Neurotransmitters and Drugs

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PREFACE

This book has been written for students of medicine, pharmacy and other biological disciplines who wish to have a working knowledge of the ways in which drugs can modify neurotransmitter activity at synapses. The actions of drugs working in both the peripheral and central nervous systems are discussed, and we attempt to relate the activity of the drug to the biology of individual neurotransmitter systems. We have written it from a mechanistic point of view in the hope that the therapeutic and adverse actions will be more readily understood.

In the first chapter the processes involved in neurochemical transmission are discussed, and subsequent chapters deal with individual neurotransmitters and neuromodulators. There is a brief section in the last chapter which considers the properties of some drugs whose mechanisms of action are not established. The discussion of each neurotransmitter is divided into six sections. The first five consider the synthesis, storage, release, receptors and inactivation of a neurotransmitter and how these processes are affected by drugs. The final section discusses the therapeutic applications and side effects of drugs described in the preceding sections.

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1 NEUROTRANSMISSION: SITES AT WHICH DRUGS MODIFY NEUROTRANSMISSION

1.1 Axonal Transport 1.2 Axonal and Other Membranes 1.3 Precursors 1.4 Synthesis 1.4.1 Control of Synthesis 1.5 Storage 1.6 Organelles and Enzymes 1.7 Release 1.8 Receptors 1.9 Postreceptor Sites 1.10 Inactivation 1.11 Neurotransmitters and Neuromodulators 1.12 How Drugs Can Be Classified

The idea that nerves may communicate with other cells by releasing small quantities of chemicals at their junctions may have arisen from observations of the effects of poisons on animals. It was found that certain poisons mimic the effects of stimulating certain nerves, and it must have occurred to somebody that nerves release chemicals in response to stimulation. Histological studies showed that there is always a gap between the nerve ending and the target tissue, and that this gap must be crossed if the signal from the nerve is to reach its target.

Otto Loewi provided the first evidence for the actual release of chemical in response to activation of a nerve. Using perfused frog hearts, he showed that a substance was released into the perfusion fluid when the vagus nerve to the heart was stimulated, and the heart slowed. If the perfusion fluid was passed into a second heart which was free of nervous stimulation, then this heart also was slowed. Loewi concluded that, when the vagus nerve was stimulated, a chemical which slowed the heart was released, and that this chemical passed into the perfusion fluid and acted to slow the second heart. More refined techniques were subsequently introduced to demonstrate this process in many organs and tissues. The process has been named **neurochemical transmission**, and the chemicals released have been called **neurotransmitters.**

Several chemicals that act as neurotransmitters have been identified, but not all substances that are found associated with nerves and are able to alter nervous activity are neurotransmitters. The criteria by which it is established that a substance acts as a neurotransmitter are as follows.

(1) The substance must be synthesised within the neurone from which it is released. Enzymes and substrates for synthesis must be present in the neurone.

(2) The substance must be present in the neurone from which it is released. A storage mechanism exists for many neurotransmitters.
(3) Calcium-dependent release appears to occur with all neurotransmitters. Such release must be shown to occur following physiological stimulation of the appropriate neuronal pathway.
(4) A synthetic neurotransmitter applied exogenously must mimic the actions of the true transmitter when the latter is released in response to physiological or electrical stimulation. The exogenously applied substance must behave identically in every regard to the endogenous neurotransmitter in respect of the potentiation by inhibitors of enzymes of inactivation or re-uptake blockers, antagonism by competitive receptor blockers or physiological antagonists and electrical phenomena such as reversal potentials in the postsynaptic tissue.
(5) There must be a mechanism for rapid termination of the action of a released neurotransmitter. The exogenously applied substance must be inactivated by the same mechanism as the true neurotransmitter.

The process of neurotransmission has many common features in neurones which use different chemical neurotransmitters. The gap between the nerve terminal and the tissue is known as the synapse, and such synapses are found at nearly all junctions of a nerve and tissue, regardless of whether the tissue is another nerve, a muscle or a secretory cell. Specialised synapses occur in some non-mammalian species, and transmission at these synapses is believed to be an electrical process. Such synapses are not considered in this text.

The mechanisms involved in chemical transmission at the synapse are vulnerable to the actions of drugs, and it is possible to identify specific sites in the synapse which are sensitive to the action of specific drugs. These sites of action of drugs are shown in Figure 1.1.

1.1 Axonal Transport

Axonal transport is a general term which refers to bulk axoplasmic flow, and to the specialised microtubule system found within axons. Materials are transferred along the axon by these processes, from the nerve-cell body to the nerve terminals. Axonal transport is needed since the nucleus which holds the genetic information for making enzymes is frequently far from the terminals at which the enzymes work. Axoplasmic transport both moves the enzymes necessary for transmitter synthesis and in the case of certain transmitters or neuro-

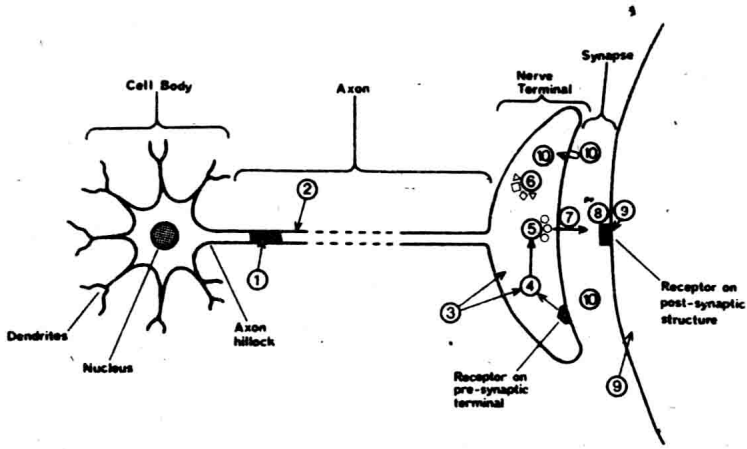

Figure 1.1: Sites at Which Drugs Can Act to Modify Neurotransmission. 1, axonal transport; 2, axonal membrane; 3, precursor availability; 4, neurotransmitter synthesis; 5, storage; 6, intracellular organelles; 7, neurotransmitter release; 8, receptor sites; 9, postsynaptic mechanisms; 10, neurotransmitter-inactivating mechanisms.

modulators, transports the active molecules themselves or their precursors. Some transmitter synthesis might occur during axonal transport, but this is not believed to be a major contribution to the total neurotransmitter found in the nerve terminal. The enzymes and organelles needed for the metabolic activity of the nerve are also carried by axoplasmic flow to the nerve terminal. Axonal transport is well illustrated by experiments in which axons are ligated (tied), and materials such as enzymes, granules and neurotransmitter accumulate on the nerve-cell body side (proximal side) of the ligature.

Substances which non-selectively interfere with axonal transport include **vinblastine**, **vincristine** and **colchicine**, all of which affect spindle formation in dividing cells and, as these structures have some features in common with neurotubules, they are also disrupted. Whereas these compounds have chemotherapeutic applications, they are only used to prevent axonal transport experimentally. Neurotoxicity due to neurotubule damage is a side effect of treatment with these compounds.

1.2 Axonal and Other Membranes

The axonal membrane (and the cell membrane of many other cells) is

only semipermeable to ions. By means of ionic pumps, ionic concentration gradients are maintained between the outside and the inside of the axon. The ionic concentration gradients result in electrical polarisation of the axonal membrane and a potential difference between the inside and outside of the axon. Transient changes in the ionic permeability of the axon membrane allow ions to flow down their concentration gradient, thus depolarising the axon. If the depolarisation is of sufficient size, the ionic permeability of adjacent sections of the axon increases, and a propagated action potential passes down the axon. Local anaesthetics (also called membrane stabilisers) prevent depolarisation of axons and prevent action potentials being propagated down axons. Local anaesthetic activity is not confined to neuronal membranes; most biological membranes across which an ionic potential is maintained are stabilised by local anaesthetics. Whereas prevention of the perception of painful stimuli is a major use of local anaesthetics, they are also used to control cardiac arrhythmias, and substances with local anaesthetic action can prevent the release of neurotransmitters. General anaesthetics are believed to stabilise neuronal membranes in addition to having actions at synapses.

1.3 Precursors

Two types of precursor appear to be used as sources of neurotransmitter. The transmitter may be synthesised in the nerve terminal from which it is released. At such nerve terminals there is an active-transport system in the cell membrane which carries the precursor into the nerve from the extracellular space. Examples of such precursor-uptake systems include tyrosine uptake into noradrenergic neurones, tryptophan uptake into 5-hydroxytryptamine neurones and choline uptake into cholinergic neurones. Increased precursor availability is the basis of some forms of therapy. Inhibitors of the precursor uptake systems are of experimental interest.

In those neurones in which the transmitter is not synthesised in the nerve terminal, a larger precursor molecule may be synthesised in the nerve-cell body and then carried by axonal transport to the nerve terminal. The large precursor molecule is broken down enzymatically into a smaller molecule, which is then released. This appears to be the mechanism by which peptides are brought to nerve terminals. Inhibitors of axonal transport are the only drugs which can modify the availability of these precursors.

1.4 Synthesis

Enzymes found in nerve terminals, together with any cofactors and necessary ions, catalyse the synthesis of neurotransmitter from precursor. Depending on the characteristics of the enzymes involved, it may be possible to speed synthesis by increasing the availability of substrate; intraneuronal mechanisms which control the rate of synthesis may limit the effectiveness of such procedures, however. Inhibitors of the enzymes of synthesis will decrease the amount of transmitter available for release. Such inhibitors are used mainly for research purposes.

1.4.1 Control of Synthesis

The rate of synthesis of many transmitters is closely linked to the rate at which they are released; the rate of arrival of nerve impulses at the nerve terminal can speed or slow synthesis to keep pace with release.

End-product Inhibition. In many instances, end-product inhibition of the rate-limiting enzyme is of importance. High concentrations of noradrenaline in noradrenergic neurones, for example, inhibit the enzyme tyrosine hydroxylase, which determines the rate of noradrenaline synthesis.

Presynaptic Receptors. Presynaptic receptors are believed to detect the concentration of neurotransmitter present in the synaptic cleft and to control the rate of synthesis and release of transmitter appropriately.

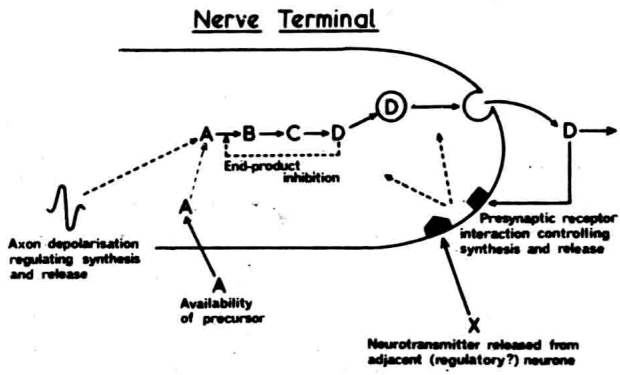

Figure 1.2: Sites of Regulation of Neurotransmitter Synthesis and Release.

This is one of the mechanisms which serve a homeostatic role in maintaining nervous activity within set limits.

Recurrent-loop Feedback. Recurrent-loop feedback by collateral processes from the postsynaptic neurone may synapse onto receptors located on the presynaptic nerve, and thus modify further release of neurotransmitter.

Availability of Precursor. This has been discussed above. If the rate-limiting enzyme is not normally saturated by substrate, then increasing the substrate will result in greater synthesis of neurotransmitter.

The above factors (summarised in Figure 1.2) are not the only influences on the rate of synthesis and release of transmitter. Availability of enzymes of synthesis, cofactors, ions and energy substrates are all of importance. Certain mechanisms have been emphasised as they have therapeutic applications.

Turnover of Neurotransmitter. This is a term used to describe biochemical measurements made in attempts to measure the rate at which neurones use neurotransmitter. Such measures are frequently and freely used to denote the functional state of a neuronal system. There are many problems in applying such a simplistic definition when the rate of transmitter utilisation by the neurone has been indirectly assessed.

1.5 Storage

There appear to be several storage forms of neurotransmitters, and a particular transmitter may be stored in more than one form. Evidence for multiple forms of storage of individual transmitters comes from anatomical, biochemical and pharmacological experiments; it is frequently not possible to obtain agreement from different experimental approaches as to which forms of transmitter storage serve which functions. What does seem to be agreed is that newly synthesised transmitter is generally released in preference to that which has been stored. Certain neurotransmitters appear to be stored in vesicles within nerve terminals. Vesicular storage has not been demonstrated for all transmitters, and indeed certain nerve terminals do not appear to contain vesicles. In such neurones, the transmitter is presumably stored in a different form – for example, in solution in the cytoplasm.

If neuronal tissue is homogenised by mechanical disruption in an isotonic medium, and the homogenate is centrifuged under appropriate conditions, it is possible to obtain fractions of the homogenate which

contain high concentrations of neurotransmitter. Examination of the neurotransmitter-rich fractions under the electron microscope shows that they are composed of what appear to be broken-off nerve endings, some of which contain synaptic vesicles. Such broken-off nerve endings are called **synaptosomes**, and it is believed that they represent presynaptic nerve terminals. Significant amounts of neurotransmitter have been detected in other fractions of homogenates, and this has been taken to indicate that transmitter may be stored in other subcellular structures.

Some neurotransmitters appear to be stored in complexes which include synthesising enzymes, structural proteins and metal ions. Drugs which decrease the stability of the storage complex may result in disruption of storage complexes, and allow transmitter to diffuse into the cytoplasm. A major function of specialised storage complexes of neurotransmitter is believed to be the protection of the transmitter from destruction by enzymes within the nerve terminal; if storage is disrupted, then transmitter will be destroyed. This may inhibit the neurotransmitter function of the nerve as a result of the decreased availability of transmitter for release.

1.6 Organelles and Enzymes

Organelles and enzymes in the nerve ending maintain processes necessary for both the metabolic and the neurotransmitter activity of the cell. The absence of energy substrates – for example, following exposure to metabolic poisons – will lead to decreased transmitter function. The inhibition of enzymes concerned with transmitter synthesis or destruction will have obvious consequences. As pointed out above, neurones maintain a controlled level of transmitter synthesis. Transmitter which is in excess of that needed for release, and which cannot be stored (because storage capacity is exceeded), is usually inactivated intraneuronally by enzymes, resulting in a product which is biologically less active. Inhibition of such enzymes may increase the amount of transmitter stored in the nerve ending.

1.7 Release

At least two processes may operate during the release of neurotransmitter. Some transmitters appear to be released by a process of exocytosis which involves the fusion of vesicular membrane with the presynaptic nerve membrane. Other transmitters are released by less well-defined processes; diffusion through presynaptic membranes and passage through special channels have been suggested. The released

transmitter is then free to diffuse across the synaptic cleft. The process of transmitter release seems to be universally **calcium-dependent.** Calcium enters the nerve terminal in response to an action potential and initiates the process of release. This has been called 'stimulus-secretion coupling'.

Drugs which can influence transmitter release in one of the following three ways are known.

(1) Disruption of storage will cause intraneuronal liberation of transmitter, and this is destroyed by enzymes. No receptor activation may occur following such disruption of stores.
(2) Drugs which release transmitter into the synaptic cleft will cause stimulation of receptors. Drugs which do this are called **indirect receptor stimulants.** Drug-induced transmitter release is calcium-dependent.
(3) Drugs may prevent the release of neurotransmitter without disrupting storage. It has been suggested that such drugs work by stabilising the presynaptic membrane (a local anaesthetic action), or by interfering with calcium entry.

1.8 Receptors

Receptors are discrete regions of postsynaptic membranes which can selectively bind molecules of a specific structure. The act of combining transmitter and receptor is the first step of initiating a response in the postsynaptic neuro-effector tissue.

There is no agreement as to whether the response is initiated by the process of occupation of a receptor or by the whole process of binding onto and the detachment from the receptor; these are, respectively, the occupation and rate theories of receptor activation. Irrespective of the exact process involved, binding of drug and receptor initiates mechanisms which lead ultimately to permeability changes, secretion, muscle contraction and activation or inhibition of enzymes, which are observed as biological responses. Certain mechanisms which are becoming understood are discussed in Section 1.9.

Agonists are substances which initiate a response in the neuro-effector tissue. Tissues generally have a maximal response which cannot be exceeded; substances which can initiate the maximal response are known as **full agonists.** A substance which initiates a response in the tissue but which cannot initiate the maximal response is known as a **partial agonist.** An alternative term for agonist is **stimulant.**

Substances which prevent an agonist from initiating a response are