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## Hormones Found That Influence Appetite

Researchers may be on the verge of explaining how changes in the brain caused by hunger can lead to increased appetite.

In the February 20 issue of the journal *Cell*, researchers from the Howard Hughes Medical Institute at the University of Texas Southwestern Medical Center at Dallas, announced the discovery of proteins found in nerve cells that stimulate appetite in laboratory rats. The discovery of the nerve cell hormones, called neuropeptides, and their receptors may help explain how the brain senses hunger signals and responds by increasing appetite.

"These newfound hormones and receptors influence feeding behavior," said Masashi Yanagisawa, a Hughes investigator at UT Southwestern. Yanagisawa's team called the neuropeptide hormones "orexins," a name which is derived from orexis, the Greek word for appetite.

### **Risky Strategy Pays Off**

Yanagisawa readily admits that the discovery was serendipitous. The members of his laboratory were searching for molecules that look like cell surface receptors for novel peptide hormones by using computer software to comb through online expressed sequence tag (EST) databases. These databases contain thousands of short sequences from genes that are expressed in a variety of organisms. Many ESTs

represent genes that have not yet been cloned or are of unknown function. Once an EST is identified by researchers, they can easily generate a full length DNA sequence for the gene.

The researchers decided to limit their database search to sequences that were likely to code for G protein-coupled receptors. Proteins from this large family are involved in a wide range of biological processes, including vision and olfaction. Yanagisawa went after these receptors because they are mostly "orphan receptors" those with no known ligand. He suspected that the ligand for many of these receptors would turn out to be a peptide hormone.

The computer research yielded about 50 sequences that the group felt were likely to be G protein-coupled receptors, and then set about using those receptors as bait to capture peptide hormones, their true quarry. This strategy is known in the field as "reverse pharmacology."

"In traditional pharmacological research, the hormone is identified first," Yanagisawa said. "That hormone is then used as a tag to pull out the receptor molecule. We're doing this in reverse."

The grasp of traditional pharmacology is evident to those, like Yanagisawa, who want to pursue more experimental approaches. While it can be fruitful, reverse pharmacology is not without its risks, Yanagisawa said. "This type of research can be very difficult to do because it's difficult to get funding to do it," he said. "Whether a person's an academic scientist or an industry researcher, they can't really do too many risky things."

After identifying the sequences of interest, Yanagisawa's team prepared to introduce the receptors into mammalian cells to see if they could snare the hormone ligands. Their somewhat risky strategy

paid off when they found that one of the receptors reacted with a compound contained in a brain tissue extract. Continuing the "muscle work," the team purified a peptide from the brain tissue fraction and showed it to be a completely novel peptide hormone.

### **Controlling Appetite**

Further work demonstrated that the hormone is expressed in the lateral hypothalamus, an area of the brain known to be involved in feeding behavior. "That immediately led us to think that this peptide might be involved in regulating feeding," Yanagisawa said.

Earlier, now-classical experiments in mice showed that if an animal's lateral hypothalamus is destroyed, the animal eats less, loses weight, and, in extreme cases, may starve to death. These experiments led to the suspicion that the lateral hypothalamus controls appetite in some way, but the molecular basis for this observation has never been found.

After synthesizing large quantities of the newfound hormone, Yanagisawa's team injected orexin directly into the brain of rats to observe if it had any effects on feeding. "Acute feeding increased about six-fold within hours of injection," Yanagisawa said. "We then thought that if this hormone was truly a physiological regulator of feeding, then the synthesis of the hormone should be influenced by the animal's nutritional state."

To test this hypothesis, rats were deprived of food for two days, after which the researchers measured the level of mRNA for orexin in the brains of the animals. "We found that under fasting conditions, orexin mRNA is significantly increased," Yanagisawa said. "It may be that neurons expressing orexin are sensing some signal from the

periphery that says the animal is starved and needs to eat."

The next step of the research, Yanagisawa says, is to try to identify the signal that causes neurons to produce orexin, which in turn leads to increased feeding. Given the pharmaceutical industry's track record in developing drugs that modulate the activity of G protein-coupled receptors, Yanagisawa said he wouldn't be surprised to see a new class of compounds that target the orexin receptor. "This receptor is an ideal target for future drug development," he said.