MECHANISMS OF DRUG ACTION

Volume I

Edited by

G.N. Woodruff

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Preface

Drugs are used to treat, and often to cure, a wide range of illnesses in man and in his domestic animals. A knowledge of the mechanisms involved in the therapeutic actions of these drugs is likely to throw light on the underlying biological disorders involved in the disease and help in the development of new and better treatments. Furthermore, drugs are used to probe biological mechanisms and an understanding of mechanisms of drug action has proved to be of immense value in basic research in medicine, neuroscience, physiology, biochemistry, anatomy, histology, histochemistry, microbiology, genetics, botany and other disciplines.

Several excellent textbooks of pharmacology are available and the authors of these works are confronted with an ever-expanding literature as new drugs are developed and new uses are found for old drugs, and as the biological mechanisms of action of existing drugs become more fully understood. The aim of this series is to supplement the standard textbooks. It is hoped that the series will provide an up-to-date account of the latest theories of drug action. In this, the first of the series, the emphasis is on drugs that act on the nervous system.

Drugs can affect synaptic transmission in a number of different ways. The conduction of nerve impulses down axons involves the movement of ions into and out of the nerve cell. The negative resting potential is, to a large extent, determined by the relative concentrations of K^+ ions across the membrane. Excitation or inhibition of neurons can be brought about by changes in permeability of the neuronal membrane to sodium, calcium or potassium ions. Drugs affecting the permeability of neuronal membranes to ions might be expected to have a wide variety of actions in different parts of the brain, in the periphery, in a wide variety of species.

The mechanisms by which excitation in a neurone is transferred into excitation or inhibition of a second neurone or muscle fibre, or a gland, has been the subject of much scientific debate (Bacq, 1975). It is now well-established that neurones influence the activity of other neurones or muscles by the liberation of small amounts of a chemical transmitter. These chemical neurotransmitters can be either excitatory or inhibitory. It is clear that the mammalian brain uses

a host of different chemical transmitters. Selectivity of drug action can be achieved by drugs which interact only with one neurotransmitter system. For example, a drug structurally related to glycine might influence transmission at synapses which utilise glycine as a transmitter, but be inactive in modifying cholinergic or adrenergic transmission. In practice, drug selectivity is relative rather than absolute, but high degrees of selectivity can be achieved. For example, the affinity of the dopamine antagonist (+)-butaolamol is 100 000 times higher for dopamine receptors than for muscarinic receptors in rat brain (Seeman, 1980).

It is possible for drugs to interfere with the process of chemical transmission by several different mechanisms. The processes responsible for the production, storage and release of the transmitter in the neuronal terminals are, of course, likely points of attack.

Another major site of action is at the receptors. Substances that are chemically related to the natural transmitter can interact with common or adjacent binding sites on the membrane. This can result in the drug behaving as an agonist or antagonist, that is, mimicking or blocking the effect of the natural transmitter. Partial agonists can have agonist or antagonist actions, depending upon factors such as receptor reserve and 'tone' in the system.

By producing drugs that can act at the level of the receptor we can achieve a considerable selectivity of action. Most, if not all, neurotransmitters appear to have associated with them more than one type of receptor. It has long been known that acetylcholine acts on 'muscarinic' and 'nicotinic' receptors and that adrenaline acts on α and β receptors. More recently, further subdivisions have been discovered, e.g. α_1, α_2, β_1, β_2. Thus it is possible to devise drugs which will block or mimic only some of the actions of the natural neurotransmitter or hormone.

Receptor activation is followed by a number of biochemical changes which translate receptor occupancy into initiation of a biological response. One commonly utilised 'second messenger' is cyclic AMP, produced from ATP in a reaction catalysed by the enzyme adenylyl cyclase, the activity of the enzyme itself being modified by the neurotransmitter or hormone. Phosphodiesterase inhibitors such as caffeine inhibit the breakdown of cyclic AMP, the second messenger.

Another mechanism of translating receptor occupation into an intracellular signal involves the generation of inositol triphosphate by hydrolysis of phosphatidylinositol-4,5-biphosphate. This system is certainly susceptible to modification by drugs, both at the receptor level and at the level of the secondary messengers. For example, inositol-1-phosphatase, the enzyme catalysing the conversion of inositol-1-phosphate to inositol, is blocked by lithium (Berridge and Irvine, 1984). Drugs can enhance the actions of a transmitter, and thus potentiate or inhibit synaptic transmission, depending on whether the transmitter is excitatory or inhibitory at that synapse, by preventing its inactivation. Transmitters can be inactivated by enzymic destruction or removed from the synaptic

cleft by uptake into neurones or glia. An example of the former is the hydrolysis of acetylcholine by acetylcholinesterase and inhibition of the enzyme by physostigmine or nerve gases. The well-known ability of many of the antidepressant drugs to inhibit the neuronal uptake of noradrenaline and 5-hydroxytryptamine is discussed in chapter 6.

In this volume, the emphasis is on drugs which act on the nervous system by an action at the synapse, in the peripheral and central nervous systems.

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Chemical Transmission in the Central Nervous System : Amino Acids, Acetylcholine and Amines

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ABBREVIATIONS

ACh, Acetylcholine; AHP, after-hyperpolarisation; ASP L-aspartic acid; BTX, α-bungarotoxin; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; CNS, central nervous system; DA, dopamine; DBH, dopamine β-hydroxylase; EPSP, excitatory postsynaptic potential; GABA, gamma-amino-butyric acid; GLU, L-glutamic acid; GLY, glycine; G_x, conductance for ion x; 5-HT, 5-hydroxytryptamine; IPSP, inhibitory postsynaptic potential; KA, kainic acid; NA, noradrenaline; NMDA, *n*-methyl-D-aspartic acid; 6-OHDA, 6-hydroxy-dopamine; QUIS, quisqualic acid; TTX, tetrodotoxin; $[\ ]_i$, intracellular concentration.

INTRODUCTION

SCOPE OF THE CHAPTER

The topic of this chapter has been growing by leaps and bounds in the last decade. An enormous amount of information has been generated which cannot be reviewed comprehensively in a relatively short space. (A recent survey of transmitters in only a small region of the brain, the olfactory bulb (Halasz and Shepherd, 1983), covers 40 pages and lists 320 references!) The aim therefore will be to summarise the main points of importance concerning the probable

transmitters and their modes of action. No attempt will be made at a systematic historical survey: for greater details of the earlier history, readers are referred to some comprehensive reviews that appeared 12 years ago (Curtis and Johnston, 1974; Krnjević, 1974a; Tebecis, 1974).

WHAT IS CHEMICAL TRANSMISSION?

Since the turn of the century, it has been generally accepted that the nerve cells of the brain and spinal cord (the CNS) are independent units (Sherrington, 1906; Ramon y Cajal, 1909). Hence, although most neurons are probably capable of generating self-propagating electrical signals (action potentials) that can travel the full length of the cells, the question arises how such signals are transferred from one cell to the next?

During the first half of the century, the majority opinion among electrophysiologists was that the action potential generated sufficient current across the junctional region (the 'synapse': Sherrington, 1906) to excite the following nerve cell (Eccles, 1936). But a different viewpoint was developing at the same time from experiments on peripheral organs: various endogenous chemicals extracted from tissues could be tested reliably on structures such as muscle, glands, the heart, etc., whose function (contraction, secretion, heart beat) could easily be measured. Elliott (1904) was the first author to propose explicitly that a nerve may exert its effects by releasing a specific chemical (adrenaline); but the first concrete evidence in favour of such a mechanism came only 14 years later, with Loewi's (1921) demonstration that vagal inhibition of the heart beat was mediated by the release of a humoral factor (Vagusstoff), which proved to be ACh. A systematic investigation of skeletal neuromuscular transmission, by Dale and his colleagues, provided strong evidence of its cholinergic nature (Dale, 1938).

At a quite early stage, ACh was convincingly shown to be concentrated in certain regions of the brain (MacIntosh, 1941). Significant progress in studies of central synapses, however, was blocked by the split between, on the one hand, the 'pharmacologists', who believed that chemical transmission was highly probable at central synapses (with a strong bias in favour of ACh, catecholamines and histamine as the probable transmitters because they played an important role in peripheral structures) but who lacked the electrophysiological expertise needed to record synaptic potentials, and, on the other hand, the electrophysiologists, who knew their way about the CNS, but felt no need to invoke any other than purely electrical mechanisms in synaptic transmission. Experiments crucial for any advance required that sophisticated electrophysiological techniques be combined with a pharmacological approach.

Such experiments began soon after the first intracellular recordings of neuronal potentials in the spinal cord (Eccles, 1953). It was now possible to analyse with some precision the various electrical manifestations of synaptic action in the light of the recently discovered excitatory and inhibitory synaptic potentials at

the vertebrate and invertebrate neuromuscular junction (Fatt and Katz, 1951, 1953); moreover the observations could be interpreted at least semi-quantitatively in a comprehensive theoretical framework – that of Hodgkin and Huxley's (1952) empirical description of ionic conductances and the action potential.

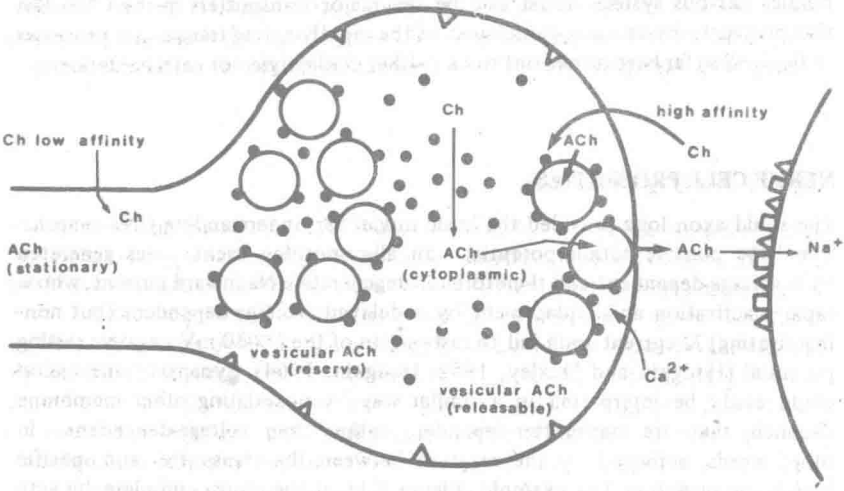

Figure 1.1 Model of a cholinergic nerve terminal, illustrating the synthesis, storage and release of ACh. Black dots, cholinacetyltransferase; triangles, acetylcholinesterase, inside terminal and on postsynaptic cell; squares, postsynaptic ACh receptors. (Figure 11a from MacIntosh and Collier, 1976)

An early result of Eccles's studies was his conversion to the idea that synaptic transmission must be mediated chemically. According to this concept – which was soon widely accepted – a specific transmitter is synthesised and stored in the nerve ending; it is released by the arrival of action potentials, which trigger a Ca^{2+} influx. After diffusing across the minute synaptic gap, the transmitter causes excitation or inhibition of the 'postsynaptic' cell by activating specific receptors, and thus initiating a selective opening of ionic channels. As a result there is enhanced flux of ions along their electrochemical gradient – inward for Na^+ and/or Ca^{2+} (hence depolarisation and excitation), and outward for K^+ or Ce^- (hence hyperpolarisation and inhibition). The transmitter is rapidly removed from the synaptic gap, either by direct uptake into the nerve ending or neighbouring glia, or by hydrolysis. The cholinergic synapse (illustrated in Figure 1.1) provides the classical example of the latter mechanism, choline – the product of hydrolysis of ACh – being then transported back into the nerve ending for resynthesis of ACh (MacIntosh and Collier, 1976).

Very conveniently, the motoneuronal recurrent inhibitory pathway – consisting of motor axon collateral branches and the inhibitory Renshaw cells – was particularly amenable to selective stimulation, and it could readily be shown to be cholinergic (Eccles *et al.*, 1954). This was fully in keeping with expectations that ACh and other transmitters of the peripheral (including the autonomic) nervous system would also be the major transmitters in the CNS. But this proved to be an exception: most of the rapidly-acting transmitter processes in the CNS so far have turned out to be neither cholinergic nor catecholaminergic.

NERVE CELL PROPERTIES

The squid axon long provided the basic model for understanding CNS neurons. The large positive action potential – an all-or-nothing event – was generated by a voltage-dependent and therefore self-regenerative Na inward current, whose rapid inactivation and replacement by a delayed, voltage-dependent (but non-inactivating) K current soon led to restoration of the 60–80 mV negative resting potential (Hodgkin and Huxley, 1952; Hodgkin, 1964). Synaptic transmission could easily be interpreted in a similar way by postulating other membrane channels that are transmitter-dependent rather than voltage-dependent – in other words, activated by the reaction between the transmitter and specific membrane receptors. For example (Figure 1.1), at the motor end-plate, by activating a relatively large increase in G_{Na} (Fatt and Katz, 1951; Takeuchi and Takeuchi, 1960), ACh generates a depolarising end-plate potential. A transmitter that evoked a selective enhancement of either G_{Cl} or G_K, on the other hand, would hold the membrane potential near the resting level and thus produce inhibition (Fatt and Katz, 1953; Eccles, 1953, 1964).

In the last decade, new information from both invertebrate and vertebrate studies has greatly complicated the 'simple' picture. In the first place, the action potential of most nerve cells probably involves a sizeable component of Ca-inward current (Baker, 1972; Hagiwara and Byerly, 1981; Llinas, 1983). The corresponding Ca channels are voltage-dependent, though much less prone to voltage-dependent inactivation than are typical Na channels. The full manifestation of Ca currents, however, is normally prevented by opposing K currents, of which there appear to be several kinds (Llinas, 1983). Especially prominent is a Ca-dependent K current, which is triggered by an increase in intracellular free Ca^{2+} (Krnjević and Lisiewicz, 1972; Meech, 1972, 1978); being generated by membrane channels with a particularly high unitary conductance (Sakmann and Neher, 1984), it readily dominates the cell's behaviour. Since it is both voltage- and Ca-sensitive, this current can be activated by both Ca influx and large depolarisations, and it causes an after-hyperpolarisation (AHP) that is often conspicuous and plays an important role in controlling on-going firing (Baldissera and Gustaffson, 1974; Krnjević *et al.*, 1978). The neuronal firing characteristics also depend on some other K currents, such as the fast-inactivating

A current (Connor and Stevens, 1971) and the non-inactivating M current (Brown and Adams, 1980). Both are voltage-dependent and sensitive to quite small depolarisations (unlike the delayed rectifier) – and therefore tend to prevent cell firing.

The present situation, therefore, is that quite a large number of both inward and outward ionic currents have been found in CNS neurons that have distinct voltage and time dependencies (Llinas, 1983). It is not certain, however, that they really represent channels with unique properties rather than the particular conditions of testing (cf. the comparable complicated situation in cardiac tissue: Noble, 1984). It is by no means clear, for example, that all the different K currents are not generated by essentially one basic type of K channel that appears to be more or less voltage-, time- or Ca-dependent, according to experimental conditions.

Any one of these 'currents' is potentially susceptible to enhancement or depression by transmitter action. The possible repertoire of transmitter mechanisms is therefore extremely large and varied. It is known, for example, that the facilitatory action of muscarinic agents is mediated by a depression of K-outward currents (Krnjević *et al.*, 1971; Brown, 1984) – a similar effect is produced by a variety of agents, including peptides (Krnjević, 1977; Nowak and Macdonald, 1981; Adams *et al.*, 1982) and monoamines (VanderMaelen and Aghajanian, 1980). Some transmitters, on the other hand, may selectively depress Ca-inward currents (Dunlap and Fischbach, 1978, 1981), thus providing a powerful mechanism for regulating transmitter release from nerve terminals (as in pre-synaptic inhibition).

An increase in intracellular free Ca^{2+} concentration ([Ca]) may occur without any alteration in membrane Ca currents, either by a release of internally bound Ca^{2+} or a reduction in Ca^{2+} -outward transport. A slowing of metabolic activity owing to hypoxia, lack of glucose, low temperature, or by various drugs can thus be expected to raise $[Ca]_i$ and therefore G_K (Krnjević, 1975). Whereas a state of low G_K is characterised by a high responsiveness to various inputs, a high G_K reduces responsiveness to a minimum: these two extremes may provide a basis for the contrasting behavioural states of arousal on the one hand, and sleep or narcosis on the other.

This brief survey can give only a perfunctory indication of the many ways in which synaptic transmitters (and modulators) may affect neuronal behaviour. Intracellular Ca^{2+} has been emphasised because of its manifold involvement in neuronal function.

ORGANISATION ACTION OF CNS

There are several ways of looking at the CNS in broad terms. Like many other kinds of cells, neurons are secretory cells. As far as is known, secretion is universally triggered by a rise in [Ca] (Rubin, 1974). Although neurons are specialised so as to transmit electrical signals, sometimes over very long distances, the

ultimate aim is to release transmitters (and probably other significant messengers), especially, though not exclusively (Cuello, 1983), from their nerve endings.

The CNS can also be viewed as made up of successively higher levels of organisation (spinal cord, brainstem and basal ganglia, sensorimotor cortex and prefrontal cortex) representing an evolutionary hierarchy (Jackson, 1887). This has some relevance for transmitter studies, because certain transmitters are more prominent at certain levels: for example, ACh as a nicotinic agent at the peripheral and the spinal levels; glycine at the spinal and brainstem levels; GABA in the forebrain and cortex.

The CNS has also been seen as consisting of a diffuse, reticular core (or 'isodendritic core': Ramon-Moliner and Nauta, 1966), which may be principally involved in the control of the internal economy of the organism (metabolism, blood supply, water balance, temperature, etc.) and related basic drives (hunger, thirst, reproduction); and an outer, more differentiated portion – with well-defined nuclei, tracts, cortical structures – responsible for the interaction between the organism and the environment (Yakovlev, 1948). ACh and monoamines may be particularly important within the core and at the interface between the core and the outer system (including the limbic brain), where psychic states may be generated (Yakovlev, 1948; Gray, 1982; S. D. Iversen, 1984).

Inhibition

A most important feature of CNS organisation is the preponderant role of inhibition. Inhibitory cells and synapses probably make up the largest single population of central neurons (Iversen and Bloom, 1972; L. L. Iversen, 1984). At most sites in the CNS, inhibitory postsynaptic actions (IPSPs) are a prominent feature both of on-going activity and of responses evoked by natural or electrical stimulation (Eccles, 1969). Central neural activity is everywhere under a tight control, which is itself modulated by inhibitory pathways, releasing the activity of limited groups of effector cells only as required for function (Roberts, 1976). Activation by disinhibition – effectively releasing the brake – is a particularly safe mechanism for initiating and executing any action, because it is inherently self-limiting. This may well account for the preponderance of GABAergic synapses and the surprising variety of mechanisms that modulate either the release of GABA or its synaptic efficacy. The cerebellar cortex and the striatum provide outstanding examples of neuronal circuits whose outputs are mediated by GABAergic inhibitory cells (Eccles, 1969; Penney and Young, 1983). Figure 1.2 illustrates the complex interactions between cortex, striatum and thalamus, in which chains of inhibitory cells play a major role.

Presynaptic Inhibition

The most prominent inhibitors are mediated postsynaptically, by transmitters which raise G_{Cl} or G_K and thus reduce excitability. Some transmitters, on the

other hand, act on presynaptic terminals, to reduce Ca influx and therefore reduce transmitter release. Whether this effect is secondary to terminal depolarisation (Eccles, 1964) or whether it is caused by a direct interference with the Ca current (Dunlap and Fischbach, 1981) is not yet certain. When the transmitter acts on the nerve endings from which it is released, the result is autoregulation (Starke, 1981); when it acts on others, it causes presynaptic inhibition (Eccles, 1964).

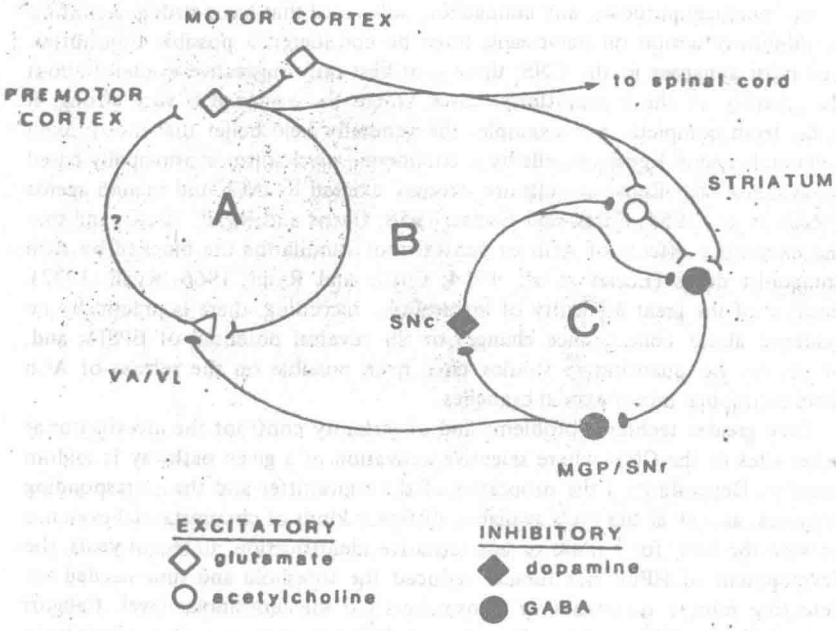

Figure 1.2 Diagram of basal ganglia pathways, indicating the probable transmitters. Note positive feedback loops between cortex and thalamus (A), and between cortex and striatum via thalamus (B); and negative feedback loop between substantia nigra and striatum. Abbreviations: MGP, medial globus pallidus; SN_r, substantia nigra reticulata; SN_c, substantia nigra pars compacta; VA/VL, ventral anterior and ventral lateral thalamic nuclei. (Figure 1 from Penney and Young, 1983)

IDENTIFICATION OF TRANSMITTERS

Synaptic transmitters are by definition endogenous substances that are released by nerve endings and have potent excitatory or inhibitory actions on nerve cells. The essential criteria for identifying the (or a) transmitter at a particular synapse are therefore the following.

(1) Activation of the nerve terminals should evoke release of the putative transmitter in sufficient quantity to produce the observed synaptic potentials.

(2) It should have a postsynaptic action identical in all its characteristics (excitation or inhibiton, conductance and potential changes, reversal potential and time course) with the physiological synaptic action.

(3) Synaptic transmission should be facilitated or diminished by drugs which have a corresponding selective action on the synthesis, removal or postsynaptic action of the putative transmitter.

For practical purposes, any endogenous substance that has a strong excitatory or inhibitory action on nerve cells must be considered a possible transmitter. For most synapses in the CNS, there is at best only suggestive evidence about the identity of the transmitter(s). Even where the evidence is very strong, it is far from complete. For example, the generally held belief that motor axon collaterals excite Renshaw cells by a cholinergic mechanism is principally based on evidence that Renshaw cells are strongly excited by ACh and related agents (Eccles *et al.*, 1954; Curtis and Eccles, 1958; Curtis and Ryall, 1966), and that the excitatory effects of ACh or ventral root stimulation are blocked by ACh antagonist drugs (Eccles *et al.*, 1954; Curtis and Ryall, 1966; Ryall, 1972). Because of the great difficulty of intracellular recording, there is practically no evidence about conductance changes or the reversal potential of EPSPs; and, of course, no quantitative studies have been possible on the release of ACh from intraspinal motor axonal branches.

Even greater technical problems and uncertainty confront the investigator at other sites of the CNS, where selective activation of a given pathway is seldom possible. Depending on the properties of the transmitter and the corresponding synapses, as well as the tools available, different kinds of circumstantial evidence provide the basis for a more or less tentative identification. In recent years, the development of HPLC has further reduced the threshold and time needed for detecting minute quantities of transmitters (to the fentomolar level: Caliguri and Mefford, 1984); and voltametric techniques now permit a continuous monitoring of extracellular concentrations of monoamines (Lane *et al.*, 1976; Lamour *et al.*, 1983). There has been a tremendous growth of immunohistochemical techniques which have greatly boosted studies on the cellular localisation of enzymes involved in transmitter synthesis (for GABA, Ribak *et al.*, 1978; for monoamines, Molliver *et al.*, 1982; and for ACh, Mesulam *et al.*, 1984), even transmitters themselves – not only peptides, but also much smaller molecules, such as 5-HT (Steinbusch, 1981), glutamate and GABA (Storm-Mathisen *et al.*, 1983).

The demonstration of a variety of 'neuropeptides' in many central neurons and fibres has had a particularly wide impact. Because peptides are often present in cells and terminals together with other agents (Hökfelt *et al.*, 1978; Cuello *et al.*, 1983; Chan-Palay and Palay, 1984), an important question that arises is whether or not all the putative transmitters that can be detected in nerve termi-

nals are necessarily released. In at least one instance, there is compelling evidence that this is not the case: noradrenaline is not released from sympathetic fibres grown in culture under conditions that favour a cholinergic function, *even though the noradrenaline-producing enzymes remain present* (Furshpan *et al.*, 1982).

The possible 'co-release' of two or more neuroactive substances at a given synapse could pose a serious problem in the identification of transmitters. For technical reasons (transient intracellular recording, electrotonically distant synapses, etc.), it is difficult enough to apply the criterion of 'identity of action' (Werman, 1966) rigorously, even if only a single transmitter is released. If two or more agents (having different actions) are involved in the generation of a PSP, the difficulties may well be practically insurmountable (especially if one agent increases and the other reduces the membrane conductance: Werman, 1980).

On the other hand, some notable technical advances have greatly facilitated the study of transmitter action. They include the advent of brain slices (Yamamoto and McIlwain, 1966; Lynch and Schubert, 1980; Dingledine, 1984) and nerve cells in culture (Nelson and Lieberman, 1981) – which provide much more stable intracellular recording – as well as microelectrode voltage-clamp techniques, applied to neurons *in situ* (Schwindt and Crill, 1980), in slices (Johnston *et al.*, 1980, 1984; Halliwell and Adams, 1982) or in cultures (Macdonald and Barker, 1981), or even to small patches of cell membranes (Neher and Sakmann, 1976; Sakmann and Neher, 1984). The voltage clamp is essential for the identification of voltage-dependent conductances, either in the 'whole cell' or at the level of individual ionic channels (with the patch clamp). Radioligands have become available for every possible variety of transmitter agonist and antagonist, permitting a wide range of studies on binding and uptake of relevant agents and the distribution and characteristics of corresponding receptors (Seeman, 1980; Olsen and Leeb-Lundberg, 1981; Roberts, 1981; Leysen and Tollenaere, 1982; Birdsall *et al.*, 1980, 1984; Foster and Fagg, 1984; Snyder 1984). A serious drawback of many binding studies, however, is the uncertain correlation with a significant physiological function (for example, in the case of α-bungarotoxin binding sites: Oswald and Freeman, 1981). What determines the synthesis, distribution and functional significance of receptors is still largely a mystery. The recent demonstration (Miledi *et al.*, 1983b) that amphibian oocytes can be induced to manufacture functional receptors to several transmitters by the intracellular injection of foreign messenger RNA, even from human brain (Gundersen *et al.*, 1984a, b), opens the way to systematic studies of this problem.

CNS TRANSMITTERS

It is now widely believed that – as was proposed two decades ago (Krnjević, 1965, 1970) – some monocarboxylic and dicarboxylic amino acids are the