Neurotransmitters, Drugs and Brain Function.
Edited by Roy Webster

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Section C

NEUROTRANSMITTERS IN DRUG ACTION AND DISEASE STATES

In the preceding sections we have outlined and evaluated the methods by which NT function may be studied and considered the general pharmacology of the major NTs. This should enable us to consider the possible role of NTs in disease states and drug action.

The object is to determine not only whether the symptoms of a particular disorder of the CNS can be explained by the malfunction of a certain NT but whether drugs which are known to be effective in a disorder have a distinct effect on one NT system. These objectives are not unrelated since if a disorder is shown to be due to the increased activity of a particular NT then at least some drugs which are effective in its treatment could be assumed to work by decreasing the function of that NT. Similarly if a whole range of drugs are found to be effective in a certain disorder and all increase the activity of one NT then the disorder could in turn be due to a reduced function of that NT. Ideally one would hope to establish the NT malfunction that causes the disorder and then develop appropriate drugs to counter that malfunction and treat the disorder. In practice this has rarely happened, mainly because of the difficulty of establishing a true NT malfunction in humans.

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14 Study and Manipulation of Neurotransmitter Function in Humans

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MEASUREMENTS OF NEUROTRANSMITTER FUNCTION IN HUMANS

BIOCHEMICAL APPROACHES

Before it can be established that a disorder is related to a change in the activity or use of a given NT it must be shown that one (or more) of the following measurements, made in patients with that disorder, is significantly different from the same measurements obtained in appropriately matched controls. Such measurements include (a) plasma, urine, CSF or post-mortem brain levels of the NT, or its metabolites, (b) the activity of its synthesising or degrading enzymes in post-mortem brain tissue and (c) the number or affinity of its receptors in the brain. Needless to say, any change observed must only be found in patients with that disorder and it must not be a consequence of drug therapy, diet or other identifiable factors. Even then it is still necessary to establish whether the change causes the disorder or results from it. The objective is clearly demanding and yet the methodology to realise it is often unreliable.

The value of using measurements of a NT, or its metabolites, in blood and urine as an index of its function in the CNS must be questionable. In fact their value depends to some extent on the NT being studied. ACh is rapidly metabolised synaptically and the choline quickly re-used, so there is no end-product metabolite to pass into the blood. The amino acids GABA and glutamate are not only rapidly taken up into neurons and glia but incorporated into various biochemical pathways and so are not likely to influence plasma levels. Peptides are also broken down rapidly. For these reasons much attention has been focused on the monoamines since despite uptake and re-use their metabolites can be measured in blood and urine, even if their origin is not easily established. Thus one may be interested in 5-HT within the CNS but since over 90% of the body's 5-HT is in the gut, its activity there will have much more effect on plasma levels than any alteration in its central function. The same could apply to NA except that 3:4 dihydroxyphenylglycol (MOPEG) is thought to be the main metabolite of brain NA, while 3-methoxy-4-hydroxymandelic acid (VMA) comes predominantly from peripheral NA. The restriction of DA primarily to the CNS gives it a somewhat unique position but very little metabolate reaches the plasma. If plasma concentrations are of little value then analysis of the urine is even more pointless. Because of these problems much hope was placed on the analysis of CSF but this can only be obtained by the

relatively dangerous procedure of lumbar puncture and as the composition of CSF at the base of the spine may not be the same as that in higher brain areas, yet alone reflect synaptic levels, it is rarely used. Microdialysis has been used in humans but raises ethical issues.

The value of direct studies on brain tissue itself depends to some extent on whether the disorder has a distinct neuronal lesion or just a biochemical malfunction with no clear neuronal degeneration. Post-mortem studies in Parkinsonian patients show a clear degeneration of the substantia nigra and while this was observed long before the corresponding loss in DA it has been possible to link them. On the other hand, a clear link between the long-established and diffusely distributed neurofibrillary tangles and cholinergic dysfunction in the brains of Alzheimer's patients has been more difficult to identify. Even when a distinct focus can be established, as in some forms of epilepsy, the results of studies on changes in NT levels have been equivocal.

In psychiatric disorders there is no clear neurodegeneration and the assumed biochemical fault has been even more difficult to identify. In schizophrenia the impetus to study the involvement of dopamine came from the discovery that all anti-schizophrenic drugs were dopamine antagonists but clear evidence of any malfunction of that NT, apart from a possible increase in receptor number, has not been forthcoming (see Chapter 17). Similarly with depression, the knowledge that most anti-depressant drugs increase NA (and/or 5-HT) function was a valuable lead but again direct attempts to implicate a reduced activity for either NT in humans have been disappointing (see Chapter 20).

Such difficulties prompted research workers to look for some other index of NT function in humans. These range from studies on platelets, such as abnormalities in their amine uptake and MAO activity in depressed patients, to changes in the secretion of a hormone known to be controlled by a particular NT. Thus if NA controls growth hormone release, and the secretion of the hormone is changed in depressed patients, does that confirm a role for NA in the mediation of depression?

BRAIN IMAGING

There is one experimental approach to the study of NT function that has probably advanced more in humans than in animals and that is the visualisation (imaging) of brain structures and chemicals *in situ*. Certainly the stimulus has come from clinical studies. Such neuroimaging began just over two decades ago with the use of X-ray computerised tomography (CT) which distinguishes between different brain regions through variations in their density, as measured by the differential attenuation of X-rays passed through the brain at different angles and, after elaborate computerised analysis, gives a clear image of the brain. Nuclear magnetic resonance (NMR) which utilises the signals given out by hydrogen nuclei in tissue when irradiated with radio-frequency energy provides better pictures. Both of these approaches provide anatomical detail but direct assessment of chemicals in the brain relies on positron emission tomography (PET).

This measures the distribution of a previously administered positron-emitting isotope. PET could be regarded as a form of *in vivo* autoradiography except that the radioligand is not [^{3}H] but [^{15}O], [^{13}N], [^{11}C] or [^{18}F], all of which have short half-lives (2, 10, 20 and 110 min respectively) and so the labelled ligand can only be prepared just before use. After intravenous injection the ligand can be located in the brain in a particular place

within a plane (the equivalent of a slice) by positron cameras (gamma detectors) arranged around the subject's head at the appropriate level. The positron emitted from the proton of the isotope collides with an electron in the atomic orbit so that two gamma-rays are given out simultaneously at 180° to each other. Since the detectors only respond when they make two simultaneous detections, i.e. from the two emitted rays, they can record their precise origin and thus the location of the labelled ligand. The intensity of the detected emission is colour coded and reflects the concentration of ligand (Fig. 14.1). Of course, a low level of emission will be detected throughout the brain from the presence of the labelled substance and its metabolites in the blood and extracellular fluid, as well as that non-specifically located in all neuronal and glial tissue and such background activity must be distinguished from the more specific labelling.

PET has two basic uses in studying NT function.

(1) Localisation of specific NT terminals. After its injection a labelled precursor should be taken up and detected in appropriate nerve terminals (and possibly cell bodies) so that the intensity of emission reflects the density of nerve terminals and the innervation. Using this procedure it has been possible to show that very little [^{18}F] fluorodopa is concentrated in the striatum of Parkinsonian patients, compared with normals (Fig. 14.1). Whether the label remains on dopa or is transferred to dopamine will not greatly affect the result since both will label DA neurons although some will occur in noradrenergic nerve terminals.
(2) Labelling NT receptors. The injection and subsequent detection of an appropriately labelled ligand can give an indication of the density of the receptors to which it is bound. Various ligands have been used in this way to label and measure the number of DA and 5-HT receptors in schizophrenics (see Chapter 17) and the extent to which they are occupied by drugs used to treat the disorder. As with any binding study the validity of the approach depends on the specificity of the ligand for its receptor. Of course, there will always be the background activity mentioned above, but the extent of specific binding may be gauged by comparing the density of emission in any area where the NT is likely to function (e.g. striatum for DA) with that from one where it is not normally found (e.g. cerebellum). The difference between these two levels should in fact increase as unbound drug is lost (excreted). To determine the precise number of receptors and see if that varies from brain to brain (e.g. between normal and schizophrenic) is somewhat more difficult. Normally the estimation of receptor number requires a measure of specific binding at two or more ligand concentrations under equilibrium conditions (see Chapter 3), which will clearly be difficult *in vivo*, not least because the effect of different doses may be unacceptable to the patient or subject. Appropriate quantitative analysis has, to some extent, overcome this. It must also be remembered that much of the *in vivo* binding can be to presynaptic receptors and uptake sites as well as postsynaptic receptors, although drugs specific for those sites can be used to label nerve terminals.

IMPLICATIONS OF NEUROTRANSMITTER MALFUNCTION FROM DRUG STUDIES

It is perhaps not surprising, in view of all the problems associated with studying NT function directly in humans, that much attention has been focused on evaluating how

drugs which are therapeutically effective in a particular disorder may modify the function of a NT. That NT may be implicated in the disorder if it can be shown that the relative therapeutic effectiveness of a range of drugs correlates significantly with some action they have on it. This requires that all the compounds must have a similar chance of reaching the CNS, or at least of providing appropriate plasma levels, and also that compounds with little or no clinical efficacy in the disorder do not modify the NT in the same way as effective drugs. Such approaches have proved useful. Thus there is a clear correlation between DA receptor antagonism, as measured by ligand binding studies and the anti-schizophrenic activity of a wide range of phenothiazine and butyrophenone derivatives (see Chapters 7 and 17). Good correlations between the analgesic potency of morphine derivatives and displacement of the labelled morphine antagonist, naloxone, helped not only to formulate the concept of opioid receptors and hence of endogenous opioids to occupy them but also the actual discovery of the enkephalins. Displacement of labelled diazepam by a wide range of other benzodiazepines, in an order in keeping with their clinical efficiency as anxiolytics, led to the realisation of endogenous benzodiazepine receptors.

Unfortunately a significant correlation between the clinical efficacy and a particular pharmacological effect of a range of drugs may give the impression that that is the only way in which that disorder may be treated. This can be counterproductive. When drugs are evaluated for therapy in a peripheral malfunction, the tendency is to try to show that they work through different mechanisms. Indeed it is considered desirable, if not essential, to develop anti-hypertensive drugs with different actions, e.g. α_1 and β antagonists, α_2 agonists, vasodilators, calcium channel blockers, etc., and then to use them in sensible combinations. Yet remarkably, when dealing with the CNS, the tendency has been to try to treat a disorder by manipulating just one NT. Consequently the assumption arose that all anti-depressant drugs must augment NA function and all neuroleptic anti-schizophrenic drugs must be DA antagonists, etc. Certainly the introduction of some drugs that are effective in depression without blocking NA uptake, which was the conceived mode of action of all the earlier tricyclic anti-depressants, not only gave a new approach to therapy but also questioned the long-held view that depression is due only to reduced NA function. There could be as many ways of controlling depression as there are of reducing blood pressure and a number of different NTs involved.

ANIMAL MODELS OF HUMAN DISORDERS

Neurotransmitter malfunction in a disorder could be studied in animals in which:

(1) the disorder has been induced by CNS lesion or changes to the animal's environment
(2) the function of a particular NT has been manipulated to see if it produces symptoms of the disorder
(3) there are spontaneous signs of the disorder or they have been produced by genetic manipulation

Specific animal models are discussed in some detail in the appropriate chapters on epilepsy, depression, schizophrenia, etc.

All such animal procedures suffer from the obvious and basic problem that laboratory animals do not behave like humans and that humans cannot reliably interpret their reactions and behaviour. Thus we know that Parkinson's disease is caused by a degeneration of the dopaminergic nigrostriatal tract but its lesion in animals does not produce any condition which resembles human Parkinsonism, except in primates, even though there are functional tests (e.g. rotational movements) which readily establish that loss of dopamine function and also respond to its augmentation (Chapter 15). By contrast, there are many ways, e.g. electrical stimulation and the administration of certain chemicals, to induce convulsions in animals and a number of effective anti-epileptic drugs have been introduced as a result of their ability to control such activity. Indeed there are some tests, as well as animals with varied spontaneous seizures, that are even predictive of particular forms of epilepsy. But then convulsions are a very basic form of activity common to most species and epileptic seizures that are characterised by behavioural rather than motor symptoms are more difficult to reproduce in animals.

It is a statement of the obvious to say that we cannot tell when a rat is anxious or depressed, assuming that they can even experience such human reactions, but they can be subjected to conditions that would make us anxious or depressed. Whether animals then react in the same way as humans is not certain but we have to assume that to be the case if the animal is to be used to detect changes in NT function that can be related to humans. This may be a more acceptable assumption if the animal model responds to drugs that are effective in humans, although it can prejudice thinking and perhaps restrict it to a consideration of just one NT. Thus since all drugs with anti-schizophrenic activity are dopamine antagonists, at least to some extent, the predictive value of any model of that condition has been evaluated by its responsiveness to DA antagonists. Consequently such tests only yield more DA antagonists—a property that can be established in the test tube. Of course, changes in other NTs may be found in such animal tests and a really genuine model of schizophrenia could generate totally different drugs. These problems are well known to experimental psychopharmacologists whose studies are becoming more sophisticated and, hopefully, more appropriate and predictive.

APPROACHES TO THE MANIPULATION OF NEUROTRANSMITTER FUNCTION IN HUMANS

Once the malfunction of a particular NT has been established in a disease state, we need to find ways by which its activity can be restored to normal. The approaches used are indicated in Fig. 14.1 and outlined below. It is assumed that no NT crosses the blood-brain barrier and so its activity must be modified indirectly.

METHODS OF INCREASING NT FUNCTION (Fig. 14.1(a))

(1) ***Increase synthesis***. This may be achieved by giving the precursor, if it crosses the blood-brain barrier. Whether this works will depend on (i) how many neurons remain to synthesise the NT, unless this can be performed extraneuronally and (ii) the availability of synthesising enzymes. Thus if synthesis is a complicated multi-stage process or is controlled by the availability of enzymes that are already reduced or working maximally in remaining neurons, this approach may prove difficult.

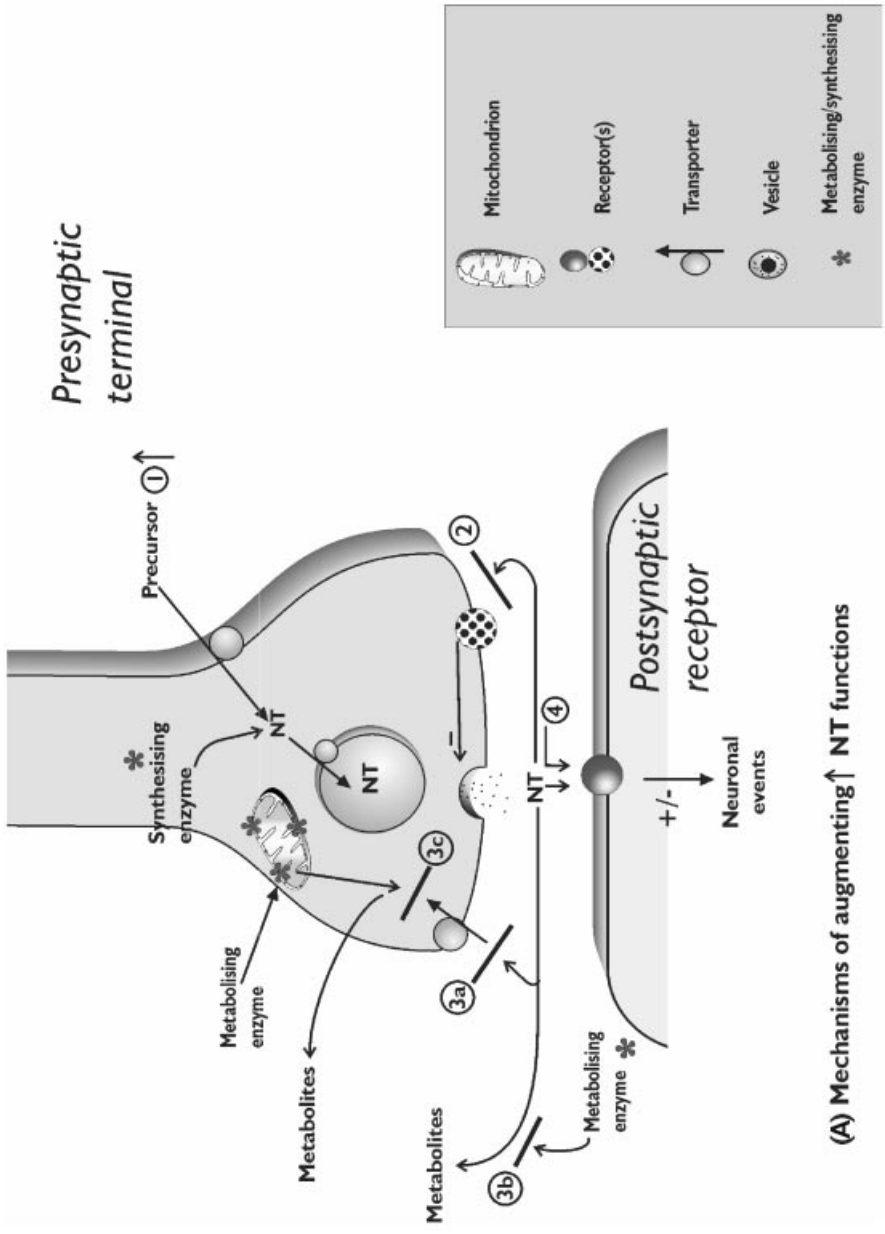

Figure 14.1 *Caption opposite*

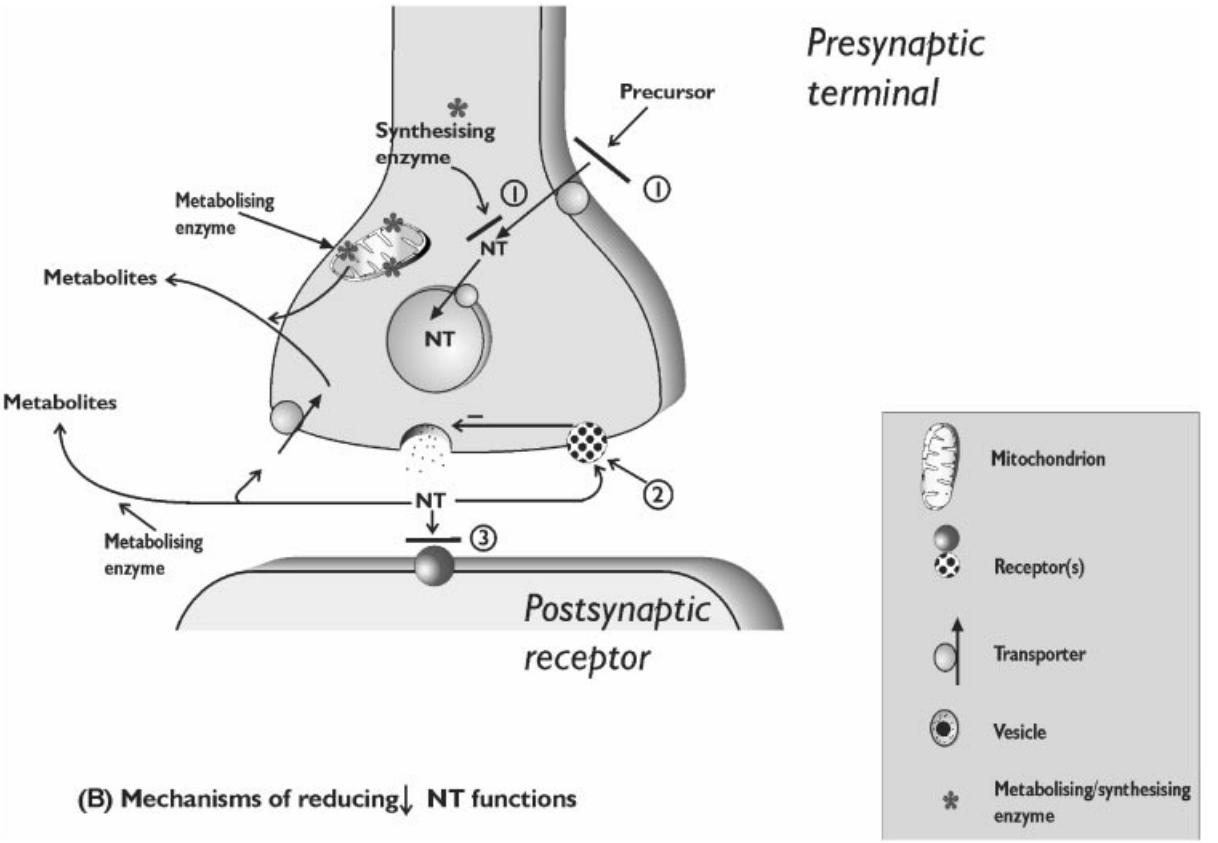

Figure 14.1 *continued* Diagrammatic representation of a neuronal synapse and the mechanisms by which the action of a neurotransmitter may be either augmented (A) or reduced (B). Augmentation of a NT (A) could involve; providing the precursor (1), increasing release by blocking autoreceptors (2), reducing destruction by blocking its reuptake (3a) or extra- (3b) or intraneuronal (3c) metabolism, or providing appropriate postsynaptic agonists (4). Reduction of NT function (B) follows blocking synthesis (1), reducing release by stimulating autoreceptor (2), or using antagonists (3). For further details see text

(2) ***Increase release***. This should follow block of any presynaptic inhibitory autoreceptors. It is not practical at present to increase the vesicular release of a particular NT.
(3) ***Reduce destruction***. This may be achieved by blocking the neuronal or glial uptake (3a) of the NT or its extra- (3b) or intraneuronal metabolism (3c). Its success depends on there still being an adequate, even if reduced, release of the NT, and the protected NT being able to work postsynaptically and not stimulate autoreceptors to reduce the synaptic release of the endogenous NT even further. If the uptake sites are outside the synapse then the protected NT may not easily gain access to the receptors located postsynaptically.
(4) ***Give an appropriate agonist***. Many of the problems associated with the above approaches may be circumvented by administering an appropriate agonist. This could be designed chemically so that it crosses the blood–brain barrier, has a long half-life, and works on the most appropriate subset of receptors, although experience has shown that sometimes more than one effect (receptor action) of the NT may be required. It would be counterproductive if the drug activated the presynaptic autoreceptors unless they happen to augment release. The synaptic action of a NT may also be increased by drugs that have an allosteric action on the receptor to increase its affinity or response to the endogenous NT, e.g. benzodiazapines at the GABA receptor.

Approaches (1)–(3) clearly depend on there being some residual neuronal function and NT release.

METHODS OF DECREASING NT FUNCTION (Fig. 14.1(b))

(1) ***Stop synthesis***. This may be achieved by inhibiting the appropriate enzyme. Its value depends on all, or at least one stage, of the synthesis being sufficiently specific to the NT involved so that only its synthesis is affected. A good example would be choline acetyltransferase in the synthesis of ACh or glutamic acid decarboxylase in the synthesis of GABA. By contrast, inhibiting amino acid decarboxylase could reduce NA, DA and 5-HT synthesis. It may be possible to reduce the neuronal uptake of a precursor if this requires a specific transport mechanism. Thus the synthesis of ACh can be reduced by blocking the uptake of precursor choline with hemicholinium.
(2) ***Reduce release***. This is most likely to be achieved by stimulating inhibitory presynaptic autoreceptors (2a). Some drugs may reduce storage (2b) and hence release, although it is unlikely that this can be targeted at just one NT.
(3) ***Give an appropriate antagonist***. As with agonists, these have the advantage that they can be designed to have a long half-life and act specifically on one type of receptor.

Currently it is not possible to increase the rate of removal (uptake) or metabolism of a NT.

RELATING NT MANIPULATION TO THE CAUSE OF THE DISORDER

To what extent the above approaches can provide successful therapy will depend on both the cause of the disorder and the manner in which the NT is used in normal neuronal function. Thus the disorder could be due to:

(1) An actual degeneration of a NT pathway or
(2) No actual degeneration but a biochemical abnormality or some circuitry failure, leading to inadequate or excessive activity of the NT.

The requirement in respect of NT function may be:

(a) That it must be released physiologically from its nerve terminals by appropriate synaptic activity in order to produce the desired effect or
(b) That it is sufficient merely to provide the NT at the synapse, without the need for it to be released physiologically.

Clearly a disorder combining (1) with (a) would mean that little improvement could be expected by manipulating the lost NT, since the nerves are no longer there to release it physiologically. The main hope then would be to try to replenish the neurons with transplants (regeneration may be possible one day) and hope they become appropriately innervated, or modify the action of some other NT which has become exaggerated (or reduced), as a result of the primary NT loss. By contrast it is easier to treat a disorder, whether characterised by neuronal degeneration ((1) above) or not (2), if it is sufficient just to provide NT (b), as appears to be the case in Parkinsonism.

Of course, the effectiveness and specificity of any of the above manipulations will depend on how widely the NT is distributed and used and whether the malfunction applies only to one area or activity. Thus trying to increase (or decrease) the activity of a NT in only one area will be difficult if it has actions elsewhere which have not been affected by the disorder. The nervous system also has remarkable adaptive powers so the synaptic loss (or increase) of a NT is generally followed by a local compensating increase (decrease) in postsynaptic receptor number. This can be a useful response initially but it will be negated by the therapeutic provision of more (less) NT. Also a change in the activity of one NT can lead to desirable compensating changes in the function of other NTs either working in conjunction with it or normally controlled by it. These will be lost by replenishment of the NT.

It must also be remembered that some NTs, like ACh, NA and 5-HT, have important peripheral as well as central roles and any attempt to modify them centrally will affect those peripheral effects as well. This may be avoided, or reduced, by utilising the blood–brain barrier. Thus if attempts made to increase the central action of a NT result in peripheral effects, these may be counteracted by using an appropriate antagonist that does not cross the blood–brain barrier. It is less easy to overcome peripheral side-effects caused by using a drug that antagonises the action of a NT, although in theory drugs that mimic or augment its action and do not cross the blood–brain barrier could be used. In fact this approach has proved valuable in treating the peripheral neuromuscular disorder of myasthenia gravis which presents as a muscle weakness caused by insufficient cholinergic activity at skeletal neuromuscular junctions. The function of ACh can be increased and the symptoms alleviated, without central side-effects, by reducing the destruction of ACh by giving the anticholinesterase drug neostigmine, which does not cross the blood–brain barrier. Of course, nothing is perfect and anti-muscarinic drugs may be needed to overcome the accompanying increased peripheral parasympathomimetic effects of ACh.

Despite all these problems there has been considerable progress in the treatment of disease states through NT manipulation. Before the advent of levodopa therapy in Parkinsonism the treatment of neurological and psychiatric disorders had little

scientific basis but the initial and striking success with levodopa in Parkinsonism perhaps raised false expectations. In respect of drug therapy, Parkinsonism presented with a number of advantageous features that are unlikely to be repeated in other conditions. It involves a relatively specific degeneration of one particular NT (DA) pathway, DA has a precursor (levodopa) that readily crosses the blood–brain barrier and its peripheral metabolism to DA can be stopped by decarboxylase inhibitors that themselves do not cross the blood–brain barrier. Although DA is used elsewhere in the brain, nowhere is it concentrated to the same extent as in the striatum, where the degeneration occurs, and it has few peripheral effects. Thus side-effects are relatively few. Fortunately DA also appears to be synthesised from levodopa even in the absence of DA neurons and it does not appear to have to be released synaptically in a physiological way in order to control striatal activity and reduce the symptoms of rigidity and akinesia. Even so, long-term therapy with levodopa has not been without its problems and disappointments and highlights the difficulties of replacement therapy.