II

Specific Neuronal Participants and Their Physiological Actions

As we learned in the first series of chapters, each neuron manufactures and stores one chemical transmitter. This becomes its biochemical fingerprint and its principal method of communicating with other neurons. Unfortunately, the transmitter is not known for the vast majority of neurons in the CNS. This is at once the greatest shortcoming in our understanding of the brain, and at the same time the greatest challenge. Far more is understood of the anatomical connections and physiological functions of neurons than is understood of their individual neurotransmitters. And yet when this information has become available, impressive horizons have frequently opened up. New understandings of the integrated action of the brain, new insights into disease states, and new approaches to drug development have resulted.

In this next section we describe the known neurotransmitters, the types of cells that use them, and the physiological actions that can be observed. We also describe another method of neurotransmission, not covered in the first section, which involves a second messenger in the postsynaptic cell. This is covered in detail in Chapter 8. The second messenger, a cyclic nucleotide possessing an unusually powerful high-energy phosphate bond, triggers off a series of chemical reactions with a variety of results. They may be short term, altering the membrane potential, or longer term, bringing about trophic and plastic changes.

Neurotransmitters, therefore, fall into two categories. The first we refer to as *ionotropic* because the principal function of the transmitter is to open ionic paths across the postsynaptic membrane. The action is rapid and produces large conductance changes. Acetylcholine at the neuromuscular junction and at Renshaw cells (Chapter 4); glycine in the spinal cord (Chapters 4 and 6); GABA at higher levels in the CNS (Chapters 4 and 6); and glutamate as a putative excitatory transmitter (Chapter 7) are some examples.

The second we refer to as *metabotropic* because these neurotransmitters

stimulate cyclic nucleotide production and consequent metabolic changes in the postsynaptic cell (Figures 8.9 and 8.11, Section 8.2.3; and 8.13, Section 8.2.4). They are slower acting and do not produce large conductance changes in the neuronal membrane. Dopamine in SIF cells and in the striatum (Chapter 8); noradrenaline (Chapter 8) and serotonin (Chapter 9) at most CNS sites; and acetylcholine (Chapter 5) at some muscarinic sites are examples. Information on the physiology of metabotropic neurotransmitters is scanty compared with the ionotropic ones, as we shall see in succeeding chapters. It is probable that some transmitters function in both capacities depending upon the nature of the receptors, i.e., acetylcholine in the superior cervical ganglion (Figures 8.9 and 8.11, Section 8.2.3; and 15.9, Section 15.4).

Each transmitter has an extraordinarily sophisticated and delicate task to perform. It is the final end product of all the efforts of a neuron. The complex anatomical connections faithfully produced during embryological development and the intricate firing patterns precisely executed by a neuron can be utterly defeated by the failure of the transmitter to finish the job. On the other hand, fine tuning of transmitter action through the use of drugs can sometimes compensate for neuronal malfunctioning in disease states.

Transmitter action can be modified in many ways. Its synthesis, storage, or release may be impaired. Its receptor sites may be blocked, or fooled by a competitor. Its synthesis may be stimulated or its destroying enzymes impaired. The reuptake mechanism which sweeps it away from receptor sites may be interfered with or the receptor sites themselves may be occupied by other, more effective agents. Knowledge of the chemistry and pharmacology of neurotransmitters can thus be a powerful tool in the hands of the neuroscientist or physician.

The first step is to know whether a compound is a transmitter at all. In the past, attempts have been made to set down definite criteria for neurotransmitter status. These may work well in some situations, however, but be of little value in others. All that really can be said is that many tests may be applied to determine whether a material is *the* specific molecule stored in vesicles and released from the terminals of a given neuron. When a certain number of tests are satisfied, then the molecule can be regarded as a bona fide transmitter. If there is reasonable doubt, it is described only as a putative transmitter. If the information is speculative, it is usually referred to as a transmitter candidate.

The following are the more important tests:

Anatomical

1. The substance must be present in the nervous system in reasonable concentration. This is an obvious but necessary condition. There are many compounds that can be isolated from the nervous systems of submammalian species, or from other tissues of mammalian species, which have biological activity. But they are of no interest if they are absent from brain or occur only in miniscule concentrations.

2. The substance should occur in the nerve ending fraction of subcellular homogenates (Figure 5.8, Section 5.3.3). Since a transmitter must be stored in nerve terminals for there to be an adequate physiological supply, failure to find a concentration in this compartment is regarded as important negative evidence.

3. The substance may be distributed unevenly in brain. This is important because it suggests an association with particular neurons. Such uneven distribution was a key point in the identification of dopamine, glycine, and several other transmitters.

4. There may be a drop in concentration of a substance following lesions to known or suspected brain pathways. Since degeneration always takes place distal to a lesion, and also proximally whenever there are no sustaining axon collaterals (chromatolysis), such a concentration change is strong presumptive evidence of association of a substance with a pathway. The technique is almost invariably used in establishing the biochemical nature of long-axoned pathways, but is of no value for short-axoned neurons.

5. A histochemical or immunohistochemical method for the substance may establish its localization to specific neurons and pathways of the brain. This is extremely strong evidence. Histochemistry was the crucial technique for the establishment of the catecholamines and serotonin as transmitters (Chapters 8 and 9). Unfortunately, histochemical methods are extraordinarily difficult to develop and they exist for very few compounds in the CNS. Immunohistochemistry is possible if antibodies can be developed for the material as, for example, with substance P (Chapter 10).

Chemical

1. The presence of enzymes for synthesis and destruction should be identifiable. A method must exist for supplying large quantities of the transmitter to the nerve ending, and then rapidly disposing of it. The synthetic enzymes obviously need to be more specific than the destroying enzymes, and need to be localized to the terminal fraction. The destroying enzymes may be in dendrites or glial cells. The synthetic enzymes should also behave as does the transmitter by dropping in concentration following lesions. Tyrosine hydroxylase, the key enzyme for catecholamine synthesis, for example, is found in the nerve ending fraction, and decreases in concentration following lesions to known pathways, while catechol-O-methyltransferse (COMT), one of the destroying enzymes, is found in the soluble fraction and is not affected by the lesions.

2. Immunohistochemical localization of the synthetic enzymes to specific neurons may be possible. Immunohistochemistry is a powerful new technique which works well with enzymes because of their high molecular weight and the relative ease of developing antibodies to them. It can be effective at the electron microscopic level. As with the transmitters themselves, this cellular and subcellular localization can constitute the most persuasive evidence of all. Immunohistochemical localization of glutamic acid decarboxylase (GAD), and

choline acetyltransferase (CAT), was critical for the establishment of GABA and ACh pathways in brain.

3. The material, or its precursor, should be taken up into nerve endings by a pump, or high-affinity uptake system (Figure 5.7, Section 5.3.3). This permits it to be accumulated against a concentration gradient, and is necessary if the nerve ending is to obtain an adequate supply for physiological purposes. Pumping is a selective, but not absolutely specific, process, so that nontransmitters may be pumped.

4. The material should be released from suitable tissue preparations by a K^+ stimulation in a Ca^{2+}-dependent process. These conditions stimulate *in vivo* nerve stimulation. K^+ is a nonspecific neuronal depolarizer, while Ca^{2+} mobilization in the nerve ending is necessary for transmitter release.

5. The material should be bound to intrasynaptosomal vesicles. Since the binding is reversible, being used only for temporary storage purposes, this is sometimes hard to demonstrate.

6. The molecule, as well as its synthetic enzymes, may be transported by the process of axoplasmic flow. Techniques now exist for demonstrating this in central as well as peripheral pathways, but this is one of the less valuable methods.

Physiological

1. A transmitter must have action at receptor sites. Therefore the material should show definite physiological action when administered by various techniques. Ideally, it should exactly duplicate the effects of stimulation of the nerve or pathway in which it is believed to be the transmitter. Classical tests of neurotransmitter status using peripheral systems required such duplication (Chapter 4), but it is almost impossible to achieve centrally. Iontophoretic application is the most widely used technique, and, while quantitative comparisons are seldom possible, excellent correlation can often be found in postsynaptic neurons between stimulation of a pathway and the iontophoretic application of the presumed transmitter. The iontophoretic technique has been instrumental in turning up GABA, glycine, glutamic and aspartic acids, and many transmitter candidates.

2. A transmitter must be released upon nerve stimulation. Again, such release is relatively easy to achieve peripherally by perfusing isolated organs or ganglia and collecting the released agent. Centrally, it is much more difficult. Cups applied to the surface of the brain, however, or push–pull cannulae embedded in tissue, have been successfully applied to collecting increased quantities of transmitters such as ACh or dopamine following appropriate stimulation.

Pharmacological

1. It should be possible to find agents which interfere with the transmitter at any of the stages of synthesis, storage, release, or action at receptor sites.

Specificity is critical, and yet extremely hard to prove. Selective blockers were extremely valuable in confirming GABA and glycine as transmitters and would be extremely valuable if they could be found for putative excitatory transmitters such as glutamic acid.

2. It should also be possible to find agents which mimic the proposed transmitter at receptor sites, or indirectly enhance its actions in other ways. This could be by stimulating its synthesis or release, or by inhibiting its reuptake or destruction.

In each chapter of Part II the manner in which transmitters came to the attention of neuroscientists is described, followed by the anatomical, chemical, physiological, and pharmacological evidence upon which their proposed roles are based. There are many chapters in such a section which cannot yet be written because it is only possible to present an account of those few transmitters and few pathways which have been biochemically fingerprinted. We have tried to do this with some perspective, realizing that present information is poorly balanced. For example, there are probably fewer than 20,000 catecholamine neurons in the rat brain, and at most a million in the human brain. Yet the data regarding them are immense. On the other hand, the transmitters for most of the 10 billion cells of the human neocortex are unknown. Because there is so much more to learn, stress has been placed on how the known transmitters have been discovered and what techniques have been used to verify their roles.

Although we have grouped neurons according to the transmitter they supposedly manufacture and use, this is a generality which must be pursued with caution. The cells themselves may be very different in nature. For example, there is little in common from a functional point of view between the large, long-axoned neurons of the substantia nigra and the small, intensely fluorescent (SIF) interneurons of the superior cervical ganglion. Yet, because they both use dopamine as their neurotransmitter, they may find common ground in the action of drugs which affect dopamine. Here detailed knowledge can be extremely helpful in understanding the totality of action of a drug.

The section starts with Chapter 5 on acetylcholine, the compound which "rang up the curtain" on chemical transmission in the nervous system. It is the classical ionotropic excitatory neurotransmitter, although, as we will describe, it is by no means certain that it always acts in this fashion in the brain. ACh receptors vary considerably, and there is good evidence for metabotropic action as well.

Chapter 6 is a brief chapter on the ionotropic excitatory amino acids glutamate and aspartate. The evidence that these compounds are neurotransmitters is slim, but they may be the "workhorses" that operate the great majority of the excitatory synapses.

Chapter 7 deals with the classical inhibitory amino acids GABA and glycine. Their ionotropic mechanism of action is reasonably well understood.

Chapter 8, on the catecholamines, focuses on the completely different

aspects of neuronal activity suggested for metabotropic neurotransmitters. They do not seem to obey the microphysiological rules followed by the previously described ionotropic transmitters, but on the other hand they stimulate second messenger production in the postsynaptic cell. Their neurons have tremendous divergence (each dopaminergic neuron of the substantia nigra has roughly 500,000 axonal varicosities for releasing transmitter) so that interference with the transmitter produces widespread effects.

Chapter 9 on serotonin neurons presents even more puzzling data on neurotransmitter action, again emphasizing a possible metabotropic role.

Chapter 10 describes the promising peptides. For modern neuroscientists this is a "hot" field of investigation because the diversity of agents being discovered lends interest to the speculation that many of the neurons not yet fingerprinted may turn out to be peptidergic.

Chapter 11 deals with putative transmitters and mentions a large number of transmitter candidates. The evidence that these compounds are neurotransmitters ranges from very strong in the case of histamine to highly doubtful in many others. Nevertheless, it is from such data that future transmitters will emerge.