21 September 1984, Volume 225, Number 4668 SCIENCE

Neurosciences: An Integrative Discipline

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One of the major questions in all biology is just how discrete portions of the body come to be where they are and adopt their characteristic appearance and function. Although this issue of *Science* deals with several facets of neurosciences, cell-cell recognition is a central theme. What tells a group of cells in an embryo to sprout an arm? Why do some cell groups develop into the liver, others into the adrenal glands, and yet others into the gonads? The brain is a single organ which, in many ways, displays greater complexities than the entire rest of the body. In embryonic life thousands of discrete neuronal pathways must meander through often convoluted itineraries before reaching their adult locations. It is difficult to conceive how the body can possess signaling mechanisms with such precision to direct this morass of cellular traffic.

The article by Cowan *et al.* (*1*) demonstrates one major principle of organization in the nervous system. The embryonic and newborn brain extends many more neuronal processes than will ultimately be employed by the adult organism. Subsequently, inappropriate neural connections are pruned away. In some parts of the nervous system large numbers of excess neurons simply degenerate and vanish. Black *et al.* (*2*) review research from their own laboratory indicating specific cellular mechanisms that can accomplish some of this synaptic organization and reorganization. One neuron synapsing upon a second one "entrains" it to produce a particular neurotransmitter and to suppress the production of others. Loss of innervation "releases" expression of other transmitter systems. These regulatory mechanisms are retained, in part, even in the adult nervous system and may play a role in the brain's adaptation to injury. Such principles also operate outside the brain as is evident in the adrenal gland, which in the rat produces large quantities of epinephrine or adrenalin and very little of the opiate-like peptide enkephalin. The nervous input to the adrenal normally suppresses the formation of the enkephalins. Interruption of this innervation prompts a vast augmentation of enkephalin biosynthesis, which begins with the processing of genetic information at the messenger RNA level.

The way in which cell-cell contact restricts the expression of genetic information in neurons may provide clues for the control of cell growth throughout the body. One is reminded that cell-cell contact inhibits the proliferation of most cells in culture. The itinerary of neurons during development and cell-cell interactions can be mapped with still greater precision in invertebrates such as the fruit fly, as reviewed by Goodman *et al.* (*3*).

Neuronal cells are directed to grow toward appropriate targets by specific growth factors. The best known of these is nerve growth factor. Recently, numerous other neural growth factors have been identified. Brockes (*4*) addressed these questions by first exploring mechanisms regulating the regeneration of limbs in amphibia. Using monoclonal antibodies, he showed that one of the key stem cells for the new limb is a type of glial cell called a Schwann cell. Glia are generally regarded as "supporting" cells in the nervous system, subsidiary in importance to neurons, although they constitute 85 percent of all cells in the brain. The work reviewed by Brockes suggests that they are principal actors in developmental neurobiology. The stimulation of Schwann cells and eventual limb growth may be regulated primarily by a recently isolated protein referred to as glial growth factor. When Brockes and his colleagues isolated glial growth factor, they noted striking resemblances to platelet-derived growth factor. Part of this latter protein has been shown to be a product of the expression of one of the well-studied oncogenes and thus may have a role in tumorigenesis. The roles of growth factors and of other agents in regulating synaptic plasticity and regeneration are reviewed in the article by Cotman and Nieto-Sampedro (*5*). They have been used to identify neuron populations, cell lineages in development, and mechanisms of axon growth. Reichardt (*6*) reviews the uses of monoclonal and other antibodies to identify functionally related neurons and molecules involved in cell aggregation, migration, position, and growth and to map the distributions of receptors, ion channels, and other proteins in the brain.

Lessons from Genetic Engineering

Techniques of genetic engineering have influenced neurobiology. Many of the major neurotransmitters in the brain are peptides, which are formed from large precursor proteins. Gene cloning techniques have revealed the sequences of several precursors, often with surprising results. For instance, the precursors of the enkephalin peptides have as many as seven copies of enkephalin in each precursor molecule. In this issue Scheller *et al.* (*7*) using the relatively simple nervous system of the snail-like sea slug *Aplysia* describe a striking application of genetic engineering to neurobi-

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ology. Cloning the genes for the slug's egg-laying hormone led to the discovery of a sequence of several peptides in the precursor protein. These various peptides play distinct but closely coordinated roles in the egg-laying process. Thus a single protein contains a series of separate hormones that work together to orchestrate the complex behaviors involved in egg-laying. Sutcliffe *et al.* (*8*) review their work identifying brain specific messenger RNA's. Their hope was that some of these might reveal previously unrecognized neurotransmitter peptide sequences, which has indeed been the case. They found a series of "identifier" gene sequences that occur in almost all genes that are specific for the brain. These identifier sequences are apparently responsible for coordinating the expression of these genes in the brain but not in other tissues. Presumably other organs have analogous tissue-specific identifier sequences, which may help explain why different tissues express different genes.

Rosenfeld *et al.* (*9*) describe their application of genetic engineering to neuroendocrinology. Like Sutcliffe *et al.*, they identified precursors to hormones, in this case calcitonin. In the process, they detected a novel peptide, calcitonin gene–related peptide. At the same time, they also worked out novel principles of genetic organization. Specifically, a single messenger RNA may be transcribed quite differently in various tissues.

Genetic engineering has also found clinical application in the neurosciences. Gusella *et al.* (*10*) describe how they localized the abnormal gene responsible for Huntington's disease to a particular region in a specific chromosome. Huntington's disease is a genetically dominant neurologic disorder that does not become manifest until adult life, when it causes movement abnormalities, dementia, and death. Each offspring of a patient with Huntington's disease has a 50 percent chance of developing the disorder. These gene markers may eventually be used to identify in peripheral blood samples those individuals at risk who will or will not develop the disease.

Synapses

All information processing in the brain involves neurons "talking" to each other at synapses. Accordingly, research in synaptic transmission is central to neurobiology. One area of major advance in neurobiology relates to the number of neurotransmitters in the brain. Chemically, neurotransmitters may be amines, amino acids, or peptides. The greatest proliferation of neurotransmitter candidates has occurred with peptides. At present more than 50 neurotransmitter peptides have been identified. They occur by themselves or are stored together in the same neurons with other transmitters. Co-storage of peptides with amines, amino acids, or other peptides has been revealed largely through immunohistochemical mapping of transmitter containing neuronal pathways. Tomas Hökfelt, whose original research is seminal to this area, reviews its implications for synaptic function (*11*). The interaction of a neurotransmitter with its receptor site and the subsequent translation of this recognition information into changes in ion movement or the formation of second messenger molecules is key to synaptic activity.

Changeux *et al.* (*12*) describe recent advances in the isolation and molecular characterization of the best studied neurotransmitter receptor, the nicotinic acetylcholine receptor obtained from the electric organ of certain fish. This acetylcholine receptor is only 1 of 50 to 100 or more neurotransmitter receptors that have been characterized in recent years and whose properties have been recently reviewed (*13*). Gene cloning techniques have revealed the complete amino acid sequence of the five subunits of the nicotinic acetylcholine receptor. Models have been developed showing how these subunits are folded and interact with each other to provide the recognition site for acetylcholine and the associated channel through which sodium ions flow. Ion channels may be regulated by neurotransmitters, as with the sodium channel of acetylcholine receptors, or controlled by voltage. Stevens (*14*) reviews new developments in characterizing ion channels, especially the use of patch clamp techniques, a most important breakthrough in neurophysiology. This procedure enables researchers to measure electrical changes associated with the opening and closing of a single ion channel. Briefly, a microelectrode is inserted into cells so that a tight seal occurs between the outside of the electrode and the cell membrane, greatly reducing electrical "noise." Then, a recording can be made from the cell containing the implanted electrode. Alternatively, the small patch of membrane impaled by the electrode can be "sucked" out from the cell and inserted into a buffer solution. In this way the effects of carefully controlled environmental manipulations upon the behavior of single ion channels can be measured.

Opening or closing of ion channels follows directly from neurotransmitter recognition at some receptors as is the case for the nicotinic acetylcholine receptor and sodium channels or for gamma-aminobutyric acid (GABA) and chloride ion channels. In other instances "second messenger" metabolic alterations appear to be the first events that follow transmitter-receptor interactions. Cyclic adenosine monophosphate (AMP) is the best known second messenger system. Schramm and Selinger (*15*) review the well-characterized systems whereby hormones or neurotransmitters stimulate or depress the activity of adenylate cyclase, the cyclic AMP–forming enzyme. Cyclic AMP occurs throughout the animal kingdom and even has important functions in bacterial metabolism.

Protein phosphorylation is another universal regulatory system. Some of the best known protein phosphorylation or "kinase" enzymes are stimulated by cyclic AMP. However, as Nestler, Walaas, and Greengard (*16*) show, protein kinases exist which are regulated by calcium, calmodulin, phosphatidylserine, rhodopsin, cyclic GMP, and other mediators. Since phosphorylation of proteins is of such importance in communicating the influence of hormones, neurotransmitters, and other mediators to the interior of cells, it became necessary to identify just which proteins are the normal substrates for these phosphorylating enzymes. Over the years, Greengard and his associates have pioneered in identifying and isolating several specific protein kinase substrates. A number of these proteins are highly selective. For instance, when dopamine interacts with one subtype of dopamine receptor, the D_1 receptor, it initiates phosphorylation of a protein designated DARPP-32. Immunohistochemical studies reveal that DARPP-32 is localized only to neurons that receive input from dopamine-containing nerve endings.

An equally important second messenger system regulates turnover of inositol phospholipids. Nishizuka (*17*) reviews the breakdown of phosphatidylinositol biphosphate releasing diacylglycerol and inositol triphosphate and its role in cellular alteration.

Neuropeptide Synthesis: Enkephalin Convertase

The behavior of neurotransmitter receptor systems is quite reminiscent of enzymes. Receptors are proteins with specific recognition sites for neurotransmitters, just as enzymes recognize their substrates. The only fundamental differ-

ence seems to be that enzymes cause metabolic alterations in the substrates, which does not take place in the case of receptors. Many advances in receptor research have made use of the great wealth of information about enzymes that has been accumulated over the years.

Recently developed tools in receptor research have helped to characterize neurotransmitter-related enzymes. Neuropeptides are initially contained within large protein precursors. Within the precursor they are flanked on both sides by pairs of basic amino acids, arginine or lysine. Their biosynthesis from the precursor involves two successive enzymatic cleavages. First a trypsin-like enzyme cleaves to the right of basic amino acids. This liberates the other end (the amino terminal) of the neuropeptide but leaves a single basic amino acid attached to the carboxyl terminal. Then, an enzyme with carboxypeptidase B–like activity removes the remaining basic amino acid. This pattern of formation has been accepted for both hormonal and neurotransmitter peptides, since the pioneering work of Steiner in 1967 showed that insulin is formed in this way from proinsulin (*18*).

Since peptide bonds are essentially the same for all biological peptides, a few generalized trypsin-like or carboxypeptidase B–like enzymes could serve to generate all neurotransmitter peptides. Alternatively, highly specific enzymes might exist for the generation of individual peptides. Whether or not neuropeptide-specific–synthesizing enzymes exist has not been clear. Recently my colleagues and I identified a novel carboxypeptidase B–like enzyme with a high affinity for the precursors of enkephalin and with a regional distribution in the brain resembling that of the enkephalins (*19*). The difference between this enzyme and other carboxypeptidases was highlighted by the finding that certain chemicals were a thousand times more potent in inhibiting the enkephalin-forming carboxypeptidase, designated enkephalin convertase, than in affecting other carboxypeptidase enzymes. With one such potent inhibitor guanidinoethylmercaptosuccinic acid (GEMSA), we used receptor-binding technology and were able to show that enkephalin convertase occurs in specific neuronal systems (*20*).

Measuring the binding of a radioactive form of the transmitter or related drug to tissue membranes is a means of identifying the receptor recognition site for a neurotransmitter. The key element in the procedure is to use a ligand with very high affinity for the receptor, which minimizes the binding to nonspecific sites of lower affinity. Since GEMSA has affinity in the $10^{-9}M$ range for enkephalin convertase, we could measure selectively the binding of ^{3}H-labeled GEMSA to enkephalin convertase. The technique of autoradiography permitted us to visualize enkephalin convertase by applying [^{3}H]GEMSA to brain slices on a microscope slide and then exposing the slices to tritium-sensitive film. The observed localizations of enkephalin convertase were virtually identical to those of enkephalin-containing neurons, establishing that this enzyme is selectively involved with the biosynthesis of the enkephalins although it may also have other functions (Fig. 1).

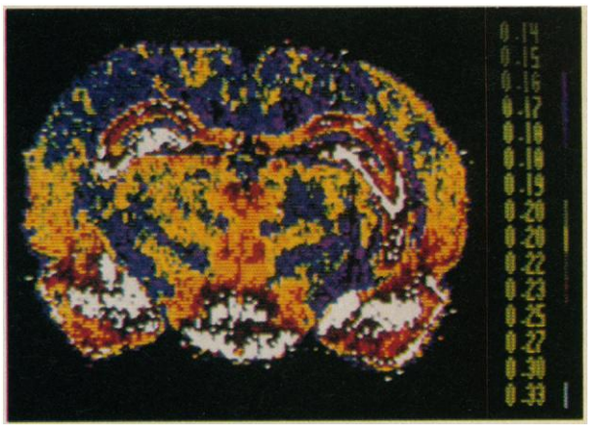

Fig. 1. Locations of the enzyme enkephalin convertase: autoradiography of the enzyme inhibitor [^{3}H]GEMSA. The highest enzyme concentrations, indicated by the white color, include the central nucleus of the amygdala and the hypothalamus.

If specific enzymes exist for the formation of enkephalins, it is likely that similarly selective enzymes synthesize other neurotransmitter peptides. Some of the most important advances in understanding neurotransmitter function have come from drugs that selectively inhibit their biosynthesis. In GEMSA we already have an extremely selective inhibitor of an enkephalin-forming enzyme. This particular compound cannot be readily used for studies of brain enkephalins, since its highly charged character precludes penetration into the brain, a problem that may be overcome with more lipophilic derivatives. Analogous drugs inhibiting the formation of other neuropeptides may have similar utility. Besides their importance for understanding the functions of neuropeptides, such drugs might be valuable therapeutic tools. Numerous effective agents for treating depression, anxiety, and schizophrenia are now available to the physician. These drugs achieve their therapeutic ends by influencing only three or four of the "older" biogenic amine or amino acid neurotransmitters. The neuropeptides have been so recently discovered that the pharmaceutical industry is just beginning to seek psychotherapeutic drugs that work by way of the neuropeptides. The existence of specific peptide-forming enzymes may afford a powerful approach in attaining such therapeutic goals.

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