as a probe of where in the brain the enzyme was. "The pictures exceeded our fondest expectations," says Snyder. "I couldn't believe it. It looked just like a map of enkephalin neurons. It was just fantastic."

The implications of the finding are many. If researchers could stop enkephalin formation, the drugs they used could be useful as appetite supressants, to relieve constipation, to bring patients out of shock, and to regulate blood pressure, among other things. These are a few of the clinical applications of opiate antagonists, which are compounds that compete with enkephalins and thereby block their effects. These and other medical uses of the antagonists are now under intensive development, but Snyder points out that antagonists will almost certainly have a different clinical profile than drugs that block the enkephalin-forming enzyme and that both will be medically important.

The applications to basic research are twofold. If enkephalin formation can be specifically blocked, investigators will be able to learn at least just what is the function of these neuropeptides. Enkephalins seem to have a wide variety of effects, but it still is not clear what they really do in the brain. Secondly, the finding strongly indicates that other neuropeptides have their own peptidases too. And if a specific inhibitor of the enkephalin peptidase can be found, specific inhibitors of these other peptidases might be found also.—**GINA KOLATA**