high in liver and kidney and low in brain. As liver and kidney may be more efficient than brain in permitting access of the heavy metal to the cell interior, the apparent cell specificity of the response may be more reflective of the tissue's characteristics than of the gene's. Brinster notes, "Although the pattern of expression is what you would expect of metallothionein, we can't really say yet that it is expressed in a tissue-specific way."

Some puzzles remain about the expression of the hybrid metallothionein -tk gene in mice. For one, the degree of its expression is not proportional to the number of copies per cell. Like the other investigators, Brinster and Palmiter often find multiple copies of the transferred gene per cell. Usually these copies are arranged in a head-to-tail tandem array. But tissues from animals that have many copies per cell do not necessarily make more enzyme than tissues from animals that have only a few copies per cell, and may make less.

For another, the ability to make the viral tk enzyme may not be stably inherited. Offspring carrying the gene sequences may express the enzyme to a greater, lesser, or the same degree as their parents. If the parent was a mosaic, with the foreign gene in only some of its cells, the level of expression of the gene might appear higher in the tissues of its progeny, who would carry the gene in all their cells. Both Mintz and Costantini and Lacy also found evidence for mosaicism in animals produced by egg injection experiments. The loss of expression is more difficult to explain, however.

The Mintz group, in a very early experiment, found production of the viral tk enzyme in one of five fetuses that developed from injected eggs and carried the foreign gene. But preliminary efforts to detect expression of transferred hemoglobin genes have proved less successful. Mintz, Stewart, and Wagner did not find the human β-globin gene sequences to be transcribed in mice that had acquired them.

Costantini and Lacy detected transcripts of the rabbit β-globin gene in muscle tissue from two mice and possibly in testis from another. But all other tissues, including those of the hematopoietic system, which normally make hemoglobin, gave negative results. Nor did they find evidence of transcription of the hybrid gene, even though it carried mouse sequences thought to be involved in initiating gene transcription. "The tentative conclusion," Costantini says, "is that this gene isn't working any better than the rabbit gene."

The gene works in cultured mouse cells, however. Tom Maniatis of Harvard University, who supplied Costantini and Lacy with the hybrid gene, reported at the meeting on work done in collaboration with Richard Axel of the College of Physicians and Surgeons of Columbia University. They transferred the gene into cultured mouse erythroleukemia cells, where the gene was transcribed. The transcription could be increased by treating the cells with dimethyl sulfoxide, as is that of the endogenous mouse gene, although the level of transcription of the transferred gene is much lower. "There seems little doubt that the cell-specific induction of the globin gene can be achieved and studied," Maniatis concluded.

Several theories, which are not mutually exclusive, have been put forth to explain the difficulties in obtaining expression of genes transferred into mice. Methylation of the foreign sequences might occur during early embryonic life and prevent their expression. Detlev Jähner from Rudolf Jaenisch's laboratory at the Heinrich-Pette Institüt of the University of Hamburg, Germany, presented results showing that methylation prevents the expression of viral DNA that is incorporated into the genomes of mice early in development.

The position of the integrated foreign gene sequences is another factor that may influence their transcription. Generally, the transferred DNA integrates randomly throughout the genome, rather than in the normal location for the particular gene. Transferred genes may be missing remote control sites needed for the normal initiation of their transcription.

Control of genes such as the globin gene may be complicated and easily disrupted. Brinster notes, "The β-globin gene is one of the more fastidious genes. It is cell-specific and developmentally specific, expressed only in certain cells at certain times." Still, the gene transfer work is moving rapidly ahead, and reliable expression seems to be an advance that will come with time.

—JEAN L. MARX

Brain Receptors for Appetite Discovered

Amphetamines and their derivatives bind to the receptors, and the strength with which they bind is directly related to their ability to suppress appetite

Three neuropharmacologists have found what appear to be appetite receptors in the brain. Steven M. Paul and Bridget Hulihan-Giblin of the National Institute of Mental Health and Phil Skolnick of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases report their work in this issue (p. 487). The finding should enable pharmaceutical firms to search quickly for new drugs that suppress the appetite without producing undesirable side effects. It also may explain how amphetamines work and may lead to a coherent theory of how appetite is regulated.

There has been no lack of research on the control of appetite but the field has so far been muddled by a surplus of data and a lack of unifying hypotheses. It is known, for example, that eating is somehow regulated by the hypothalamus—destroy part of the hypothalamus and a laboratory animal will overeat until it becomes obese.

The hypothalamic area of the brain contains the neurotransmitters epinephrine, norepinephrine, serotonin, and dopamine—all of which have been implicated in the control of appetite. For example, several groups of researchers have reported that when they inject norepinephrine directly into animals' brains, the animals overeat and that serotonin injections cause the animals to undereat. A few years ago Sarah Leibowitz of Rockefeller University found that when she injected dopamine or epinephrine directly into animals' brains, they underate.

In addition, drugs that alter the concentrations of these neurotransmitters alter eating patterns. Tricyclic antidepressants cause patients to gain weight apparently, Leibowitz says, by increasing the amount of norepinephrine in their

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hypothalamuses. Methysergide (which is used to treat migraine headaches) alters or interferes with brain serotonin and leads people to gain weight. Leibowitz reports that L-dopa, which is converted in the brain to dopamine and which is used to treat patients with Parkinson's disease, causes animals to eat less and may cause Parkinson's disease patients to lose weight. But investigators have not been successful in attempts to use L-dopa as an obesity treatment, so the effects of this drug on appetite are not clear.

Another substance that may affect eating is cholecystokinin. This hormone was first found in the gut and later discovered throughout the nervous system. When cholecystokinin is injected directly into the brain, animals stop eating, indicating that the hormone affects satiety.

Paul, Hulihan-Giblin, and Skolnick came to their present focus on appetite in a roundabout way. As neuropharmacologists interested in the biochemical basis of such conditions as anxiety and seizures and psychoses, they set out to study amphetamine binding to the brain. Amphetamines and their derivatives cause increased motor activity and at high doses they cause stereotyped repetitive movements. A rat will sit in a corner and lick its paw or nod its head over and over again, for example. The drugs also raise body temperature and constrict blood vessels.

Investigators studying other drugs, most notably the anxiety-reducing benzodiazepines, had learned how the drugs work by doing binding studies. So, says Skolnick, he and Paul set out to do amphetamine-binding studies in the hopes of gaining some insight into the drug's effects on motor activity. Much to their surprise, however, they found two binding sites for the drug, neither of which appears to have anything to do with motor activity. The role of the higher affinity site is unknown. But the lower affinity site, which is concentrated in the hypothalamus and brainstem and which appears on nerve endings, apparently is related to the appetite-suppressing effect of amphetamines. Paul and Skolnick found that the relative abilities of the amphetamines and their derivatives to bind to the low-affinity site are highly correlated only with their abilities to cause loss of appetite.

Hormone and neurotransmitter receptors on cell surfaces usually are regulated—when more are needed, more appear and when fewer are needed, fewer appear. Skolnick and Paul asked, then, What happens to these amphetamine

Jackson Laboratory

A genetically obese mouse with a normal littermate. The obese mouse may have too many appetite receptors.

binding sites when animals are deprived of food and what happens to the sites in genetically obese animals? "We have preliminary evidence that the sites do change as a function of satiety," Paul says. When animals are deprived of food for several days they lose sites.

The next step is to learn what exactly the amphetamine binding sites do. Paul and Skolnick looked first to see if they are actually well-known receptors for other drugs or neurotransmitters and found that they are not. Next they looked to see if they are uptake sites for serotonin or norepinephrine, or dopamine. Drugs, such as the tricyclic antidepressants, that block serotonin uptake do not bind to the amphetamine receptors. Nor does cocaine, which blocks norepinephrine uptake.

It is Skolnick and Paul's belief that the amphetamine binding sites may be neurotransmitter release sites, most likely for the release of norepinephrine or serotonin. Although the involvement of both these neurotransmitters in appetite seems a bit complex, it is unlikely that the two substances act independently. "Neurotransmitter systems communicate. If you affect serotonin you affect norepinephrine. We don't think the systems are as disparate as people once believed," says Skolnick.

To test their hypothesis that these are neurotransmitter release sites, Paul and Skolnick are starting lesioning experiments. They destroy with toxins nerves that contain serotonin, norepinephrine, or dopamine and then see if the amphetamine receptors also are destroyed.

The implications for the drug industry of this finding of amphetamine receptors are important. Increasingly in the industry there has been a movement away from animals and toward in vitro methods to screen for new drugs. Since the ability of amphetamines and their derivatives to bind to their receptors seem unrelated to their abilities to elicit motor activity, it should be possible to screen in vitro for new drugs that bind tightly to the amphetamine receptor and so cause loss of appetite but do not have the side effects of amphetamines.

The analogy is with benzodiazepines—antianxiety drugs such as Valium and Librium. About 6 years ago, investigators found benzodiazepine receptors in the brain and they now are using those receptors to screen new drugs in vitro. Recently, pharmaceutical firms have found substances that have some specific properties of benzodiazepines, such as reduction of anxiety or sedation, without the other properties of these drugs. Yet, says Skolnick, "Until 5 or 6 years ago, people were convinced you couldn't make a better benzodiazepine. They were convinced the side effects were always connected to the drug."

The discovery of opiate receptors in the brain led to the search, and eventually to the discovery of endogenous opiates. The discovery of benzodiazepine receptors led to a search for endogenous benzodiazepines. Is it possible that the brain makes its own amphetamines that control appetite? "We might fantasize about endogenous amphetamines," says Skolnick, "but before we look for them we first must better define what these sites are doing."

Since neither of the amphetamine receptor sites seems related to motor activity, it seems likely that there may be no simple explanation of these behavioral effects of the drugs. The amphetamine binding sites, however, are all over the brain, even though they are concentrated in the hypothalamus and brainstem, so it is possible that, through complex interactions in other areas of the brain, the drugs affect activity. "It is clear that the hypothalamus is involved in eating behavior," says Skolnick. "But it is much more difficult to put your finger on motor activity. It may involve more areas of the brain. Amphetamines may affect serotonin pathways in one area of the brain and norepinephrine or dopamines pathways in another and together these changes may affect different behaviors." In other words, the simple concept of one receptor that affects one behavior may be too simple in this situation.

The function of the second receptor for the amphetamines—the high-affinity receptor—remains a mystery. Skolnick and Paul are not even sure whether it is on or in or near nerve endings. But the amphetamine receptor research has hardly begun and, at this point, surprises are to be expected.—GINA KOLATA