Neurotransmitters, Drugs and Brain Function.
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20 Depression

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INTRODUCTION

Depression and mania are both 'affective disorders' but their symptoms and treatments are quite distinct. Mania is expressed as heightened mood, exaggerated sense of self-worth, irritability, aggression, delusions and hallucinations. In stark contrast, the most obvious disturbance in depression is melancholia that often co-exists with behavioural and somatic changes (Table 20.1). Some individuals experience dramatic mood swings between depression and mania. This is known as '*bipolar disorder*' which, like mania itself, is treated with lithium salts or neuroleptics.

The focus of this chapter is '*unipolar depression*' in which patients suffer long bouts of depression interspersed with periods of remission. Unipolar depression is regarded as either '*reactive*' or '*endogenous*': whereas the former is apparently triggered by a recent life event involving 'loss' (e.g. bereavement or redundancy), the latter has no obvious origin, although it is often associated with disease (e.g. viral infection, diabetes, neoplasia), drug use/misuse or chronic pain.

A major difficulty in unravelling the neurobiological basis of depression is that it is not a simple, unitary disorder. Also, whereas about 33% of patients recover spontaneously, about the same proportion do not respond to any treatment and only about 60–70% of patients who do respond show any improvement with the first drug of choice. So far, there is no explanation for these different prognoses. There is an urgent need to find a solution to these problems, though, because this is a debilitating disorder that affects about 1 in 10 women and 1 in 50 men at some stage in their life. In over half of these cases, the depression recurs and about 15% of depressives commit suicide. A particular concern is that, in the UK, about 1 in every 200 depressed patients attempts suicide by overdosing on their drug treatment (Henry and Rivas 1999).

These statistics alone stress the urgency of finding more effective and safer treatments for depression but this requires a better understanding of its underlying neurobiology and the mechanism(s) of action of existing drug treatments. This chapter outlines some of the progress that has been made so far.

LINKING DEPRESSION AND CENTRAL MONOAMINES

A link between the central monoamines, 5-hydroxytryptamine (5-HT) and noradrenaline, and depression was forged some 40 years ago and arose from clinical experience with the drugs, reserpine and iproniazid. At that time, reserpine was used as an

Table 20.1 Symptoms and signs of depression

Psychological	Depressed mood
	Anhedonia
	Low self-esteem
	Guilt
	Cognitive deficits
	Poor concentration
	Feeling of 'hopelessness'
	Suicidal thoughts
	Hypochondria
Somatic	Unintentional weight change
	Fatigue
	(Appetite and sleep disturbance)
	Aches and pains
Behavioural	Psychomotor agitation or retardation
	Self-neglect
	Sleep disturbance

antihypertensive and neuroleptic agent but a major problem was that it induced suicidal depression in some (but, interestingly, only about 5–10%) patients. From studies in rats it emerged that reserpine consistently caused behavioural changes, such as motor inactivity and sedation (the 'reserpine syndrome'), which resemble certain features of depression in humans. These behaviours, and consequently those in humans, were attributed to the depletion of neuronal vesicular stores of monoamines and the reduction in monoamine transmission caused by this drug.

In contrast, iproniazid, introduced in 1951 for treatment of tuberculosis, induced euphoria and was described as a 'psychic energiser'. In fact, these patients, when given iproniazid, could become quite disruptive and this action was regarded as an undesirable side-effect! However, its beneficial effects in depression were soon recognised and it was regarded as the first effective antidepressant drug. Studies of peripheral sympathetic neurons, later extended to noradrenergic neurons in the brain, showed that iproniazid irreversibly inhibits the catalytic enzyme, monoamine oxidase (MAO). Because only cytoplasmic monoamines are accessible to MAO, inhibition of this enzyme first increases the concentration of the pool of soluble transmitter but this leads to a secondary increase in the stores of vesicle-bound transmitter: i.e. the pool available for impulse-evoked release (Fillenz and Stanford 1981).

Iproniazid also prevents the 'reserpine syndrome' in rats. Reserpine blocks vesicular uptake of monoamines which, as a consequence, leak from the storage vesicles into the cytosol. Although these monoamines would normally be metabolised by MAO, they are conserved when a MAO inhibitor (MAOI) is present, and so co-administration of reserpine and a MAOI leads to accumulation of monoamines in the neuronal cytosol. It is now known that, when the concentration of cytoplasmic monoamines is increased in this way, they are exported to the synapse on membrane-bound monoamine transporters. The ensuing increase in monoamine transmission, despite the depletion of the vesicular pool, presumably accounts for the effects of iproniazid on the behaviour of reserpine-pretreated rats.

In 1958, another agent, imipramine, was discovered by chance to have beneficial effects in depression. This compound is not a MAOI and its actions were first described as 'a complete riddle'. Axelrod's group in Washington (Hertting, Axelrod and Whitby

1961) later found that imipramine blocked neuronal reuptake of noradrenaline from the synapse. This blockade prolongs and augments the actions of released transmitter and was assumed to explain imipramine's antidepressant effects.

Drawing all this evidence together, Schildkraut (1965) concluded that depression was caused by a functional deficit of noradrenergic transmission in the brain. He also thought that the rebound depression and fatigue, which are experienced after the arousing effects of amphetamine have worn off, were due to depletion of neuronal stores of noradrenaline. However, Schildkraut made a clear distinction between the effects of antidepressants and the arousal induced by amphetamine, describing the latter as 'stimulation' and 'excitement'. To this day, there is controversy over whether or not amphetamine has a beneficial effect in depression.

Later theories continued to focus on the monoamines. One suggested that there is a malfunction of neurons which release 5-HT (Coppen 1967). Another proposed that a deficit in both noradrenergic and serotonergic transmission is to blame (Maas 1975). Others have argued that an imbalance in the functional output of these two systems is the key factor (Ricci and Wellman 1990). However, they all share a common theme: that disruption of some aspect of monoaminergic transmission in the brain is a causal factor in depression. It is remarkable that, although this theory is often challenged, it has not yet been replaced by a validated alternative and, to this day, central noradrenergic and/or serotonergic systems are primary targets for all established antidepressant drugs.

THE NEUROBIOLOGY OF DEPRESSION

Attempts to find the cause(s) of depression have adopted two main approaches. One is to look for the neurobiological basis of depression in human subjects and animal models of this condition. The second is to investigate the pharmacology of established antidepressant agents to see whether they consistently augment some, and ideally the same, neurobiological targets in the brain.

HUMAN STUDIES

The objective of these studies is to find a neurochemical marker for depression. For obvious reasons, the majority has looked for changes that might affect monoamine function and so the following sections concentrate on these neurotransmitters. (Evidence suggesting that a dysfunction of the gluocorticoid hormonal system could be involved is discussed later.) Most techniques compare depressed and non-depressed (control) subjects and measure:

(1) The concentration of monoamines and their metabolites in accessible tissue samples (e.g. blood and urine).
(2) Indices of neurotransmitter function on lymphocytes (e.g. β-adrenoceptor binding) or platelets (e.g. α_2-adrenoceptor binding, 5-HT uptake).
(3) Monoamine concentrations or receptor binding in brain tissue post-mortem.
(4) Changes in neuroendocrine responses to challenges with drugs that target specific monoamine receptors (e.g. the α_2-adrenoceptor agonist, clonidine, stimulates growth hormone secretion while β-adrenoceptor activation stimulates melatonin secretion from the pineal gland).

Table 20.2 Neurochemical markers for depression

Marker	Tissue		Usual finding in depression
NA or MHPG concentrations	Post-mortem brain, urine, CSF, plasma	NCC	
Tyrosine hydroxylase immunoreactivity	Post-mortem brain	NCC	
α_1-adrenoceptor binding	Post-mortem brain	↓	Some brain areas
α_2-adrenoceptor binding	Post-mortem brain	↑	High-affinity site in some brain areas
	Platelets	NCC	
β_1-adrenoceptor binding	Post-mortem brain	↓	Certain cortical areas of suicide victims
β_2-adrenoceptor binding	Lymphocytes	↓	
—cAMP response		↓	
5-HT/5-HIAA concentration	Post-mortem brain, CSF, urine	NCC NCC	
5-HT uptake	Platelets	$\downarrow V_{max}$	
[^{3}H]imipramine binding	Platelets	NCC	
5-HT_1 receptor binding	Post-mortem brain	↓	Hippocampus
5-HT_{1A} receptor binding	Post-mortem brain	NCC	
5-HT_{1D} receptor binding	Post-mortem brain	↑	Globus pallidus
5-HT_2 receptor binding	Platelets	↑	Suicides
	Post-mortem brain	NCC	
D_1-/D_2-receptor binding	Post-mortem brain	No change	Drug-free suicides
GABA receptor binding	Post-mortem brain	NCC	
NMDA receptor	Post-mortem brain	↓	Affinity of glutamate site ligands and allosteric coupling to high-affinity glycine sites (frontal cortex of suicides)
Cortisol concentration	Plasma	↑	
Dexamethasone response		↓	

Notes:
Measurements in depressed patients compared with normal subjects, euthymic controls or patients suffering from an unrelated psychiatric disorder. NCC: No consistent change. The changes indicated are based on the most frequently published findings.

Unfortunately, such measurements are fraught with difficulties. For instance, it is not at all certain that neurochemical changes in the plasma or urine give any reliable indication of what is happening in the brain. Measurements in post-mortem brain tissue do not have this problem but the unavoidable delay in collecting tissue samples introduces another. Confirmation of the diagnostic status of the subjects is often difficult (especially retrospectively) and any drug treatments they had taken could distort the results. Another confounding factor is the possibility that any neurochemical changes are expressed only while the patient is experiencing a depressive phase ('state') rather than persisting during remission or recovery ('trait').

So far, evidence for abnormal peripheral (Elliott 1992) or central (Horton 1992) monoamine function in depression is equivocal, and no consistent biochemical markers have emerged to provide a firm link between the two (Table 20.2). One widely cited finding is that subjects who have attempted violent suicide form a neurochemically distinct group because the concentration of the 5-HT metabolite, 5-HIAA, in their CSF is lower than normal, suggesting that a deficit in 5-HT release is associated with suicide

(Åsberg, Traskman and Thoren 1976). However, this abnormality is now believed to be associated with a deficit in control of behavioural impulsivity, rather than depression.

Evidence for a link between monoaminergic transmission and the therapeutic effects of antidepressant agents is more convincing. Depletion of noradrenaline stores (achieved by administration of the noradrenaline synthesis inhibitor, α-methyl-*p*-tyrosine) causes a resurgence of depression in patients who are in remission following treatment with antidepressants that selectively target noradrenergic neurons. However, patients who respond to antidepressants that act primarily on serotonergic neurons are unaffected (Delgado *et al*. 1993). Conversely, a deficiency of tryptophan in the diet, which depletes 5-HT stores in the brain, reinstates depression in patients who have responded to drugs presumed to increase serotonergic transmission, but not those acting on noradrenergic neurons (Salomon *et al*. 1993). It seems that the therapeutic effects of different antidepressants could well rest on augmenting particular components of central monoamine transmission, whether or not depression itself is explained by a deficit in the functional output of these neurons.

ANIMAL STUDIES

The use of animal models for depression has two main objectives. One is to provide a behavioural model that can be used to screen potential antidepressant treatments. For this, the behaviour does not have to be an animal analogue of depression: all that is needed is for it to be consistently prevented by established antidepressant agents (i.e. no false negatives) but not by drugs which have no antidepressant effect in humans (i.e. no false positives).

A second objective is to produce behavioural changes in animals that are analogous to depression so that the model can be used to discover its neurobiological cause(s). This is a far more demanding problem and its success rests on satisfying at least three criteria (see Willner 1984): *face validity* (i.e. the behaviour looks like depression), *construct validity* (i.e. the causes and consequences of the behavioural change are the same as in depression) and *predictive validity* (i.e. the behaviour is reliably prevented only by drugs which have antidepressant effects in humans).

Procedures that have been suggested as models of depression and used to look for neurochemical changes that parallel the onset of the behavioural change, as well as to test how antidepressants affect the behaviour, are listed in Table 20.3. Those that have been used most, either as a drug screen or in research into the neurobiology of depression, are as follows.

Table 20.3 Procedures that have been used as animal models for depression or as a preclinical screen for novel antidepressant drugs

Procedures that have been widely studied:
Forced swim test ('behavioural despair')
Inescapable shock ('learned helplessness')
Olfactory bulbectomy
Novel (or controversial) models of depression that await further validation:
Chronic isolation housing
Chronic mild stress
Raphé lesion-induced muricidal behaviour
Tail suspension test

Olfactory bulbectomy

The olfactory bulb has multiple connections with limbic areas of the brain and receives afferent neurons from both the Raphé nuclei and the locus coeruleus. Bilateral removal of the olfactory bulbs in rats induces many behavioural, neuroendocrine and immunological changes including: hyperphagia, decreased rapid eye movement (REM) sleep, hyperactivity and deficits in the acquisition of conditioning behaviour (passive avoidance) (Van Riezen and Leonard 1990). These changes echo many of the problems experienced by depressives (disruption of appetite and sleep patterns and cognitive deficits). Also, as in many depressed patients, the concentration of plasma corticosteroids is increased in rats after olfactory bulbectomy (see later). This pattern of changes suggests that bulbectomy disrupts links between limbic areas of the brain and the hypothalamus. Its validity as a model of depression is supported by findings that all physiological and behavioural changes resulting from bulbectomy, that have been reported so far, are normalised by antidepressants from different generic groups. Only tranylcypromine, a MAOI, is ineffective in this model and this could be because it is metabolised to the CNS stimulant, methamphetamine, which is also ineffective.

Learned helplessness

This model was developed after pioneering experiments carried out in the USA by Overmier and Seligman (1967) who reported profound behavioural changes in dogs after their exposure to inescapable, uncontrollable stress (footshock). Subsequent work has concentrated on rats and mice, which show a similar behavioural response. This is expressed as appetite and sleep disturbance, general passivity and, on re-exposure of subjects to the stress, a failure to attempt to escape ('escape deficits'), even when this is feasible.

This behavioural syndrome, rather emotively called '*learned helplessness*', is widely believed to share many features of depression, not least because both culminate in psychomotor retardation and both are linked with experience of uncontrollable, unpredictable stress. Whether or not learned helplessness really is an analogue of depression remains controversial (Maier 1993). Nevertheless, escape deficits in rats are prevented by pretreatment with antidepressants from different generic groups. Other psychotropic agents, such as CNS stimulants and neuroleptics, are generally ineffective.

One of the earliest and most consistent findings with this model was a marked depletion of noradrenaline stores in certain brain regions, particularly the cortex, hippocampus and hypothalamus, of mice that have been exposed to inescapable shock. Moreover, when noradrenaline stores are depleted by other means (e.g. by administration of reserpine or α-methyl-*p*-tyrosine) the development of escape deficits is accelerated. One school of thought proposes that exhaustion of releasable noradrenaline in the neurons projecting to these brain regions underlies learned helplessness (Anisman and Zacharko 1991). Others highlight the depletion of noradrenaline stores in the locus coeruleus and suggest that a reduction in the release of noradrenaline in this nucleus diminishes the α_2-autoreceptor-mediated feedback inhibition of neuronal firing and that the resulting neuronal hyperactivity explains learned helplessness (Weiss *et al.* 1981). These two theories obviously differ in respect of whether it is an increase or a decrease in noradrenergic transmission in the terminal field that could account for depression. However, they are both consistent with Schildkraut's theory in that

depression could arise from a deficit in noradrenaline release, be it in either the terminal field or the cell body region.

One problem with both these theories is that disruption of noradrenergic transmission by selective adrenoceptor antagonists has little impact on the development of escape deficits. However, such antagonists do prevent the reversal of learned helplessness by antidepressants (reviewed by Stanford 1995). Also, it would be most unlikely that a deficit in only one neurotransmitter system fully accounts for learned helplessness. Indeed, there is plenty of evidence for a role for 5-HT in learned helplessness: for instance, this behaviour is reversed by microinjection of 5-HT into the prefrontal cortex (Davis *et al*. 1999). Finally, it is clear that opioid, GABAergic and cholinergic systems (among others) are all linked with this behavioural deficit and even dihydropyridine antagonists of Ca^{2+} channels prevent its development.

In short, the widespread neurochemical disruption during learned helplessness suggests that antidepressant drugs could prevent this syndrome by targeting any of several different neurotransmitter systems.

The swim test

Another behavioural model focuses on the immobile, floating posture (sometimes called '*behavioural despair*') which develops in rodents during a brief swim when they are put into a pool of water from which they cannot escape. Many antidepressants delay the onset and duration of immobility and this action has been widely adopted as a preclinical screen for novel compounds (Porsolt *et al*. 1979). However, drugs that selectively inhibit reuptake of 5-HT (SSRIs, see below), and which are highly effective antidepressants, are generally ineffective at diminishing immobility in the swim test, a finding that somewhat undermines its validity as a model of depression. Also, the many false positives (e.g. anticholinergics, amphetamine, sodium valproate) emphasise why it is important to distinguish drug effects on the emotional impact of this test from their non-specific effects on animals' motor activity.

SUMMARY

All these animal models express behavioural deficits that are paralleled by some abnormality in noradrenaline and/or 5-HT function but it is unlikely that the monoamines are the only neurotransmitters to influence these complex behaviours. Nevertheless, the behavioural deficits all respond, with varying degrees of specificity, to established antidepressants and central monoamines appear to have a crucial role in the therapeutic effects of these drugs. For a more detailed review of this subject see Stanford (1995).

NEUROCHEMISTRY OF ANTIDEPRESSANTS

The monoamine hypothesis predicts that drugs which increase the concentration of noradrenaline and/or 5-HT in the synapse should relieve depression. This could be achieved in two ways, as illustrated in Figure 20.1:

(1) The first is by preventing their intraneuronal destruction, thereby making more transmitter available for release (e.g. the MAO inhibitors). Some antidepressants

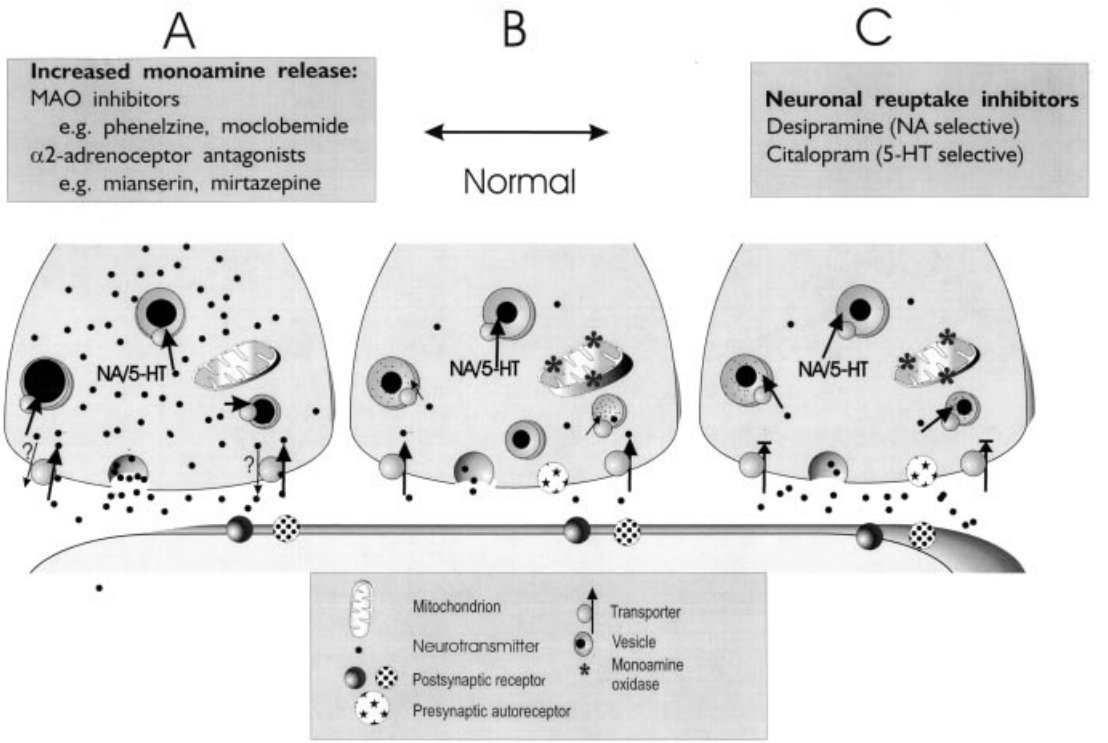

Figure 20.1 Schematic diagram illustrating how antidepressants increase the concentration of extraneuronal neurotransmitter (noradrenaline and/or 5-HT). In the absence of drug (b), monoamine oxidase on the outer membrane of mitochondria metabolises cytoplasmic neurotransmitter and limits its concentration. Also, transmitter released by exocytosis is sequestered from the extracellular space by the membrane-bound transporters which limit the concentration of extraneuronal transmitter. In the presence of a MAO inhibitor (a), the concentration of cytoplasmic transmitter increases, causing a secondary increase in the vesicular pool of transmitter (illustrated by the increase in the size of the vesicle core). As a consequence, exocytotic release of transmitter is increased. Blocking the inhibitory presynaptic autoreceptors would also increase transmitter release, as shown by the absence of this receptor in the figure. In the presence of a neuronal reuptake inhibitor (c), the membrane-bound transporter is inactivated and the clearance of transmitter from the synapse is diminished

are α_2-adrenceptor antagonists and the ensuing increase in monoamine release is thought to account for their antidepressant effects (e.g. mianserin).

(2) A second way of increasing synaptic concentrations of noradrenaline and 5-HT is to block their neuronal reuptake. Several groups of compounds act in this way and can be classified according to their relative selectivity for the noradrenaline and 5-HT transporters.

As described in the introduction, the first generation of antidepressant drugs comprised the MAO inhibitors and the reuptake blockers (e.g. imipramine) which became known as the tricyclic antidepressants (TCAs). The following section starts with a discussion of these two groups of compounds. Subsequent research concentrated on developing drugs that prevent the reuptake of either noradrenaline or 5-HT, like imipramine, but which lack its side-effects. Some drugs even combine reuptake inhibition with actions which increase transmitter release. Examples of all these types of compounds are given in Table 20.4. Whereas the newer antidepressants are a great improvement in terms of safety and tolerability, imipramine still remains the benchmark for efficacy. Full appraisals of antidepressants that are already in the clinic and those that are currently under development, together with their likely clinical and commercial impact, are to be found in Cheetham and Heal (2000) and Heal and Cheetham (1999).

MAO INHIBITORS (MAOIs)

With the exception of tranylcypromine (a phenylcycloalkylamine), the first MAOIs (e.g. iproniazid, isoniazid, phenelzine, isocarboxazid) were derivatives of hydrazine (originally used as a rocket fuel) (Fig. 20.2). All are irreversible inhibitors of the enzyme and restoration of MAO activity requires the synthesis of new enzyme.

As described above, because MAO is bound to mitochondrial outer membranes, MAOIs first increase the concentration of monoamines in the neuronal cytosol, followed by a secondary increase in the vesicle-bound transmitter. The enlarged vesicular pool will increase exocytotic release of transmitter, while an increase in cytoplasmic monoamines will both reduce carrier-mediated removal of transmitter from the synapse (because the favourable concentration gradient is reduced) and could even lead to net export of transmitter by the membrane transporter. That MAOIs increase the concentration of extracellular monoamines has been confirmed using intracranial microdialysis (Ferrer and Artigas 1994).

The main problems with early, irreversible MAOIs were adverse interactions with other drugs (notably sympathomimetics, such as ephedrine, phenylpropanolamine and tricyclic antidepressants) and the infamous '*cheese reaction*'. The cheese reaction is a consequence of accumulation of the dietary and trace amine, tyramine, in noradrenergic neurons when MAO is inhibited. Tyramine, which is found in cheese and certain other foods (particularly fermented food products and dried meats), is normally metabolised by MAO in the gut wall and liver and so little ever reaches the systemic circulation. MAOIs, by inactivating this enzymic shield, enable tyramine to reach the bloodstream and eventually to be taken up by the monoamine transporters on serotonergic and noradrenergic neurons. Like amphetamine, tyramine reduces the pH gradient across the vesicle membrane which, in turn, causes the vesicular transporter to fail. Transmitter that leaks out of the vesicles into the neuronal cytosol cannot be metabolised because

Table 20.4 Main groups of antidepressant drugs affecting monoamine uptake, release or receptors

Group	Specific mechanism	Examples	Additional notable actions
Inhibition of momoamine uptake			
Tricyclics	Preferential inhibition of noradrenaline *in vivo* (except clomipramine)	*Imipramine* *Clomipramine* *Desipramine* *Dothiepin*	Potent antagonists of: M-receptors, α_1-adrenoceptors and H_1-receptors. Some are antagonists of 5-HT_2 receptors
'NARI'	Inhibition of noradrenaline reuptake	*Maprotiline* *Viloxazine* *Reboxetine*	H_1- and α_1-antagonism
'SSRI'	Inhibition of 5-HT reuptake	*Citalopram* *Fluoxetine* *Fluvoxamine* *Paroxetine* *Sertraline*	Various
SNRIs	Inhibition of noradrenaline and 5-HT reuptake	*Venlafaxine* *Milnacipran*	
Increased monoamine release			
MAOIs	Irreversible, non-selective inhibition of MAO (causes secondary increase in monoamine release)	*Phenelzine* *Pargyline* *Isocarboxazid* *Tranylcypromine*	
RIMA	Reversible, selective inhibition of MAO_A (causes a secondary increase in noradrenaline and 5-HT release)	*Moclobemide* *Pirlindole*	
Tetracyclic	α_2-adrenoceptor antagonist	*Mianserin* *Mirtazepine*	H_1-antagonist, 5-HT_1, 5-HT_2 and 5-HT_3 antagonist and some α_1-antagonism (especially mianserin)
	α_2-adrenoceptor antagonist and some 5-HT uptake inhibition	*Trazodone* *Nefazodone*	5-HT_{1A} and 5-HT_2 antagonist and some α_1- (especially trazodone) and H_1 antagonism
Mechanism unknown			
'Atypical'		*Iprindole*	

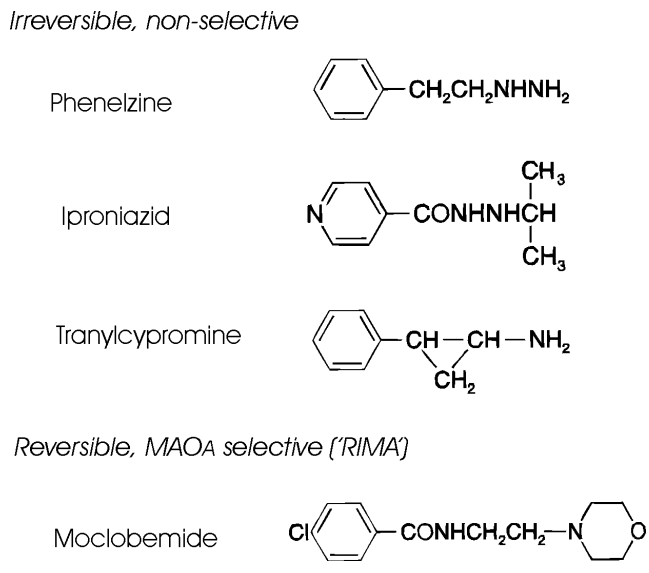

Figure 20.2 The chemical structure of some well-known MAO inhibitors. Most of these drugs irreversibly inhibit both MAO_A and MAO_B but reversible inhibitors (RIMAs), such as moclobemide, inhibit MAO_A only

MAO has been inhibited. As a result, transmitter accumulates in the cytoplasm and is exported into the synapse via the membrane-bound transporter. The ensuing (impulse-independent) sympathetic arousal can be disastrous, culminating in a hypertensive crisis and stroke. Although this process is a pharmacological curiosity and certainly contributed to the demise of MAOIs, it is possibly overrated (Tyrer 1979): it has been estimated that the number of deaths associated with the use of the MAOI, tranylcypromine, amounts to only 1 per 14 000 patient years. However, this sequence of events echoes exactly the acute actions of methylenedioxymethamphetamine (MDMA, 'Ecstasy') and undoubtedly accounts for some of the deaths attributed to this drug.

The discovery that MAO has two isoenzymes with different distributions, substrate specificity and inhibitor sensitivity has helped to rehabilitate the MAOIs to some extent. These isoenzymes are the products of different genes on the X-chromosome and share about 70% sequence homology. Whereas noradrenaline and 5-HT are metabolised preferentially by MAO_A, tyramine and dopamine can be metabolised by either isoenzyme. Selective inhibitors of MAO_A (e.g. moclobemide; Da Prada *et al.* 1989) should therefore be safe and effective antidepressants whereas the selective MAO_B inhibitor, selegiline, should not have any appreciable antidepressant activity (Table 20.5).

Both these predictions are borne out by clinical experience despite the snag that only MAO_B is found in serotonergic neurons (Saura *et al.* 1996). So far, there is no explanation for this anomaly. However, the lack of a tyramine-induced pressor effect with moclobemide probably owes more to the fact that it acts as a reversible inhibitor of $\underline{M}AO_{\underline{A}}$ (RIMA) than to its isoenzyme selectivity. Its reversible inhibition of MAO_A means that, should tyramine ever accumulate in the periphery, it will displace

Table 20.5 Irreversible MAO inhibitors and RIMAs

	MAO_A	Non-selective	MAO_B
Substrates	Noradrenaline 5-Hydroxytryptamine		β-Phenylethylamine Benzylamine
		Tyramine Dopamine	
Inhibitors			
Irreversible	Clorgyline	Iproniazid Isocarboxazid Phenelzine Tranylcypromine	Selegiline Pargyline
Reversible ('RIMA')	Befloxaton Brofaromine Moclobemide Pirlindole Toloxatone		

RIMA: Reversible Inhibitor of Monoamine $Oxidase_A$.

moclobemide from the enzyme, thereby ensuring that it is metabolised before reaching sympathetic neurons (Benedetti *et al.* 1983).

TRICYCLIC ANTIDEPRESSANTS (TCAs)

The tricyclic antidepressants (TCAs) derive their name from their three-ringed molecular structure (Fig. 20.3) and emerged, in 1958, from a search for better neuroleptics than chlopromazine among the phenothiazines. The prototype, imipramine, turned out to be ineffective in treating the positive symptoms experienced by schizophrenics but it did relieve their depression (negative symptoms). In fact, imipramine is still the standard agent against which novel antidepressants are compared in clinical trials.

All TCAs are either secondary- or tertiary-amines of a dibenzazepine nucleus (Fig. 20.3), and they all inhibit neuronal reuptake of noradrenaline and/or 5-HT but are much less potent as dopamine reuptake blockers. A common claim is that secondary amines (e.g. desipramine) are preferential inhibitors of noradrenaline uptake whereas the tertiary derivatives (e.g. imipramine, doxepin and amitryptyline) preferentially inhibit 5-HT uptake. However, when Richelson and Pfenning (1984) actually compared the effects of a wide range of antidepressants on the synaptosomal uptake of [^{3}H]monoamines *in vitro*, and compared their K_is, instead of merely ranking IC_{50}s collected from different studies, they found that tertiary- and secondary-substituted compounds were equipotent inhibitors of [^{3}H]noradrenaline uptake. Moreover, all the TCAs turned out to be more potent inhibitors of [^{3}H]noradrenaline than of [^{3}H]5-HT uptake. Tertiary amines are even less convincing inhibitors of 5-HT reuptake *in vivo*, because any such action is diminished by their metabolism to secondary amines (e.g. imipramine to desipramine; amitriptyline to nortriptyline). Only clomipramine retains any appreciable 5-HT uptake blocking activity *in vivo* with (an unimpressive) five-fold selectivity for 5-HT versus noradrenaline.

Set against this background is the finding that the inhibition of [^{3}H]noradrenaline uptake by the neuroleptic, chlorpromazine, is even greater than that of imipramine and yet chlorpromazine has no apparent antidepressant effects. This serves as a testimony

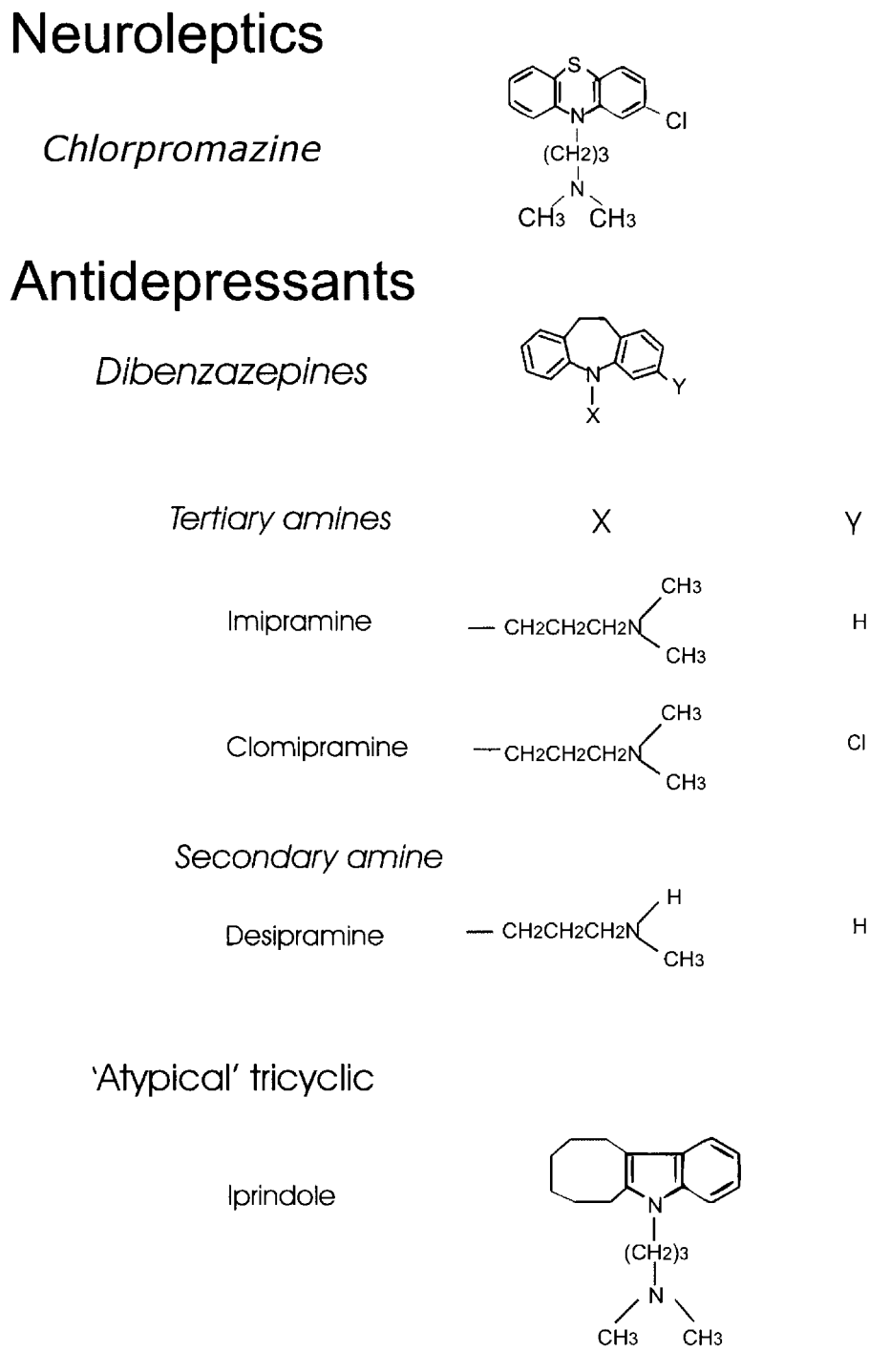

Figure 20.3 The basic structure of tricyclic antidepressants with some well-known examples

to the complex pharmacology of chlorpromazine and also as a warning that studies *in vitro* can be poor predictors of drug effects on patients' mood and behaviour.

The major drawback of the TCAs is their adverse side-effects. These are explained by their high affinity for histamine H_1- and α_1-adrenoceptors and all five of the muscarinic (M-) receptor subtypes. They consequently induce sedation (possibly through H_1-receptor antagonism), anticholinergic effects, such as dry mouth and blurred vision (M-receptor antagonism), orthostatic hypotension and dizziness (α_1-adrenoceptor antagonism). In fact, the tertiary TCA, doxepin, is considerably more potent as an H_1-receptor antagonist (K_B: 56 pM) than the standard H_1-receptor antagonist, mepyramine (K_B: 1 nM). The combination of inhibition of noradrenaline uptake and anticholinergic (antivagal) effects accounts for the pronounced cardiotoxicity of the older TCAs. This is of particular concern when treating the elderly, especially if patients have a history of cardiac problems, and, together with hyperpyrexia and convulsions, these effects explain the toxicity of TCAs in overdose. Other side-effects include loss of libido and stimulation of appetite which leads to weight gain. Little is known about the physiological bases of these actions which, although not life-threatening, are important because they undermine patient compliance.

The adverse side-effects of the TCAs, coupled with their toxicity in overdose, provoked a search for compounds which retained their monoamine uptake blocking activity but which lacked the side-effects arising from interactions with H_1, α_1-adrenoceptors and muscarinic receptors. One of the first compounds to emerge from this effort was iprindole, which has an indole nucleus (Fig. 20.3). This turned out to be an interesting compound because it has no apparent effects on monoamine uptake and is not a MAO inhibitor. This, together with its relatively minor antimuscarinic effects, led to it commonly being described as an 'atypical' antidepressant. Mechanisms that could underlie its therapeutic actions have still not been identified but, in any case, this drug has now been withdrawn in the UK.

α_2-ADRENOCEPTOR ANTAGONISTS

Another compound to emerge from the refinement of the TCAs is the tetracyclic agent, mianserin (Fig. 20.4). Like iprindole, this drug lacks antimuscarinic activity and, since it also has no significant effects on monoamine reuptake or MAO activity, it was regarded as another 'atypical' agent. However, it is now known to act as an α_2-adrenoceptor antagonist, an action that will increase the release of noradrenaline through blockade of autoreceptors on the cell bodies and terminals of noradrenergic neurons (see Chapter 8). In the sense that this process will increase synaptic concentrations of noradrenaline, and is thought to explain (or contribute to) its therapeutic effects, mianserin is not at all 'atypical'. Of course, it is likely that this drug will also block postsynaptic α_2-adrenoceptors, unless it specifically targets a different subtype of this receptor family, but this evidently does not prevent its therapeutic effects.

Mianserin will also increase 5-HT release through inhibition of α_2-heteroceptors on serotonergic neurons. Whether this contributes to its antidepressant actions is uncertain because it is a potent antagonist of 5-$HT_{2A/2B/2C}$ receptors and because, like other antidepressants (and despite being an antagonist), its chronic administration leads to downregulation of these receptors. However, this action of mianserin might well limit or reduce any co-existing anxiety and insomnia. A recent addition to this class of

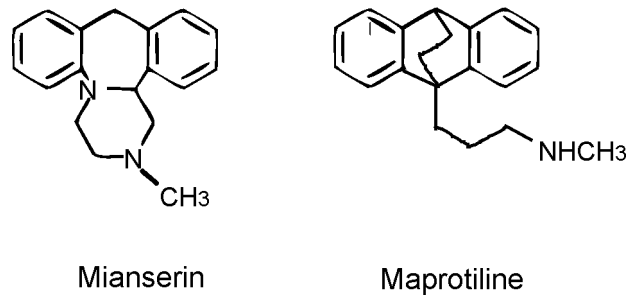

Figure 20.4 The chemical structure of mianserin and maprotiline

antidepressants is mirtazepine, which is an analogue of mianserin but with fewer pronounced side-effects.

SELECTIVE NORADRENALINE REUPTAKE INHIBITORS (NARIs)

Once it was realised that the adverse effects of the TCAs were due to their interactions with transmitter receptors, rather than their inhibition of noradrenaline reuptake, one objective in the development of novel drug treatments was to produce a 'clean' and selective noradrenaline reuptake inhibitor. The first of these was maprotiline, a bridged tricyclic agent (Fig. 20.4) which has a four hundred and fifty-fold selectivity for inhibition of noradrenaline versus 5-HT uptake *in vitro*. Although it has little antimuscarinic activity, its antidepressant activity is compromised because it is highly sedative, probably because of its appreciable H_1-receptor antagonism, and it is also an α_1-adrenoceptor antagonist. Viloxazine, an oxazine derivative of propranolol, is a bicyclic agent which similarly inhibits noradrenaline uptake more than that of 5-HT (hundred-fold selectivity *in vitro*) but which has little anticholinergic or antihistaminic activity. The latest NARI to be recruited in the clinic (1997) is another bicyclic antidepressant, reboxetine (Dostert, Benedetti and Poggesi 1997).

SELECTIVE SEROTONIN REUPTAKE INHIBITORS (SSRIs)

An alternative strategy was to develop drugs that are selective inhibitors of 5-HT reuptake but which, because they are chemically unrelated to the TCAs, would be unlikely to share their side-effects. This direction of research was prompted by the finding, in the late 1960s, that imipramine inhibited 5-HT reuptake, as well as that of noradrenaline, and was reinforced by the evidence that the TCA, clomipramine, was a preferential 5-HT reuptake inhibitor. The first selective serotonin reuptake inhibitor, zimelidine, was tested in the clinic in 1971 but, although it proved to be an effective antidepressant, it was subsequently withdrawn because it could apparently induce the serious neurological disorder, Guillain-Barré syndrome. Nevertheless, other SSRIs quickly followed and five agents are currently available in the UK: fluvoxamine, fluoxetine, paroxetine, sertraline and citalopram (Fig. 20.5).

The SSRIs are all chemically unrelated but their benefits and adverse effects are broadly similar. Their efficacy in depression is not superior to that of the TCAs but their side-effects (nausea, agitation, akathisia and sexual dysfunction), although sometimes problematic, are not life-threatening. They are also considerably safer

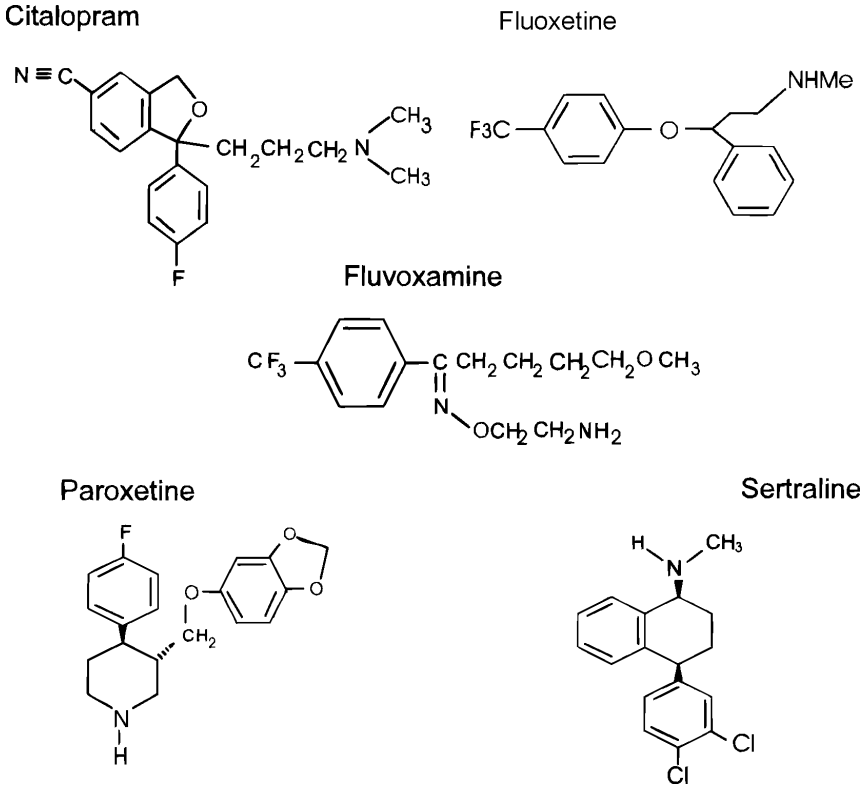

Figure 20.5 The chemical structure of the selective serotonin reuptake inhibitors (SSRIs)

than the TCAs in overdose, but the excessive activation of serotonergic systems can culminate in the 5-HT syndrome, a life-threatening delirium. All SSRIs have other clinical applications, such as in treatment of bulimia, anxiety disorders (e.g. obsessive compulsive disorder, panic disorder, social phobia) and Seasonal Affective disorder.

Paroxetine is the most potent inhibitor of 5-HT reuptake but, in terms of distinguishing one compound from another, their preferential selectivity for inhibition of 5-HT rather than noradrenaline reuptake is the key criterion. Citalopram is by far the most selective *in vitro* (1500–3000-fold) and fluoxetine, the most frequently prescribed SSRI in the UK, is the least selective of all these agents (see Stanford 1999). In fact, it is worth questioning whether fluoxetine is a true SSRI at all.

The most reliable estimates of the selectivity of fluoxetine for inhibition of 5-HT, versus noradrenaline reuptake, put this at twenty-fold, with a high K_i for noradrenaline uptake of between 1 and 10 μM. However, its active metabolite, norfluoxetine, is an even more effective inhibitor of noradrenaline uptake (K_i: 0.1 μM). After chronic administration, the concentration of fluoxetine in the plasma of patients is between 0.5 and 1.5 μM and is thought to be even higher in the brain. Thus, even accounting for pharmacokinetic factors, such as protein binding, the brain concentrations of fluoxetine and norfluoxetine could well be high enough to inhibit noradrenaline reuptake. Similarly, the plasma concentration of citalopram (285 nM) after chronic administration of the recommended therapeutic dose is about a hundred times greater than its K_i for inhibition of 5-HT uptake (1–10 nM), and its corresponding brain concentration is

ten-fold greater still. With a K_i for inhibition of noradrenaline uptake of 4 μM, even this drug, the most selective of all the SSRIs, could still express this inhibition in patients.

It is perhaps not surprising that, even after taking into account pharmacokinetic differences between these drugs, the therapeutic doses of the SSRIs do not parallel their K_i for inhibition of 5-HT reuptake. For instance, citalopram is about a thousand times more selective than fluoxetine for inhibition of 5-HT uptake, and yet their clinically effective doses are similar. In short, not only is their selectivity for the 5-HT transporter *in vitro* a poor predictor of their efficacy *in vivo* but it has to be questioned whether any of these compounds actually work by blocking 5-HT uptake alone.

Of course, it has to be borne in mind that there are functional interactions between serotonergic and noradrenergic neurons in the CNS. Indeed, intracerebral microdialysis studies in rats have confirmed that, with the exception of fluvoxamine, all SSRIs increase the concentration of extracellular noradrenaline whether they are given systemically, or by local intracranial infusion. Such an increase could result from activation of 5-HT heteroceptors on noradrenergic neurons. There is plenty of evidence that activation of 5-HT_2, and possibly 5-HT_3 receptors, in the terminal field increases noradrenaline release. There is also evidence that activation of presynaptic 5-HT_{1A}, and possibly 5-HT_2, receptors, increases the activity of noradrenergic neurons in the locus coeruleus. The complex interactions between serotonergic and noradrenergic neurons that could mediate SSRI-induced changes in noradrenaline release are discussed in more detail in Stanford (1999).

Because the SSRIs are derived from different chemical groups, their receptor interactions vary from compound to compound but, apart from paroxetine, none of them shows any appreciable binding to muscarinic receptors, a prime objective of their development. However, compared with other SSRIs, fluoxetine binds with moderately high affinity to human 5-HT_{2A} (K_i: 280 nM) and 5-HT_{2C} receptors (K_i: 55 nM); sertraline is a relatively potent ligand for α_1-adrenoceptors, α_2-adrenoceptors and D_1 receptors and citalopram shows appreciable binding to 5-HT_{1A}, α_1-adrenoceptors and H_1 receptors (Table 20.6; Stanford 1996). The extent to which any of these receptor interactions affects the efficacy of these compounds is not known.

SEROTONIN AND NORADRENALINE REUPTAKE INHIBITORS (SNRIs)

Over the last ten years or so, the emphasis on selectivity of action has waned. This is because the relative importance of blocking noradrenaline and 5-HT reuptake remains uncertain and it is possible that it could be beneficial to block both. Some drugs that act in this way have already been developed. It is hoped that this approach might increase the response rate of patients who are resistant to more selective drug treatments and even reduce the therapeutic lag that dogs their predecessors. As yet, there is not enough information on these compounds to know whether or not this has turned out to be the case.

One of these compounds, venlafaxine (licensed in the UK in 1996), is regarded as an inhibitor of both 5-HT and noradrenaline reuptake but this is based on its actions *in vitro*. At low doses *in vivo*, it is a more potent inhibitor of 5-HT (K_i: 39 nM) than noradrenaline reuptake (K_i: 210 nM). Moreover, its active metabolite, *O*-demethylvenlafaxine, is a weaker inhibitor of NA reuptake, and has a longer half-life, than its parent compound. However, at high doses, venlafaxine inhibits reuptake of both these monoamines but has negligible activity at muscarinic, H_1-receptors or α_1-adrenoceptors and

Table 20.6 Rank order of affinity for receptor binding of the SSRIs

Receptor	K_d or K_i (nM)		
	$< 1\,\mu M$	$1–10\,\mu M$	$> 10\,\mu M$
α_1	sert	zimel > cital. > fluox ⩾ parox > fluvox	
α_2	zimel	sert	fluox > fluvox ⩾ cital > parox
5-HT_{1A} (rat)			fluox > cital > fluvox > sert
5-HT_{2A} (rat)		fluox	sert ⩾ cital > fluvox
5-HT_{2C} (pig)		fluox	sert > cital ≫ fluvox
DA_2			sert > fluox > parox
Muscarinic	parox > sert	fluox > cital	zimel > fluvox
H_1	cital	zimel > fluox	parox ⩾ sert ≫ fluvox
σ_1	fluvox > sert > fluox > cital	parox	
σ_2		sert = cital > fluvox	fluox > parox

Notes:
cital = citalopram; fluox = fluoxetine; fluvox = fluvoxamine; parox = paroxetine; sert = sertraline; zimel = zimelidine.
Sequences derived from within study K_ds or K_is.

so lacks the problematic side-effects of the TCAs. Milnacipran is another agent in this group, and has only a two-fold preference for noradrenaline versus 5-HT reuptake inhibition. Finally, another SNRI, sibutramine, has been found to induce weight loss, for reasons that are not fully understood, and it is licensed for use as an anti-obesity agent rather than as an antidepressant.

SEROTONIN REUPTAKE AND RECEPTOR INHIBITORS

A final group of antidepressants targets both uptake and release of monoamines. These are triazolopyridine derivatives and include trazodone and the more recent addition, nefazodone. Trazodone is a weak inhibitor of 5-HT uptake but shows appreciable binding to 5-HT_{1A} receptors, α_1-adrenoceptors and H_1-receptors and so shares some of the disadvantages of the TCAs. It is also a 5-$HT_{2A/2C}$ receptor antagonist and an α_2-adrenoceptor antagonist, an action that is thought to contribute to its antidepressant actions. A related compound that has recently been introduced into the clinic is nefazodone. This is another weak 5-HT reuptake inhibitor with 5-HT_{2A} antagonist effects but it also inhibits uptake of noradrenaline to some extent. It has a lower affinity for the receptors that are responsible for the unwanted side-effects of trazodone, in particular α_1-adrenoceptors and muscarinic receptors.

Both these compounds have several metabolites and one of these, albeit constituting only 1% of the total, is *m*-chlorophenylpiperazine (*m*CPP). This is a 5-HT_{2C}-receptor agonist/5-HT_{2A} antagonist and has been suggested to contribute to the antidepressant effects of these compounds. In fact, 5-HT_{2C} receptor agonists are currently being explored as potential antidepressants. This is interesting because *m*CPP induces anxiety in humans (Rotzinger *et al*. 1999) and trazodone is contraindicated in the treatment of patients experiencing depression with panic attacks. The enzyme responsible for this metabolic product, CYP2D6, shows genetic polymorphism and so it is possible that the accumulation of *m*CPP is more problematic in some individuals than others.

Ultimately, agonist drugs that directly activate monoamine receptors would appear to be a logical development in this field. Unfortunately, the peripheral side-effects of such compounds could well limit their acceptability even if we were to discover what subset of receptors to target.

NEUROBIOLOGICAL CHANGES INDUCED BY CHRONIC ADMINISTRATION OF ANTIDEPRESSANTS

The actions of all the compounds described so far seem to underpin the monoamine hypothesis. Yet an outstanding problem in treating depression is that the therapeutic response is both slow and progressive: a significant improvement usually takes at least 2–3 weeks and sometimes much longer. Obviously, if we are to explain the therapeutic effects of antidepressants, we must search for long-term neurochemical changes that occur after their prolonged administration.

NORADRENERGIC TARGETS

The first indication that some neurochemical changes developed only after prolonged treatment with antidepressants came from landmark experiments carried out by Vetulani

and Sulser in the mid-1970s (Vetulani *et al.* 1976). They found that repeated, but not a single, administration to rats of any of the antidepressants which were available at that time (i.e. MAOIs, TCAs, iprindole and even simulated electroconvulsive therapy) attenuated the increase in cAMP in the cerebral cortex induced by β-adrenoceptor agonists. They suggested that antidepressants desensitised β-adrenoceptors by uncoupling the receptor from what is now recognised as the Gs-protein so that it can no longer synthesise the β-adrenoceptor second messenger, cAMP. Shortly afterwards, it was found that this desensitisation was usually paralleled by downregulation of β_1- (but not β_2-) adrenoceptors. This action is even shared by repeated electroconvulsive shock (Stanford and Nutt 1982) but not by drugs that are ineffective in relieving depression (e.g. neuroleptics).

A logical conclusion from this work was that depression is caused by hyperresponsive β-adrenoceptors. At first, this might seem to undermine Schildkraut's suggestion that depression is caused by a deficit in noradrenergic transmission. However, proliferation of receptors is the normal response to a deficit in transmitter release and so the opposite change, downregulation of β-adrenoceptors by antidepressants, would follow an increase in the concentration of synaptic noradrenaline. This would be consistent with both their proposed mechanism of action and the monoamine theory for depression.

Nonetheless, there are many reasons to be confident that β-adrenoceptor desensitisation does not explain the therapeutic effects of antidepressants. First, with the development of more selective ligands for use in radioligand binding studies, it became evident that β-adrenoceptor downregulation can occur after only 2–3 days of drug treatment (Heal *et al.* 1989). Second, maprotiline, most of the SSRIs, and even some of the newer TCAs have no effect on β-adrenoceptor binding or function. Third, and the greatest problem of all, citalopram *increases* the β-adrenoceptor-mediated cAMP response without changing receptor density. Evidently, we must look elsewhere to find an explanation for the neurobiology of depression and its treatment.

SEROTONERGIC TARGETS

There is a good deal of evidence that the therapeutic effects of antidepressants could involve adaptive changes in 5-HT_{1A} receptors. Postsynaptic 5-HT_{1A} receptor responses became implicated because the hyperpolarisation of hippocampal CA3 pyramidal neurons that follows ionophoretic administration of 5-HT was found to be increased after chronic treatment with most (but not all) antidepressants (Chaput, de Montigny and Blier 1991). Others suggested that antidepressants attenuate postsynaptic 5-HT_{1A} responses because the hypothermia, evoked by their activation, is diminished by antidepressants (Martin *et al.* 1992).

More recently, a series of studies using microdialysis *in vivo* has suggested that long-latency changes in presynaptic 5-HT_{1A} receptors could underlie the therapeutic lag in antidepressant treatment. In these experiments, a single dose of either a SSRI (e.g. fluoxetine or paroxetine), or a MAOI (e.g. tranylcypromine) increased the concentration of extracellular 5-HT in the dorsal Raphé nucleus but not in the brain areas to which these neurons project (e.g. the frontal cortex or striatum; see Hervás *et al.* 1999). The suggested explanation for this regional difference was that the accumulation of extracellular 5-HT in the Raphé nuclei, caused by the SSRIs blocking its reuptake, activates somatodendritic 5-HT_{1A} receptors and so inhibits the firing of serotonergic neurons. This results in reduced impulse flow to their terminals so that extracellular 5-HT does not increase there despite blockade of its reuptake (Fig. 20.6). Obviously, if

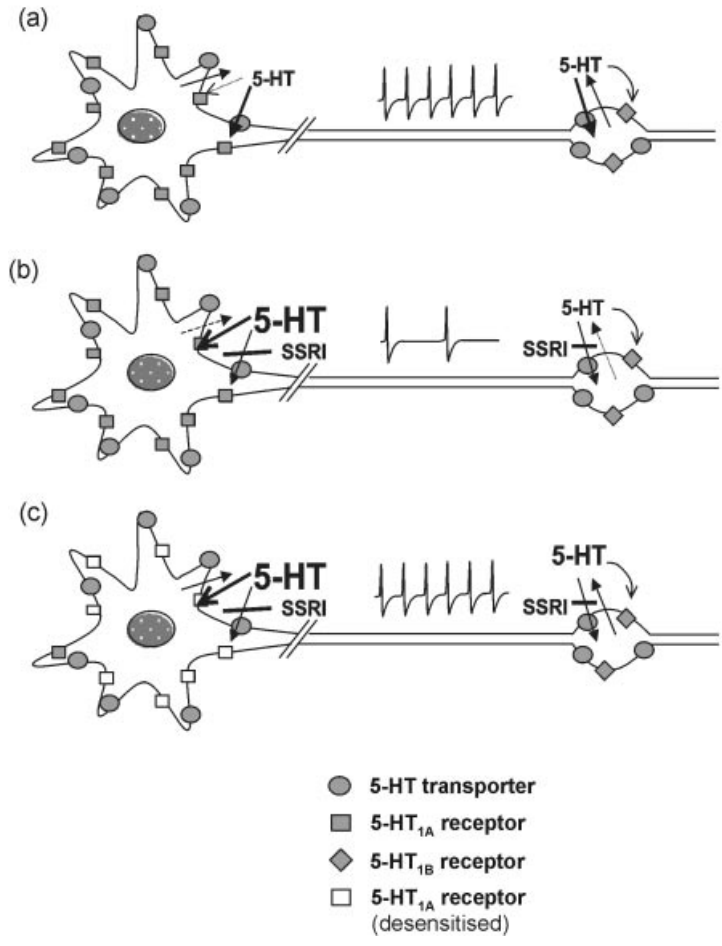

Figure 20.6 Schematic representation of the effects of 5-HT reuptake inhibitors on serotonergic neurons. (a) 5-HT is released at the somatodendritic level and by proximal segments of serotonergic axons within the Raphé nuclei and taken up by the 5-HT transporter. In these conditions there is little tonic activation of somatodendritic 5-HT_{1A} autoreceptors. At nerve terminals 5-HT_{1B} receptors control the 5-HT synthesis and release in a local manner. (b) The blockade of the 5-HT transporter at the level of the Raphé nuclei elevates the concentration of extraneuronal 5-HT to an extent that activates somatodendritic autoreceptors (5-HT_{1A}). This leads to neuronal hyperpolarisation, reduction of the discharge rate and reduction of 5-HT release by forebrain terminals. (c) The exposure to an enhanced extracellular 5-HT concentration produced by continuous treatment with SSRIs desensitises Raphé 5-HT_{1A} autoreceptors. The reduced 5-HT_{1A} function enables serotonergic neurons to recover cell firing and terminal release. Under these conditions, the SSRI-induced blockade of the 5-HT transporter in forebrain nerve terminals results in extracellular 5-HT increases larger than those observed after a single treatment with SSRIs. (Figure and legend taken from Hervás *et al*. 1999 with permission)

this is correct, then blocking 5-HT_{1A} receptors in the Raphé nuclei should prevent these changes. This was confirmed by the finding that SSRIs did increase the concentration of extracellular 5-HT in the cortex and failed to reduce neuronal firing rate if the 5-HT_{1A} receptor antagonist, WAY 100635, was co-administered, either systemically or by infusion directly into the dorsal Raphé nucleus.

More importantly for this discussion is the finding that chronic administration of an antidepressant produces a similar increase in the concentration of extracellular 5-HT in the terminal field together with recovery of neuronal firing. Presumably this is because the prolonged elevation of extracellular 5-HT around the neurons in the Raphé causes progressive desensitisation of the somatodendritic 5-HT_{1A} receptors. At this point, inhibition of their firing does not occur and so more 5-HT is released in the cortex (see Hervás *et al.* 1999).

If long-latency 5-HT_{1A} receptor downregulation explains the antidepressant therapeutic lag, then 5-HT_{1A} receptor antagonists might reduce the delay in treatment response. This prediction has been tested in the clinic using combined treatment with paroxetine and the mixed β-adrenoceptor/5-HT_{1A} antagonist, pindolol and the majority of studies report a successful outcome (see Hervás *et al.* 1999). However, it remains uncertain whether this effect of pindolol is due to its actions at presynaptic 5-HT_{1A} receptors. If, as suggested earlier, postsynaptic 5-HT_{1A} receptors are involved in the therapeutic effects of antidepressants, then co-administration of a 5-HT_{1A} receptor antagonist of this receptor might well diminish any antidepressant effect. Pindolol is said to avoid this problem by its selective antagonism of presynaptic, but not postsynaptic, 5-HT_{1A} receptors, but this is controversial.

A related strategy would be to inactivate the 5-$HT_{1B/1D}$ autoreceptors which are found on serotonergic nerve terminals and so prevent feedback inhibition of 5-HT release in the terminal field. These drugs would not prevent the impact of indirect activation of 5-HT_{1A} receptors, and the reduced neuronal firing, by SSRIs (described above), but they would augment 5-HT release in the terminal field once the presynaptic 5-HT_{1A} receptors have desensitised. Selective 5-$HT_{1B/1D}$ antagonists have been developed only recently but will doubtless soon be tested in humans.

OTHER TRANSMITTER SYSTEMS

The extensive literature on long-latency changes in neurotransmitter receptors following chronic administration of antidepressants reflects the intense effort that has been invested in the search for the cause of their therapeutic lag. Indeed, apart from developing compounds that help patients who currently do not respond to any existing treatment, the most pressing problem in this field is to reduce the delay in treatment response. Yet, despite the numerous investigations of the effects of antidepressants on a wide range of transmitter receptors, few consistent findings have emerged. Results tend to vary not only from laboratory to laboratory and between different brain regions but they also vary with the species and compound tested. The most promising changes are summarised in Table 20.7 but, so far, these do not fit into a scheme that explains either depression or its reversal by antidepressants.

Obviously one limitation of all this work is that the drug effects have been tested in 'normal' animals. So far, the neurochemical changes induced by long-term drug treatment have not been tested in combination with procedures such as learned helplessness, but it cannot be assumed that they will be the same as those in normal (non-depressed) subjects.

THE HPA AXIS AND DEPRESSION

There are clear links between depression and disruption of the neuroendocrine system. Thyroid and gonadal hormone secretion are both abnormal in depression but most

Table 20.7 Neurochemical changes generally found after chronic administration of antidepressant drugs or repeated electroconvulsive shock

Neurochemical marker	Usual finding	Exceptions
Tyrosine hydroxylase expression (locus coeruleus)	↓	
Turnover rate (noradrenaline, dopamine and 5-HT)	↓	
α_1-adrenoceptor density (cortex)	↑	
α_2-adrenoceptor density (cortex)	↓	Inconsistent effects with SSRIs. Some studies find reduction in locus coeruleus, only
β-adrenoceptor binding (cortex)	↓	β_1-adrenoceptors, only. Generally no change with SSRIs
β-adrenoceptor-mediated cAMP response	↓	Inconsistent changes with SSRIs
5-HT_{1A} receptor density (cerebral cortex)	↓	
5-HT_2 receptor binding (rat cortex)	↓	Increased with simulated ECT. No change with SSRIs. Species differences in change
DA_1 receptor-mediated responses	↓	
DA_2 receptor-mediated responses	↑	
NMDA receptor: affinity of glycine for strychnine-insensitive site	↓	

attention has been devoted to the hypothalamic–pituitary–adrenocortical (HPA) axis (Mussleman and Nemeroff 1993). This is a complex system with many interlinked feedback and feedforward controls. However, a key role is thought to be served by corticotropin-releasing factor (CRF) which is released from neurons in the paraventricular nucleus (PVN) in the hypothalamus. From here, CRF is carried to the anterior pituitary where it triggers release of adrenocortical hormone (ACTH) into the systemic circulation. In turn, ACTH promotes release of glucocortioid hormones from the adrenal cortex. Not all CRF release is directed at the HPA system: extrahypothalamic CRF is found in many limbic areas, including the locus coeruleus and Raphé nuclei (Fig. 20.7).

Normally, circulating glucocorticoids (of which cortisol is the most prominent in humans) cause feedback inhibition of ACTH release so that cortisol secretion is, to some extent, self-limiting. However, many patients suffering from major depression have an increased concentration of plasma cortisol but reduced ACTH secretion. The latter abnormality seems to be partly due to a reduction in the number of CRF receptors in the pituitary, although it is thought that decreased ACTH secretion could provoke the adrenal hyperplasia which is common in depression. This would result in excessive secretion of cortisol and contribute to the inhibition of ACTH release (Musselman and Nemeroff 1993).

Also, a high proportion of depressed patients do not show the reduction in cortisol secretion which is seen when normal subjects are challenged with the synthetic glucocorticoid, dexamethasone, that normally decreases further release through feedback

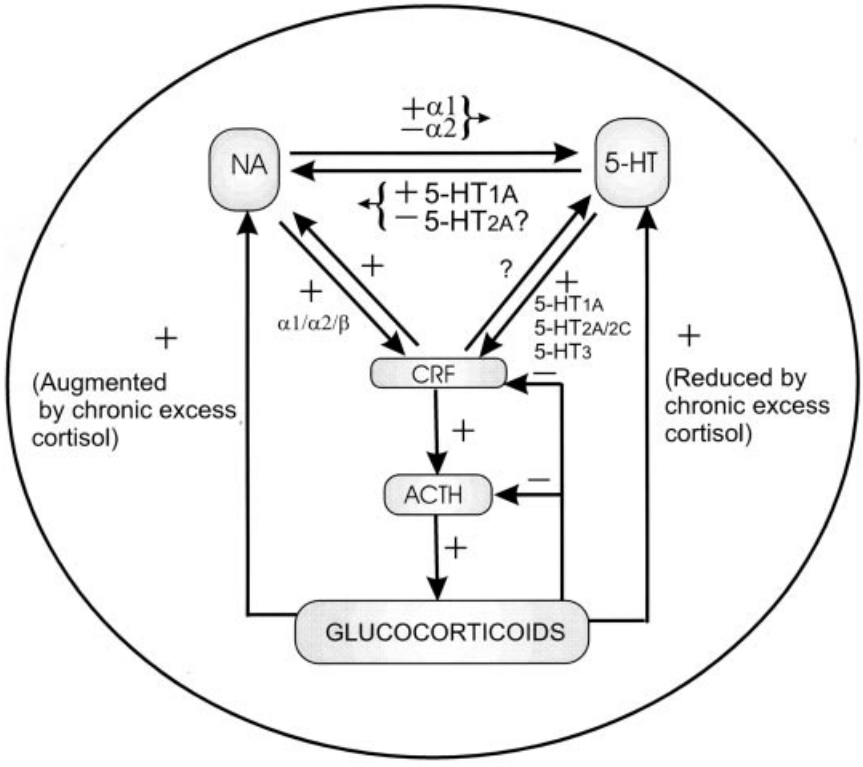

Figure 20.7 Possible interactions between hormones of the HPA axis, extrahypothalamic CRF and the central monoamines, noradrenaline and 5-hydroxytryptamine. See text for a full explanation

inhibition. This is the '*dexamethasone suppression test*' (Carroll, Curtis and Mendels 1976). For most patients whose depression is relieved by antidepressants or electroconvulsive therapy, both the raised concentration of circulating cortisol and the negative 'dexamethasone suppression' response are resolved. This suggests that depression is associated with a defect in the regulation of glucocorticoid secretion and the locus of this disorder could be glucocorticoid receptors in the hippocampus. Evidence that CRF secretion is increased in depressives supports the idea that these receptors, which depress CRF secretion, are hypofunctional in depression (Ritchie and Nemeroff 1991). Also, transgenic mice which are deficient in glucocorticoid receptors exhibit many features of depression; these extend to disruption of feeding and cognitive deficits as well as abnormal HPA function. Antidepressant treatment causes a long-latency increase in hippocampal glucocorticoid receptor binding and the concentration of mRNA for these receptors. Since this happens even in cultured fibroblasts it is thought to involve an action at the level of the genome. Increased glucocorticoid receptor function is thought to restore the feedback effects of cortisol on neurons that regulate CRF secretion (Barden, Reul and Holsboer 1995).

In addition to these actions, glucocorticoids modify the function of several transmitters and/or their receptors, notably GABA, acetylcholine, noradrenaline, 5-HT and sigma (σ)-receptors for which the endogenous ligand is unknown. Conversely, the

paraventricular nucleus in the hypothalamus receives noradrenergic and serotonergic inputs from the brainstem, both of which can activate CRF release, so there are complex reciprocal interactions between CRF and monoamine function in the brain. Electrophysiological studies of the locus coeruleus suggest that antidepressants might influence these interactions because chronic administration of antidepressants, from different generic groups, blocks the activation of noradrenergic neurons induced by CRF. However, different antidepressants seem to achieve this through different mechanisms. Some appear to block CRF release (e.g. mianserin) while others show physiological antagonism (e.g. sertraline) (Curtis and Valentino 1994).

AN OVERVIEW OF THE NEUROCHEMISTRY OF DEPRESSION

Separate lines of research have implicated either the noradrenergic, serotonergic or the HPA axis in depression, and there is more evidence, not covered here, that other neuroendocrine systems are involved as well. Yet, all this effort has so far failed to identify disruption of any single transmitter or hormone system as the sole culprit. This points to disruption of the *interactions* between these different systems as the cause of the problem.

Concentrating on the systems highlighted in this chapter, there is plenty of evidence for mutual interactions between the noradrenergic and serotonergic neuronal systems and the HPA hormones: inappropriate release or dysfunctional receptors at any point in these interactions could easily disrupt the balance between these different factors. (Figure 20.7 incorporates some of the interactions that have been characterised so far, but there are doubtless many others.) For example, it is clear that either hyperresponsive α_2-adrenoceptors or hyporesponsive α_1-adrenoceptors could diminish the release of 5-HT evoked by noradrenaline. Conversely, hyporesponsive 5-HT_{1A} receptors and possibly hyperresponsive 5-HT_2 receptors would diminish noradrenaline release from neurons in the locus coeruleus. A disorder of the HPA axis could affect the monoamines in two ways: either directly through effects of CRF on monoamine release or through its effects on glucocorticoid secretion. For instance, whereas CRF can modulate the release of these monoamines directly, 5-HT release is increased by cortisol, but this is blunted by prolonged excessive cortisol secretion (such as can occur in depression). Also, α_2-adrenoceptors, which normally limit release of noradrenaline, are desensitised after chronic exposure to excess cortisol. From this perspective, any single neurochemical factor could have far-reaching effects on all these (and other) neurohumoral systems and could lead to the mood and behavioural changes that culminate in depression. In other words, whereas the expression of an abnormal neurochemical response would be linked with one transmitter system, the problem could lie in another. If this is so, the prospects for finding either a marker for, or a definitive cause of, depression are gloomy, if not misguided.

However, experience proves that depression can be reversed by drugs that augment serotonergic and noradrenergic transmission (and reinstated by a deficit in the synthesis of these monoamines). These, then seem to be crucial targets that ultimately determine mood. This would explain why, despite numerous neurochemical options for the causes of depression, all antidepressants developed so far (and even those discovered by chance) target these neuronal systems. Whatever the cause of depression, therefore, its relief seems to rest on appropriate secretion of these monoamines. This would be entirely

consistent with their pervading influence throughout the limbic system (regarded as the governor of mood and behaviour), a characteristic not found so far for any other neurotransmitter.

What remains to be seen is whether it will be possible to accelerate the neurochemical readjustments triggered by antidepressant drugs that target these systems, so as to reduce the latency in their therapeutic effects. We also need to discover why some patients do not respond to any drug treatment. Is this because existing antidepressants simply fail to initiate the appropriate combination of changes in monoaminergic transmission in these patients or do they have a disorder that affects neuronal systems that function in parallel with (or override) the monoamines? If the former is the case, then drugs with combinations of actions which modify monoaminergic transmission in ways that differ from those of established antidepressants might prove to be effective. In the latter case, a new approach to development of antidepressant drugs, targeting completely different transmitter systems, is needed. Obviously, there is a pressing need for future research that will distinguish between these possibilities. Overall, it is 'depressing' to realise that, despite all the advances in our understanding of central neurotransmitters and brain function, the drug treatment of depression has not advanced significantly since the discovery of imipramine nearly half a century ago.

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