Molecular Biology of Brain Hormones

By using molecular probes, researchers can find which hormones are made by the brain and where

As of about 5 years ago, the very idea that peptide hormones might be made in the brain, anywhere except for the hypothalamus, was astounding. Peptide hormones, everyone thought, were made by endocrine glands and dumped into the bloodstream where they traveled to target organs. These hormones never got to the brain, it was believed, because they do not cross the blood-brain barrier.

But these assumptions about peptide hormones were questioned as laboratory after laboratory found that antiserums to peptide hormones bind to the brain, indicating that either the hormones or something that cross-reacts with the antiserums is present. By this measure, says Joel Habiner of Massachusetts General Hospital, about 35 different peptide hormones are present in the brain.

This immunological method of looking for peptide hormones, however, is imprecise. Cross-reactions are possible and the method cannot determine whether the peptides detected by the antibodies really are the hormones. Furthermore, the method cannot be used to determine whether hormones are made by the brain or are just transported there.

The new techniques of molecular biology, however, provide a way to answer these questions. It is possible to make specific complementary DNA's (cDNA's) that can serve as molecular probes to fish out messenger RNA's (mRNA's) for the peptide hormones. If brain cells are making the hormones, the cells will contain these mRNA's. If the products the brain cells make resemble the hormones but are not identical to them, then the cDNA's should still bind to these mRNA's but should not bind as tightly as they would to mRNA's for the true hormones. Researchers can then decode these mRNA's to determine just what their protein products are and how closely the products resemble the true peptide hormones.

The cDNA probes have another use as well. They often contain the sequences of hormone precursors as well as sequences of the hormones themselves and investigators can use them to piece together the complex control systems that determine which hormones are synthesized and where.

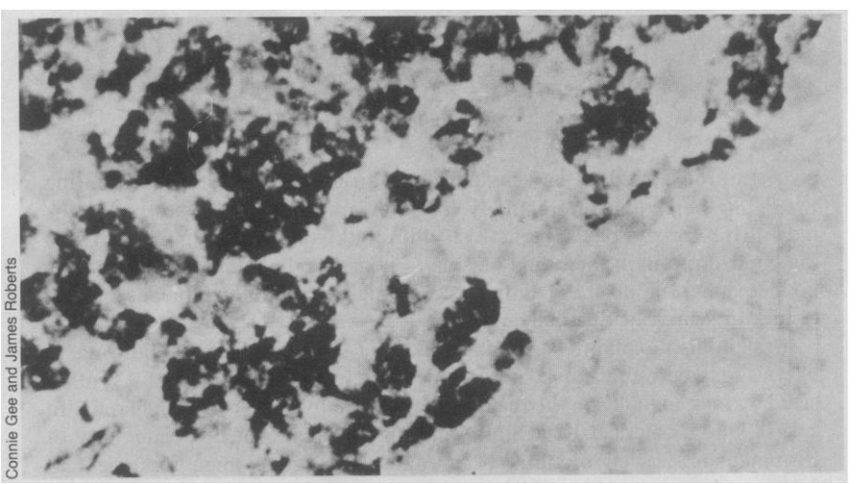
For example, Edward Herbert of the University of Oregon and James Roberts of Columbia University have recently reported that enkephalins are derived from a large precursor mRNA containing sequences for five other hormones, including ACTH, and that different cells cut up the precursor in different ways. Thus, the hypothalamus makes enkephalin and the pituitary gland makes ACTH. Pauline Kay Lund of Massachusetts General Hospital finds two glucagon genes in pancreatic islet cells, each gene containing two copies of the glucagon sequence arranged in tandem. The two copies are transcribed into one large mRNA. In the pancreas, this mRNA is cleaved to form an mRNA just for glucagon. In the intestine, less of the mRNA is cleaved, resulting in an mRNA for the hormone glicentin, which contains the glucagon sequence in its interior.

Still another advantage of the molecular approach using cDNA probes is that it is much faster than the traditional immunological methods. It can take years of tedious purifications to isolate peptide hormones and then raise antibodies to them. Roberts, who is an enthusiastic convert to molecular techniques, explains, "I was trained as a protein chemist and endocrinologist. But it became clear to me that the field of endocrinology needed molecular biology input. Those who are still grinding out protein purifications just are not moving as fast as they could. The addition of molecular biology will really break open the peptide hormone field."

A number of molecular biologists are now making cDNA probes to look for peptide hormone mRNA's in the brain, but one who has made substantial progress is Lydia Villa-Komaroff of the University of Massachusetts School of Medicine in Worcester. She has been looking for insulin mRNA in the brains of rats, mice, and humans—with surprising results.

A few years ago, when she was working in Walter Gilbert's laboratory at Harvard, Villa-Komaroff and her colleagues got a cDNA probe for rat insulin from an insulin-producing tumor of the pancreas. She decided to use this probe to look for insulin mRNA in the rat brain because there was a heated controversy between researchers using immunological methods over whether insulin is synthesized in the brain. Villa-Komaroff found that there is one, and possibly two, mRNA's in adult rat brains that bind to the cDNA insulin probe but that, clearly, are not insulin mRNA's.

Villa-Komaroff went on to study mice and humans, working with her colleagues Antonio Gonzalez, Paul Dobner, and Leslie DeLong in Worcester. She used the rat cDNA probe for the mouse studies to eventually obtain a human



Connie Gee and James Roberts

Growth hormone mRNA's in rat pituitary cells

Radioactively labeled growth hormone cDNA binds to growth hormone mRNA's and appears black in the picture. The left-hand two-thirds of the picture is the anterior pituitary where growth hormone is made. The lower right-hand section is the intermediate lobe of the pituitary and the bottom is the posterior pituitary. Growth hormone is not made in these two areas of the pituitary and the growth hormone cDNA does not bind to mRNA's there.

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insulin cDNA probe from a pancreatic tumor to use for the human studies.

Looking at fetal and neonatal mice, she finds at least three and possibly as many as five different mRNA's in the brain that resemble, yet are different from, insulin mRNA's. Moreover, she does not find these mRNA's in adult mouse brains. Human fetal brains, she has discovered, have at least two insulin-like mRNA's. And in both the human and mouse fetuses, these insulin-like mRNA's appear to be specific to the brain.

Villa-Komaroff has no information yet on what these insulin-like peptides are doing in the brain, but she does have what she refers to as a "working prejudice." She explains, "We think they are going to be growth factors. There are insulin-like growth factors in serum and there is something like them in fetal tissues. Our long-range goals are to make the proteins [coded by the insulin-like mRNA's] in bacteria and study their biology."

Other researchers, taking a similar approach, are just now gearing up to look for peptide hormones in the brain. For example, Richard Goodman of Massachusetts General Hospital is studying somatostatin, a 14-amino-acid peptide found originally in the hypothalamus and subsequently in pancreatic islets.

Goodman extracted somatostatin mRNA from the marble-sized pancreatic islets of the anglerfish. (In humans, the islets are microscopic.) Using this mRNA, he made a cDNA probe with which he looked for and found somatostatin mRNA in frog brains. Then he used the probe to extract somatostatin mRNA from a rat medullary carcinoma, a nerve-related tumor. He copied the rat mRNA into cDNA and cloned the cDNA, thereby obtaining a cDNA probe for mammalian somatostatin mRNA. Now, he says he is ready to look in rat brains for somatostatin mRNA's.

Roberts is planning to look for the peptide hormone LHRH in rat brains, using a cDNA probe. Herbert will be looking for enkephalin mRNA's in the rat brains and Lund plans to use her cDNA probes for glucagon mRNA's to look for that hormone in the brain.

If, as most neurobiologists think likely, there really are numerous peptide hormones that are made in the brain, what is their function? It is doubtful that they are all growth regulators, even if the insulin-like hormones serve this purpose. A number of researchers propose that they are used for intercellular communication in the brain. With the flexibility seen in the processing of the glucagon and opiate genes, the brain could generate a large variety of peptide signals. Says Habiner, "If, on top of the electrical circuitry of the brain you have a whole array of peptide signals that can change the qualitative reactions of cells, you could approach the complexity of signals needed for the brain to function."

Habiner, for one, is quite excited by the possibilities of using the techniques of molecular biology to study the brain. "Until now, there have been few attempts to use molecular biology to study the nervous system because people felt the nervous system was just too complex. But with the techniques of molecular biology available and developing rapidly, it has become reasonable to begin to explore brain functions on a molecular level," he says.—GINA KOLATA

The Scent Makes Sense

Pigs have been known to detect truffles buried as deeply as 3 feet below the ground by scent alone. After the truffle is detected, the sows root for the high-priced delicacy with such vigor that many truffle hunters have turned to dogs simply because they are easier to control. The ability of pigs to detect truffles results from their keen sense of smell, but the intensity with which the sows root them out has been a matter of some mystery. Now, three German investigators think they have solved this mystery with the discovery that truffles contain a pig sex pheromone [*Experientia* **37**, 1178 (1981)].

R. Claus and H. O. Hoppen of the Technical University of Munich and H. Karg of the Lubeck School of Medicine have shown by radioimmunoassay and gas chromatography–mass spectrometry that truffles contain a steroid, 5*a*-androst-16-en-3*a*-ol, that has a pronounced musklike scent. Other scientists had previously shown that this steroid is synthesized in the testes of the

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boar and transferred to the salivary gland, from which it is secreted during premating behavior. Interestingly, the concentration of the steroid in the most highly prized black Perigord truffles and white truffles is about twice the concentration in the blood plasma of boars. "The biological role of this boar sex pheromone," the authors say, "might explain the efficient interest of pigs in search of this delicacy."

It might also explain why humans like the fungus, which is said to taste like a cross between musk, nuts, and ozone. The same steroid is synthesized by human males in the testes and secreted by axillary sweat glands. In a 1978 study, Michael Kirk-Smith and his colleagues at the University of Birmingham showed pictures of normally dressed women to male and female volunteers, some of whom were exposed to the steroid during the viewing [*Res. Commun. Psychol. Psychiat. Behav.* **3**, 379 (1978)]. The volunteers were asked to score the pictures for beauty. Those who were exposed to the steroid consistently gave the women higher scores for beauty.—THOMAS H. MAUGH II

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