The medicinal chemistry of arylpiperazines with potential antidepressant efficacy
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1 INTRODUCTION

1.1 Depression

1.1.1 The Archeology of Mood Disorders

The terms mania and melancholia were originally introduced by the Greeks. The modern meaning of these terms, however, only goes back one hundred years.¹ Until early 1900, ca. 50% of the diagnoses made were for mania. Since World War I, in contrast, the diagnosis of mania has become a part of the diagnosis of manic-depression. Today no more than 1-2% of psychiatric patients are diagnosed with mania. Many terms used in psychiatry, including the terms neurosis, psychosis, mania and melancholia, changed in meaning at the turn of the 20th century. The diagnosis of depression did not even exist before 1900. Before this term was introduced, any state...
that would lead to under-activity, was diagnosed as melancholia. Conditions that are now recognized as negative schizophrenia, obsessive-compulsive disorder, social phobia, panic disorder and major depression would all attract the diagnosis of melancholia.

In 1919, the German psychiatrist Kraepelin introduced the term ‘manic-depressive insanity’. In contrast to schizophrenia, manic-depressive disorders showed a remitting course and the symptoms he described were very close to what would now be diagnosed as a major depressive disorder or endogenous or vital depression. The disorder was characterized by apathy, loss of energy, retardation of thinking and activity, as well as profound feelings of gloominess, despair and suicidal ideation. In addition, Kraepelin pointed to vegetative symptoms, such as poor sleep and loss of appetite. The development, in the 1950s, of the antipsychotics and the antidepressants vindicated Kraepelins’ point of view. Early 1960s, operational definitions to distinguish between endogenous and reactive (or neurotic) depression were proposed.

Endogenous depression was characterized by vegetative disturbances such as early morning wakening, loss of appetite, and diurnal variation of mood as well as retardation of thinking and feeling. Accordingly it was only appropriately treated pharmacologically or with electric convulsive-shock treatment (ECT). Neurotic, or reactive depression in contrast stemmed from adversity and could probably be managed psychotherapeutically in the main. This formulation fitted nicely with the amine theories of depression that emerged in the mid 1960s and with the fact that the first antidepressant was an amine reuptake inhibitor.

According to the American Psychiatric Association, major depression is a syndrome consisting of affective, cognitive, motor and somatic signs and symptoms. Symptoms should be prominent and persistent and represent a change from previous function. For clinical depression to be diagnosed, a patient will exhibit either depressed mood or a loss of interest or pleasure in activities (adhedonia), or both. Several other signs and symptoms, including decreased appetite, insomnia, fatigue, feelings of worthlessness or guilt, diminished ability to think or concentrate, or recurrent thoughts of death or suicide, also need to be present.

Patients with mania, by contrast, exhibit a triad of euphoric mood, pressured speech, and psychomotor agitation. During the period of mania, signs and symptoms such as inflated self-esteem, decreased need for sleep, pressure to keep talking, psychomotor agitation, distractibility and flight-of-ideas are evident. To treat the manic phase in manic-depression, lithium is generally used. Lithium, an alkali metal, was first isolated in 1817 from stone. Suggestions of the beneficial effects of spring waters with a high lithium content, in the treatment of mania, can be cited as a forerunner of modern lithium use. In 1949, lithium was tried on a number of manic, depressed and schizophrenic patients. The manic patients responded to the treatment. In 1952, the usefulness of lithium, using a placebo and double-blind techniques and a specially devised rating scale, was explored. This study was one of the very first double-blind randomized placebo-controlled trial in psychiatry.
Besides lithium carbonate (Eskalith/Camcolith), carbamazepine (1.1, Tegretol) and sodium valproate/valproic acid (1.2, Depakene/Epilim) are currently marketed to treat bipolar disorders or prophylaxis of recurrent disorders.

1.1.2 THE DISCOVERY OF THE FIRST GENERATION OF ANTIDEPRESSANTS

In 1952, the first antipsychotic drug, chlorpromazine (1.3, Thorazine®/Largactil®), was discovered.9 It was the critical event in the foundation of psychopharmacology. The history of the synthesis of chlorpromazine goes back to the development of coal-tar chemistry. In 1883, the first phenothiazine compound had been synthesized. Sixty years later, the phenothiazines were linked to histamine (1.4) and chlorpromazine was developed from this parent compound.

The development of imipramine (1.5, Tofranil®) also came from an interest in antihistamines. A series of forty-two related compounds, using iminodibenzyl (1.6) as the lead, was put together.10, 11 In the first clinical study of imipramine, it was examined for its antipsychotic qualities and compared to chlorpromazine. In 1955, a second study was set up, to look specifically at the effects of imipramine in patients who suffered from depression. The responses were so dramatic that there was little doubt that the treatment was effective.12, 13 The patients selected for this study, were all diagnosed with endogenous or vital depression. Treatment produced an increase in vivacity and a restoration of interest in activities in general and in social interaction in particular. Sleep was restored and would feel normal and refreshing, unlike the sleep that followed the then available hypnotics. Appetite was stimulated. Although improvement might be apparent after two to three days, it was claimed that it could take up to four weeks for the clinical effect to become established. All the side effects now associated with tricyclic use – dry mouth, a tendency to sweat more profusely, some constipation, possible drops in blood pressure and possible confusional conditions in patients with other brain disorders - were described. As a result, a dose range, that remains the same today, was put forward.

In 1937, the enzyme monoamine oxidase (MAO) was discovered, which is responsible for the metabolism of adrenaline (1.7), noradrenaline (1.8, NA), dopamine (1.9, DA) and 5-hydroxytryptamine (1.10, 5-HT or serotonin). In 1912, isoniazid (1.11) was synthesized. This compound was resynthesized in 1951, when a large stock of hydrazine that was left over from World War II became available to the different chemical companies.14 Using isoniazid as the starter, iproniazid was synthesized. Both compounds were marketed as tuberculostatics.15 In 1952, it was discovered that iproniazid, but not isoniazid, inhibited MAO and was therefore named a monoamine oxidase inhibitor (MAOI).16 In 1957, the MAO inhibitor iproniazid (1.12), whose development helped to create the antidepressant market, was introduced. The synthesis and development of iproniazid was triggered by several events.17-21
In 1953, reserpine (1.13) was isolated from *Rauwolfia serpentina*, a plant root used in India for the treatment of hypertension, snakebite and insanity.\textsuperscript{22, 23} Although reserpine was not successful as a drug, it immediately led to several biochemical and psychological hypotheses. The sedative effects of reserpine were correlated to the lowering of brain 5-HT. This was the first bridge between neurochemistry and behavior. Thus, the biochemical psychopharmacology was established.\textsuperscript{24} Observations of the effects of reserpine on the release of catecholamines further extended this bridgehead in two important ways.\textsuperscript{25} It stimulated the debate between the 5-HT and the catecholamine camp and provided the pharmaceutical industry with a principle to guide drug development. Drugs could either be designed to produce similar depletions of 5-HT or catecholamines or, alternatively, they could be designed to modify or block the reserpine effect. Hence, drug development became systematic. In 1958, Merck approached several psychiatrists to investigate amitriptyline (1.14, Elavil\textsuperscript{®}) for possible antischizophrenic properties. In the field, it was suggested that amitriptyline should also be investigated for antidepressant properties, since the molecular structure was so close to that of imipramine. Clinical studies revealed that amitriptyline was effective in much the same dose range as imipramine. It had a very similar profile of side effects and, like imipramine, took several weeks before the therapeutic effects would appear.\textsuperscript{26, 27} Amitriptyline was launched in 1961. Its discovery led to the final acceptance of imipramine as an antidepressant drug.
1.1.3 TRICYCLIC ANTIDEPRESSANTS AND MONO-AMINE OXIDASE INHIBITORS

In the 1960s, the Hamilton rating scale, that has become the standard for the assessment of depression and antidepressant effects, was introduced.\textsuperscript{28} Several compounds structurally related to imipramine have been developed - using this scale to ensure a basic level of quality of their clinical actions - and are currently marketed for the treatment of depression. They each have a three (tri)-joined ring (cyclic) structure with a side chain containing a tertiary or secondary amine attached to the central ring. The acronym used for the tricyclic antidepressants is TCA. Tertiary amine-containing TCAs include imipramine, amitriptyline, trimipramine (1.15), doxepine (1.16) and clomipramine (1.17). Secondary amine-containing TCAs are desipramine (1.18) and nortriptyline (1.19). Desipramine and nortriptyline are actually the desmethyl metabolites of imipramine and amitriptyline.\textsuperscript{29, 30} The most prominent action of TCAs is the blockade of reuptake of either NA or 5-HT from the synapse back into the nerve terminal, without blocking the reuptake of DA. The secondary amine-containing TCAs are 25- to 500-fold more potent in inhibiting NA than 5-HT reuptake as compared to tertiary amine-containing TCAs, which are only 3- to 5-fold more potent in inhibiting NA than 5-HT reuptake.

Chart 1.2 Chemical structure of reserpine (13), the TCAs amitriptyline (1.14), trimipramine (1.15), doxepine (1.16), clomipramine (1.17), desipramine (1.18) and nortriptyline (1.19) and of phenelzine (1.20), tranylcypromine (1.21), amphetamine (1.22) and moclobemide (1.23).
Although the MAOI iproniazid was effective in the treatment of depression, it is no longer used clinically because of its hepatic toxicity. Two of the MAOIs currently marketed for the treatment of depression are phenelzine (1.20), a derivative of hydrazine, and tranylcypromine (1.21), which is structurally related to amphetamine (1.22). The strongest disadvantage of the MAOIs is their interaction with tyramine containing foods, like old cheese and wine, through the inhibition of the iso-enzyme MAO-B. This lead to the development of reversible monoamine oxidase A inhibitors (RIMAs), e.g. moclobemide (1.23). The advantage of the RIMAs is the absence of this so called ‘cheese-effect’, since tyramine can still be metabolized.

It should be noted that chlorpromazine, imipramine and iproniazid were discovered by sensitive observations of their effects on patients. This method of discovery is very different from the means by which the antidepressant credentials of later antidepressants were established. Today, candidate drugs have to go through extensive in vitro (affinity studies for different receptor subtypes and effects on second messenger systems) and in vivo pharmacology, toxicology and metabolism/kinetic studies before they are even admitted into a clinical trial.

1.2 NEUROCHEMICAL HYPOTHESES OF DEPRESSION

1.2.1 THE CATECHOL-AMINE HYPOTHESIS

Julius Axelrod was the first to demonstrate the presence of a catecholamine and 5-HT uptake system and that these systems were inhibited by imipramine. He was awarded the Nobel Prize for his findings in 1970.\textsuperscript{31-33}

The demonstration that imipramine inhibited catecholamine reuptake, came several years before it was shown that the TCAs inhibited 5-HT reuptake as well. This lead to the catecholamine hypothesis of depression which was put forward in 1965 by Schildkraut in the American Journal of Psychiatry.\textsuperscript{34} The argument was that as reserpine was known to deplete catecholamines and as it had been reported that reserpine could lead to depression and in some cases make people suicidal, there were strong suggestions that the low levels of catecholamines might be associated with the generation of depressive states. Because TCAs inhibited catecholamine reuptake, it was suggested that they increase catecholamine levels in the synaptic cleft. This would subsequently lead to a functional increase in the levels of these neurotransmitters. MAOIs, by blocking the metabolism, also led to an increase in catecholamine levels. The conclusion was that both major groups of drugs acted as antidepressants through this increase catecholamine levels.

A similar argument could be, and indeed was later, made for 5-HT.\textsuperscript{35,36} By then, however, it had been discovered that both imipramine and amitriptyline were metabolized to desipramine and nortriptyline. Both metabolites are much more potent inhibitors of the catecholamine uptake than of the 5-HT reuptake, strongly suggesting that the catecholamines were the more important neurotransmitters.
There were, however, several weak points in the catecholamine hypothesis. The only prospective study where reserpine was given to patients and the level of depression and suicidal tendency were monitored, showed that reserpine acts as an antidepressant and anxiolytic.\textsuperscript{37} This was in full contrast with reports of people committing suicide after being treated with reserpine for hypertension. Also, on the basis of the catecholamine hypothesis, one would expect that antidepressants would be unhelpful in the treatment of mania. However, both ECT and lithium (and more controversially the tricyclics) were used to treat both depression and mania.\textsuperscript{39}

Schildkraut characterized his hypothesis in his 1965 article as “at best a reductionist oversimplification” that might be “of heuristic value” that “may soon require revision due to rapid progress in the basic sciences.” Despite this view and all the drawbacks, the catecholamine hypothesis persisted for more than two decades. Why did the catecholamine hypothesis hold ground despite conflicting findings? For a start, it gave the pharmaceutical industry a clear goal to aim at. Second, during the 1970s attention had switched from levels of NA and its precursors and metabolites to a focus on receptors. The introduction of receptors into the argument about the mechanism of action of antidepressants offered an answer to the fact that some of the newer antidepressants neither blocked reuptake or inhibit MAO.

The classical statements of receptor theory were formulated around 1932 by Alfred Clark, who worked out the principles whereby drugs could be expected to bind to a receptor and the conditions under which there could be competition at the receptor site.\textsuperscript{41}

\[ L + R \xrightleftharpoons[k_1]{k_{-1}} LR \]

\textbf{Figure 1.1} Schematic representation of the reversible binding of a ligand to a receptor; L = free ligand; R = unoccupied receptor; LR = ligand-receptor complex; \( k_1 \) and \( k_{-1} \) are the association and dissociation rate constants.

The first steps towards proof of this theory came in the early 1970s with the use of radio-labeled hormones, such as insulin, and radio-labeled drugs, in particular those which bound to catecholamine receptors.\textsuperscript{42} Using radio-labeled drugs, it was possible to demonstrate binding to particular receptors and to estimate both the amount of receptors in a particular piece of membrane and the affinity of a particular drug. The estimates derived were in keeping with the predictions of classical receptor theory. Radio-labeled binding studies also led to the discovery of further drugs and from there it has been possible to isolate the receptor proteins and clone them.

The antidepressants were found to down-regulate \( \beta \)-adrenergic receptors.\textsuperscript{43} Even the ones that didn’t block catecholamine reuptake acted this way. This down-regulation took up to two weeks to appear, which appeared consistent with the delay there is between taking an antidepressant and onset of clinical action.
In 1976, in the same year that the dopamine hypothesis of schizophrenia was put forward, it was proposed that down-regulation of β-adrenoceptors was precisely the mechanism of action of antidepressants. The appeal of receptors came from the fact that, from the start, they had been portrayed in terms of ‘keys fitting locks’, terminology which was in line with ‘the magic bullet of therapeutic specificity’. During the 1970s the major psychiatric disorders became defined as disorders of single neurotransmitter systems and their receptors, with depression being a catecholamine disorder, anxiety a serotonergic disorder, dementia a cholinergic disorder and schizophrenia a dopamine disorder. Thus the search had begun for receptor(subtype)-specific and selective compounds.

Later it was found that an intact 5-HT system is necessary for adrenergic down-regulation to occur. Until today, it has not been possible to set up a decisive experiment that would either confirm or deny the catecholamine hypothesis. There is no conclusive evidence of the presence of supersensitive β-adrenoceptors in depressed patients, which would have fitted with the proposed action for antidepressants. It also has never been disproved as an antidepressant principle. Generally, findings of studies of the monoamine systems of depressed patients are explained with an appeal to either the heterogeneity of depression and/or a statement that ‘the findings are empirically robust but from a theoretical standpoint difficult to explain’.

1.2.2 THE SEROTONIN HYPOTHESIS

The catecholamine theory began to fade with the introduction of the first selective serotonin reuptake inhibitors in the early 1980s. Since that time, scientific discussion on the mechanisms and backgrounds of depression has been dominated by the serotonin hypothesis.

In 1967, a 5-HT version of the Schildkrauts’ catecholamine hypothesis was proposed. This hypothesis was based on the findings that adding tryptophan, a 5-HT precursor, to a MAOI, boosted its antidepressant effect. It was also found that the concentration of the 5-HT metabolite, 5-hydroxyindoline acetic acid (5-HIAA) was apparently low in the cerebrospinal fluid of depressed patients – the implication was that there was an abnormality of 5-HT turnover in depression. Furthermore, it was argued that, if imipramine and amitriptyline were only pro-drugs of desipramine and nortriptyline, they should have been associated with a greater delay in onset of action and possibly more side effects. The only dissociation between the tertiary and the secondary amine-containing TCAs was the impression that desipramine restored drive more clearly and that imipramine had greater mood-elevating properties.

In the early 1970s, it was discovered that if a preferentially noradrenaline reuptake inhibitor was halogenated, it became a 5-HT reuptake inhibitor. Halogenating brompheniramine led to the production of zimeldine (1.24), the first specific 5-HT
reuptake inhibitor, which was marketed in Europe in 1982 as Zelmid\textsuperscript{R}.\textsuperscript{51} Before it was marketed in the USA, however, it was associated with cases of Guillain-Barré syndrome and withdrawn. In the 1970s, the antidepressant market was still very small and despite the specific development of psychotrophic drugs, psychiatry was still not part of real medicine. In 1972, fluoxetine (Prozac\textsuperscript{R}, \textbf{1.25}) was first synthesized.\textsuperscript{52} Although it was clearly a 5-HT reuptake inhibitor, the suggestion that it might be considered for the treatment of depression was not immediately appreciated.\textsuperscript{53, 54} It was only in the early 1980s, after the antidepressant credentials of zimeldine had become apparent and the perception of the size of the antidepressant market began to change, that the development of fluoxetine as an antidepressant was continued.

\subsection*{1.2.3 Involvement of Other Neurochemical Systems}

Since the implication that elevation of adrenergic and later serotonergic levels is of vital importance in the treatment with antidepressants, several other neurochemical systems have been suggested to play a role in the pathogenesis and therapy of depression. Thus, calcium channel antagonists have shown promise in both animal experiments and clinical trials as potential therapeutics in the treatment of mood disorders.\textsuperscript{55-57} Intracellular calcium plays a crucial role in multiple neuronal processes involved in such essential brain functions as excitability, re- and degeneration, and the synthesis, release and reuptake of neurotransmitters/neuropeptides. In addition, several drugs that modify mood, as well as ECT, have been shown to modify cellular calcium trafficking.\textsuperscript{58-66} Another potential target is the N-methyl-D-aspartate (NMDA) receptor. Recent studies have shown, that NMDA receptor antagonists modulate both catecholamine and serotonin neurotransmission and antagonize stress-induced increases in dopamine metabolism.\textsuperscript{67-75} Neuropeptide Y (NPY), an endogenous ligand for the \(\sigma\)-receptor, may also play a role in the mechanism of action of antidepressant drugs and the pathology of mood disorders. For example, when rats are chronically treated with antidepressants or subjected to repeated ECT, there is an increase in NPY immune reactivity in several brain regions.\textsuperscript{76-78} Conversely, NPY immuno reactivity is decreased in the brains of rats that have been subjected to bilateral olfactory bulbectomy, a procedure that has been used in the development of a rodent model of depression.\textsuperscript{79} It has also been shown that NPY can modulate the release of NA and 5-HT.\textsuperscript{80, 81}
1.3 The Second Generation of Antidepressants

1.3.1 The Selective Serotonin Reuptake Inhibitors

Today, several 5-HT reuptake inhibitors are used in the treatment of depression and other, related diseases like obsessive compulsive behaviors (OCD), social phobia, panic disorders and anorexic/bulimic nervosa. Fluvoxamine (1.26, Luvox®/Faverin®) was first marketed in 1983, citalopram (1.27, Cipramil®) in 1986, fluoxetine (Prozac®) in 1987, sertraline (1.28, Zoloft®/Lustral®) in 1990 and paroxetine (1.29, Paxil®/Seroxat®), which had first been developed in the 1970s, in 1991. In an effort to distinguish paroxetine from the other marketed antidepressants, it was referred to as a selective serotonin reuptake inhibitor (SSRI). The acronym SSRI was quickly adopted as a term to refer to all 5-HT reuptake inhibitors, and has since then been used in the distinguishing of venlafaxine (1.30, Efexor), which was introduced in 1993, a SNRI (serotonin and noradrenaline reuptake inhibitor) and reboxetine (1.31, Edronax), which was launched in 1997, a SNaRI (selective noradrenaline reuptake inhibitor).

![Figure 1.2 Putative structure of the rat serotonin transporter, showing possible phosphorylation (P) sites and glycolisation sites (on the large second extracellular loop).](image)

Compared to the tricyclic antidepressants, which were for the most part very similar molecules, the 5-HT reuptake inhibitors are structurally quite different and their range of actions across a number of receptor systems is also somewhat dissimilar. Although they all inhibit 5-HT reuptake and are termed SSRI by virtue of the fact that the parent molecules have little effect on catecholamine reuptake, they are not specific to the serotonergic system or 5-HT reuptake blockade. They also differ in potencies with which they block 5-HT reuptake. Fluvoxamine is the most potent 5-HT reuptake inhibitor of the currently marketed compounds, while citalopram is the most specific (see Figure 1.3). The variation across compounds indicates that 5-HT reuptake
inhibition might not be sufficient for antidepressant efficacy. Hence, depression might not be a disorder of one neurotransmitter or a particular receptor subtype, but rather a number of physiological systems which are compromised or shut down or desynchronized in some way.

**Figure 1.3** Bar diagram showing the affinity of 1.25 – 1.29 for the 5-HT reuptake site (black bars; right y-axis) and their selectivity, as measured by the ratio between the affinities of the 5-HT and NA transporter (gray bars, left y-axis). Redrawn from ref. 82.

**Chart 1.3** Chemical structure of the SSRIs zimeldine (1.24), fluoxetine (1.25), fluvoxamine (1.26), citalopram (1.27), sertraline (1.28) and paroxetine (1.29) and of the SNRI venlafaxine (1.30) and the SNaRI reboxetine (1.31).
1.3.2  ATYPICAL ANTIDEPRESSANTS

The tetracyclic azepin mianserin (1.32, Tolvon®), was introduced as an antidepressant by Organon in Europe in the 1970s. Its structure and in vitro profile were significantly different from the existing TCAs and MAOIs. Mianserin is devoid of anticholinergic or cardiovascular side effects that are associated with the first generation of antidepressants. The most common side effect associated with the use of mianserin is sleepiness, due to its high affinity for the histaminergic receptors. It appears significantly safer in overdose than the older drugs, which has led to its widespread use. In the mid 1980s, it was one of the two most commonly prescribed antidepressants in Britain. Despite its low affinity for the NA uptake site, the antidepressant action of mianserin is explained by a combination of inhibition of NA reuptake and α₂ adrenoceptor blockade. The successor of mianserin, mirtazapine (1.33, Remeron®) was introduced in 1994. It trades heavily on the supposed interactions between the serotonergic and noradrenergic system (Figure 1.4).

Figure 1.4  Mechanisms for noradrenergic control of 5-HT release. Activation of somatodendritic α₂ adrenergic autoreceptors decreases noradrenergic neuronal activity, and activation of terminal α₂ adrenergic autoreceptors decreases release of NA. Noradrenergic terminals innervate 5-HT cell bodies, where stimulation of postsynaptic α₁ adrenergic receptors serotonergic neuronal activity, increasing 5-HT release. Activation of α₂ adrenergic heteroreceptors on 5-HT terminals inhibits 5-HT release. Redrawn from ref. 5.
Its antidepressant actions are attributed to the fact that it is an $\alpha_2$ adrenoceptor selective antagonist with no apparent affinity for the $\alpha_1$ adrenoceptor. In vivo, mirtazapine is shown to elevate both NA levels - supposedly through blockade of the $\alpha_2$ autoreceptors located on the NA cell bodies in the locus coereleus - and 5-HT levels - supposedly through blockade of the $\alpha_2$ heteroreceptors located on the 5-HT nerve terminals.

In the course of investigating clomipramine, it was discovered that this antidepressant had an effect on sexual (dis)functioning.89-92 The SSRIs also have effects on sexual functioning, affecting well over 50% of users. However, rather than direct development for the indication of anorgasmia, impotence and loss of libido, companies have waited for off-license prescribing by clinicians of e.g. clomipramine to lead to market development. In vivo experiments with SSRIs monitoring sexual functioning in animals strongly suggests that drugs, such as buspirone (1.34) - active on the 5-HT$_1A$ receptor - and trazodone (1.35) - which blocks the 5-HT$_2A$ receptor - should be reliable as aphrodisiac.93 Trazodone even developed a reputation in this respect. Following its pre-Prozac launch in the United States as an antidepressant, its sales were substantial, partly because of its effects on sexual functioning.

![Chemical structures of various compounds](image)

**Chart 1.5** Chemical structure of mianserin (1.32), mirtazapine (1.33), buspirone (1.34), trazodone (1.35), yohimbine (1.36), viagra (1.37), nefazodone (1.38) and buproprion (1.39).
Despite the clinical evidence that the very similar compound yohimbine (1.36) has reliably aphrodisiac properties this area has been downplayed by the pharmaceutical industry right up to the introduction of viagra (1.37). For the post-SSRI compounds like nefazodone (1.38), moclobemide and buproprion (1.39) the possible aphrodisiac properties are now highlighted and this marketing pitch is likely to increase awareness of the potential to prescribe for premature ejaculation and anorgasmia, even though none of the companies may ever seek a license for these indications.

The antidepressant actions of trazodone are explained by the fact that it inhibits reuptake of both NA and 5-HT. The inhibition of 5-HT reuptake is stronger than of NA. Trazodone, induces less anticholinergic and antihistaminergic side effects than the classical TCAs, although it is known to induce sleepiness. Nefazodone, like trazodone, is a 5-HT$_{2A}$ receptor antagonist with weak affinity for the NA and 5-HT reuptake sites and is also known to be sedative. However, nefazodone causes less orthostatic hypotension than trazodone. Buproprion is relatively unique in that it more potently blocks the reuptake of DA than NA or 5-HT. In spite of this, it inhibits the firing of noradrenergic cells in the locus coeruleus more potently than that of dopaminergic cells in the midbrain.

1.4 SEROTONIN RECEPTORS IN THE BRAIN

Of the many brain neuronal systems, the serotonin system is currently the most common neurobiological target for antidepressant treatments. TCAs act on both serotonergic and noradrenergic neurons by inhibiting transmitter uptake, MAOIs increase serotonergic and noradrenergic transmission by preventing their metabolism and SSRIs selectively inhibit the 5-HT transporter thereby increasing the synaptic concentration of 5-HT. SSRIs display little affinity for aminergic receptors and lack the severe side effects associated with the first generation antidepressants. The clinical actions of SSRIs can be attributed to an enhanced activation of one or several postsynaptic 5-HT receptors. It has been postulated that the slow onset of clinical action following treatment with the current available antidepressants may be partly ascribed to the inhibition of 5-HT release by forebrain serotonergic nerve terminals after the administration of antidepressants. This negative feedback, which involves the activation of 5-HT autoreceptors, is also believed to be responsible for the attenuation of cell firing of 5-HT neurons.

1.4.1 G-PROTEIN COUPLED RECEPTORS

With the exception of the serotonin 5-HT$_3$ receptor, which is a ligand gated ion channel, all serotonin receptor subtypes belong to the super family of G-protein coupled receptors (GPCRs). Signal transduction through GPCRs requires three membrane-bound components. First, a cell-surface receptor that determines to which signal the cell can respond. Second, a G protein on the intracellular side of the
membrane that is stimulated by the activated receptor. And third, either an effector enzyme that changes the level of a second messenger or an effector channel that changes ionic fluxes in the cell in response to the activated G protein (see Figure 1.5).

**Figure 1.5** Overview of G-protein signaling to protein kinases. PLC, phospholipase C; PIP₂, phosphatidylinositol biphosphate; DAG, diacylglycerol; CaM, Ca²⁺-calmodulin-dependent; IP₃, inositol 1,4,5-triphosphate, PKA and PKC, protein kinase A and C.

There are three main families of GPCRs in mammals: the rhodopsin-like family, the metabotropic glutamate and chemosensor family and the glucagon/VIP/calcitonin family. As many as 1000 receptors for monoamines, chemokines, odorants, neuropeptides and lipid messengers couple to one or more of the 20 different G proteins.

In 1983, the first G-protein coupled molecule, rhodopsin - a light sensing molecule which binds the chromophore retinal – was cloned. The most striking structural features of this photo-receptor were the seven hydrophobic segments (see Figure 1.6). These hydrophobic regions are believed to form seven transmembral (TM) α-helices, like the seven TM helices of the proton pump bacteriorhodopsin. The GPCRs possess a substantial degree of homology in their amino acid sequences, especially in the TM regions. The seven hydrophobic stretches that span the membrane are connected by loops ranging in length from 5 to 420 amino acid residues. The amino(N)-terminal region of the protein is located extracellularly, while the carboxyl(C)-terminal is located intracellularly. The second and third intracellular loop and the cytoplasmic C-terminus are involved in the coupling to the G protein. G-proteins are turmeric structures composed of two functional units: an α subunit that catalyzes the GTPase activity and a βγ dimer that tightly interacts with the α subunit when bound to GDP. G-proteins are held in an inactive state because of very high affinity binding of GDP to
their α subunits. Coupling of the endogenous ligand to the active site of the 7TM-receptor will induce conformational changes in the receptor molecule. These conformational changes will trigger the dissociation of GDP. This permits GTP to bind to and activate the α subunit, which then dissociates from the βγ dimer. The GTP-α complex can interact with e.g. adenylate cyclase, which then catalyzes the synthesis of the second messenger cyclic AMP (cAMP) from ATP. The signaling is terminated when the α subunit hydrolyzes its bound GTP to GDP. The hydrolysis permits the GDP-α complex to dissociate from its effector and associate again with the βγ dimer.5, 94-99

Figure 1.6 G-protein mediated signal transduction across the cellular membrane. Small signal molecules, like serotonin, are bound in the cavity formed by the seven trans membrane helices of the GPCR. The signal is transmitted via cytosolic loops of the GPCR to the G protein complex. Redrawn from ref. 5.

Table 1.1 Some chemical messengers that act through the Rhodopsin family GPCRs.

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<thead>
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<th>1st Messenger</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Light/Odorants</td>
<td>Retinal/ Multiple substances</td>
</tr>
<tr>
<td>Ions/Proteases</td>
<td>Ca²⁺/thrombin</td>
</tr>
<tr>
<td>Amino acids</td>
<td>Glutamate, GABA</td>
</tr>
<tr>
<td>Mono amines</td>
<td>5-HT, adrenaline, NA, DA, melatonin, ACh, histamine</td>
</tr>
<tr>
<td>Lipid messengers</td>
<td>Prostaglandins, thromboxane, platelet activating factor, leukotrienes,</td>
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<tr>
<td>Purines</td>
<td>Adenosine, ATP</td>
</tr>
<tr>
<td>Neuropeptides</td>
<td>Substance P, gastrin releasing polypeptide, NPY, enkephalins</td>
</tr>
<tr>
<td>Hormones</td>
<td>Angiotensin, ACTH (peptides); LH/FSH, TSH (glycoprotein)</td>
</tr>
<tr>
<td>Chemokines</td>
<td>IL-8, formyl-Met-Phe-Leu, Complement C5a</td>
</tr>
</tbody>
</table>
1.4.2 The serotonergic system

It was already known in the 19th century, that blood serum contained a substance with vasoconstrictive properties. In 1948, Rapport, Green and Page isolated this compound and named it serotonin.\(^{100}\) A year later, they were able to identify the compound as 5-hydroxytryptamine (5-HT).\(^{101}\) In 1953, the presence of 5-HT in the brain was demonstrated and shortly afterwards it was recognized as a neurotransmitter\(^ {102}\). In 1957, the first two types of serotonin receptors were distinguished, which were termed D and M.\(^ {103}\) Receptors of the D-type mediated the serotonin-induced contraction of smooth muscle, which could be blocked by dibenzylin (1.40). The M-type receptors mediated the serotonin-induced release of acetylcholine (1.41) from postganglionic nerve terminals, which could be blocked by morphine (1.42). In 1979, a new classification, based on radioligand studies, was proposed.\(^ {104}\) In 1986, the two classification schemes were combined and regrouped in three main classes, designated ‘5-HT\(_1\)-like’ (corresponding to some D receptors and 5-HT\(_1\) binding sites), 5-HT\(_2\) (corresponding to most D receptors and 5-HT\(_3\) (equivalent to M receptors).\(^ {105}\)

![Figure 1.7](image.png)

**Figure 1.7** Schematic drawing of the 5-HT pathways in the human brain; serotonergic neurons are indicated in black; areas that are innervated by 5-HT axons are shown in light gray.\(^ {113}\)

This classification scheme has formed the basis for the current classification of serotonin receptors, which is based on a combination of operational, transductional and structural considerations.\(^ {106,107}\) The cell bodies of 5-HT neurons are located in the brain stem and consist of two divisions. The axons of the caudal serotonergic system - which stems from cell bodies located in the caudal raphe nuclei in the medulla oblongata and the caudal pons - project to the spinal cord. For the rostral serotonergic system, the cell bodies are located in the rostral raphe nuclei in the midbrain and...
rostral pons. These neurons have projections to several tissues in the forebrain, including basal ganglia (striatum, pallidum), limbic system (hippocampus, amygdala), hypothalamus, thalamus and several parts of the cerebral cortex. In addition to the nine 5-HT nuclei (B1-B9) originally described in the mid 1960s, immunochemical localization of 5-HT has also detected reactive cells in the area postrema and in the caudal locus coeruleus.\textsuperscript{108-111} The basal ganglia were initially thought to be involved in movement processes only, but it seems that they are also involved in several neuropsychiatric symptoms. The limbic system was originally identified as the seat of emotions, but later it was suggested to have a function in memory and aggressive behavior as well. Neuroendocrine regulation is an important function of the hypothalamus and it is involved in appetite and sexual behavior. The thalamus is important in pain perception and the cerebral cortex in numerous processes that concern cognition, skills and observation.\textsuperscript{112}

**Biosynthesis and Metabolism of Serotonin**

About one to two percent of the total amount of 5-HT in the body is found in the brain. Because 5-HT cannot cross the blood-brain barrier, it is clear that the brain cells must synthesize it \textit{in situ}.

\textbf{Scheme 1.1}\textsuperscript{3} The metabolic pathways available for the synthesis and metabolism of serotonin

\begin{figure}
\centering
\includegraphics[width=\textwidth]{serotonin_metabolism_scheme.png}
\end{figure}

\textsuperscript{3} Enzymes: a) tryptophan hydroxylase; b) L-aromatic amino acid decarboxylase; c) monoamine oxidase, aldehyde dehydrogenase; d) 5-hydroxytryptamine \textit{N}-acetylase; e) 5-hydroxy indole \textit{O}-methyl transferase.
The first step in this synthesis is the uptake of the amino acid tryptophan (1.43). Plasma tryptophan arises primarily from the diet, and elimination of dietary tryptophan can profoundly lower the levels of brain 5-HT and can even induce depression. Substrates for the active carrier pump that transports tryptophan across the blood-brain barrier are large amino acids – including aromatic amino acids, like tyrosine (1.44) and phenylalanine (1.45), and branch-chain amino acids, like leucine (1.46), isoleucine (1.47) and valine (1.48), and others, like methionine (1.49) and histidine (1.50). The competitive nature of the large neutral amino acid carrier means that brain levels of tryptophan will be determined not only by the plasma concentration of tryptophan, but also by that of competing neutral amino acids. The next step in the synthetic pathway is hydroxylation of tryptophan at the 5 position to form 5-hydroxytryptophan (1.51). The tryptophan hydroxylation step can be specifically blocked by p-chlorophenylalanine (PCPA, 1.52), which binds irreversibly to tryptophan hydroxylase. The last step in the synthesis is decarboxylation of 5-hydroxytryptophan by l-aromatic amino acid decarboxylase to yield 5-hydroxytryptamine (5-HT, 1.11).

![Chemical structures](chart15.png)

**Chart 1.5** Chemical structure of dibenzylin (1.40), acetylcholine (1.41), morphine (1.42), the amino acids tyrosine (1.44), phenylalanine (1.45), leucine (1.46), isoleucine (1.47), valine (1.48), methionine (1.49) and histidine (1.50), PCPA (1.52), clozapine (1.55) and sumatriptan (1.56).
The only effective route of continued metabolism for 5-HT is deamination by MAO-A to 5-hydroxyindoleacetaldehyde. This can be further oxidized to 5-hydroxyindoleacetic acid (5-HIAA, \textbf{1.53}) or reduced to 5-hydroxytryptophol, depending on the NAD\(^+\)/NADH ratio in the tissue.

In the pineal gland, 5-HT is converted to 5-methoxy-N-acetyltryptamine (melatonin, \textbf{1.54}), via N-acetylation and 5-hydroxy-indole-O-methyl-transferase. Melatonin plays an important role in that it can \textit{e.g.} manipulate the biological clock, regulate sleep-wake patterns and improve jet-lag. Considering the multiplicity of tissues that are innervated by 5-HT neurons and their functions, it is not surprising that 5-HT has been found to be involved in a variety of central functions and disorders. These central functions include mood, sleep, appetite, pain perception, memory and sexual behavior. 5-HT has been suggested to be involved in disorders such as depression, migraine, schizophrenia and anxiety, including obsessive-compulsive disorders. A major application of drugs that interact with the serotonergic system are the antidepressants (TCAs, RIMAs and SSRIs). Other drugs that act (partly) via 5-HT receptors are antipsychotics, like clozapine (\textbf{1.55}) and antimigraine drugs, like sumatriptan (\textbf{1.56}).

\textbf{1.4.3 SEROTONIN RECEPTOR SUBTYPE CLASSIFICATION}

The three main criteria for classifying and naming 5-HT receptors are based upon operational (recognition), transductional and structural characteristics. None of these characteristics have precedence.\textsuperscript{106, 114-116} An additional classification principle is ‘Human Species Primacy’, the same receptor subtype name should be used for all species homologues of a gene, specifying the species with brief letter prefixes.\textsuperscript{117} Each of the seven currently recognized 5-HT receptor classes exhibits a quite distinct pharmacology, even though some of these classes utilize the same transduction pathway.\textsuperscript{106} Historically, the operational characteristics of 5-HT receptors, comprising functional pharmacological and radioligand binding data, were considered sufficient for their differential classification.\textsuperscript{105} The tendency for operational profiles to become increasingly conserved within a receptor class means that sub-type differentiation within a class is increasingly difficult. The human 5-HT\textsubscript{1B} and 5-HT\textsubscript{1D} receptor subtypes are classic examples. Although these two subtypes are distinct gene products with approximately 70\% sequence homology, they exhibit pharmacological profiles that are almost indistinguishable.\textsuperscript{118} On the other hand, a high degree of sequence homology between receptors does not always result in similar operational characteristics. Hence, the rat and human homologues of the 5-HT\textsubscript{1B} receptors share 97\% overall sequence identity, yet their operational profiles are quite distinct.\textsuperscript{119, 120} Thus, they were previously seen as different receptor subtypes (h-5-HT\textsubscript{1D} and r-5-HT\textsubscript{1B}).

Application of modern molecular biology techniques and the above described considerations have presently revealed the existence of seven different classes of
serotonin receptors (5-HT_{1-7}). Some of these classes, like the 5-HT\textsubscript{1} and 5-HT\textsubscript{2} receptor families consist of several subtypes. Some 5-HT receptor subtypes have only been identified as gene products and are therefore denoted in small characters.

**SEROTONIN 5-HT\textsubscript{1} RECEPTORS**

The 5-HT\textsubscript{1} receptor class now comprises of the 5-HT\textsubscript{1A}, 5-HT\textsubscript{1B}, 5-HT\textsubscript{1D}, 5-HT\textsubscript{1E} and 5-HT\textsubscript{1F} subtypes.\textsuperscript{107} The 5-HT\textsubscript{1A} receptor is found both presynaptically (autoreceptors) on the cell bodies of 5-HT neurons in the raphé nuclei - where they regulate the 5-HT synthesis, release and electrical activity via a negative feedback control - and postsynaptically in the different projection areas, with high concentrations in the hippocampus, septum and some of the amygdaloid. Many of these regions are components of the pathways involved in the modulation of emotion, the limbic system. 5-HT\textsubscript{1A} receptors are also present in the neocortex, the hypothalamus and the substantia gelatinosa of the spinal cord.\textsuperscript{121-126} In rats, activation of central 5-HT\textsubscript{1A} receptors by agonists induces a rectal temperature decrease, a behavioral (5-HT) syndrome and inhibition of ultrasonic vocalization.\textsuperscript{127-130}

The 5-HT\textsubscript{1B} and 5-HT\textsubscript{1D} receptors may be involved in a large number of functions, e.g. depression, anxiety, migraine and movement disorders. In 1996, the nomenclature for the 5-HT\textsubscript{1B/1D} receptor subtypes was revised.\textsuperscript{131} In previous classification schemes the well-characterized rodent 5-HT\textsubscript{1B} receptor was recognized as a distinct entity. The subsequent identification of a pharmacologically different receptor with a similar distribution in non-rodents led to the recognition of the 5-HT\textsubscript{1D} class. In 1992, it was shown that the human 5-HT\textsubscript{1D} receptor in fact comprised two subtypes, encoded by distinct genes.\textsuperscript{132} These were named the h-5-HT\textsubscript{1D\alpha} and h-5-HT\textsubscript{1D\beta} subtypes. It was subsequently shown that, in spite their pharmacological differences, the rodent 5-HT\textsubscript{1B} and non-rodent 5-HT\textsubscript{1D\beta} receptors were in fact homologues. Accordingly, they are now called 5-HT\textsubscript{1B} receptors. The 5-HT\textsubscript{1D\alpha} receptor has retained the 5-HT\textsubscript{1D} notion, since its pharmacology is the same as that described for the 5-HT\textsubscript{1D} receptor from the outset. The terminal 5-HT autoreceptors are of the 5-HT\textsubscript{1B} type, whereas 5-HT\textsubscript{1D} autoreceptors are colocalized with 5-HT\textsubscript{1A} receptors in the raphé nuclei.\textsuperscript{132-135}

Postsynaptically, high densities of 5-HT\textsubscript{1B} receptor mRNA has been found in the striatum, cerebellum (Purkinje cell layer), hippocampus (pyramidal cell layer of CA1), entorhinal and cingulate cortex (layer IV), subthalamic nucleus, and nucleus accumbens but not substantia nigra.\textsuperscript{136-138} High densities of 5-HT\textsubscript{1D} receptor mRNA has been found in the substantia nigra, globus pallidus, dorsal subiculum and superior colliculi.\textsuperscript{139-141} In 1996, an endogenous tetrapeptide (Leu-Ser-Ala-Leu) was isolated from both rat and bovine brain. This peptide was designated 5-HT-moduline and it appears to function as an endogenous ligand specific for the 5-HT\textsubscript{1B} receptor. 5-HT-Moduline is released from synaptosomal preparation by a Ca\textsuperscript{2+}-dependent K\textsuperscript{+} stimulation, suggesting that the peptide is stored in excitable cells.\textsuperscript{142, 143}
The 5-HT₁E receptor was first identified in 1989 in homogenates of human frontal cortex. Until now, the 5-HT₁E receptor has not been cloned from any other species. Homogenate-binding studies indicate that the receptor is generally present in brain regions similar to those of the 5-HT₁D receptor in varying relative proportions. The function of the 5-HT₁E receptor is not yet known. The pharmacology of the 5-HT₁F receptor is solely based on the isolation and expression of the encoding cDNA. Based on the sequence homology, the 5-HT₁F receptor is closely related to the 5-HT₁E receptor. From in situ hybridization studies, the mRNA for the human receptor protein has been identified in the brain, mesentery and uterus, but not in kidney, liver, spleen, heart, pancreas or testes. In the brain, the mRNA has been shown to be concentrated in the dorsal raphe, hippocampus and cortex. This distribution indicates a role as another 5-HT autoreceptor type.

SEROTONIN 5-HT₂ RECEPTORS

The 5-HT₂ receptor subclass consists of three subtypes. The 5-HT₂A receptor, the ‘classical’ 5-HT₂ receptor, the 5-HT₂B receptor, which was formerly called the 5-HT₂F receptor - F for stomach fundus - and the 5-HT₂C receptor, which was originally designated as the 5-HT₁C receptor. The 5-HT₂A receptor has been reported to be involved in many peripheral as well as central functions, e.g. smooth muscle contraction, platelet aggregation, control of hormone or transmitter release, control of sexual activity, regulation of sleep, motor behavior and psychiatric disorders like epilepsy, migraine, anxiety, depression, schizophrenia and hallucination. Some behavioral effects in rodents, like head twitch and wet-dog shake, and neuronal depolarization have been attributed to activation of central 5-HT₂A receptors. The 5-HT₂A receptors are enriched in many areas of the cortex. In the neocortex, these sites are mainly concentrated in laminae I and IV (rat) and III and V (human). 5-HT₂A sites are also found in the claustrum (connected to the visual cortex), the limbic system (olfactory nuclei) and parts of the basal ganglia.

Due to the lack of specific 5-HT₂B receptor ligands, there is not much known about the function of 5-HT₂B receptors. The rat stomach fundic strip has been known for a long time to be extremely sensitive to 5-HT, where the main effect appears to be contraction. Although the fundus receptor shared some characteristics with the classical 5-HT₂ receptor, it was clear that it was not a 5-HT₂A receptor. Based on the rank order of potency of a variety of agonists, the fundus receptor was shown to also bear resemblance to the 5-HT₂C receptor. In 1994, the h-5-HT₂B receptor was cloned. Human 5-HT₂B mRNA has been detected in a variety of peripheral tissues and also, although in relatively low amounts, in the brain. No transcripts of 5-HT₂B mRNA have been found in the rat brain. This has led to the notion that the 5-HT₂B receptor in the human brain may be involved in higher cognitive brain functions.

5-HT₂C receptors are highly expressed in the epithelial cells of the choroid plexus, and function in the control of the exchanges between the central nervous system and
the cerebrospinal fluid.\textsuperscript{158, 159} They are also present, although at lower densities than in the choroid plexus, in the limbic system and regions associated with motor behavior.\textsuperscript{160} 5-HT\textsubscript{2C} sites appear to be more abundant in the basal ganglia of humans, particularly the globus pallidus and in the substantia nigra.\textsuperscript{124} It has been suggested that 5-HT\textsubscript{2C} receptors play a role in a variety of processes such as locomotion, feeding, anorexia nervosa, cerebrospinal fluid production, adrenocorticotropic hormone release, migraine, obsessive, compulsive disorders and anxiety.\textsuperscript{161-166} The best characterized 5-HT\textsubscript{2C} receptor-mediated effects are hypolocomotion and hypophagia.\textsuperscript{165} Other effects include penile erection, decreased social interaction and suppression of hypertonic saline consumption.\textsuperscript{167, 168}

**SEROTONIN 5-HT\textsubscript{3} - 5-HT\textsubscript{7} RECEPTORS**

The 5-HT\textsubscript{3} receptor is a ligand gated ion channel, that consists of 5 subunits (pentamer).\textsuperscript{169} In the brain, the highest densities of 5-HT\textsubscript{3} receptors are found in discrete nuclei of the lower brain stem, e.g. dorsal vagal complex and trigeminal nucleus, the area postrema en the nucleus tractus solitarius and the substantia gelatinosa of the spinal cord.\textsuperscript{170, 171} Lower, but significant densities of 5-HT\textsubscript{3} binding sites are also found in the cortex and areas of the limbic region such as the hippocampal formation, amygdala and medial nucleus of the habenula.\textsuperscript{171-176} In the periphery, 5-HT\textsubscript{3} receptors are located on pre- and postganglionic autonomic neurons and neurons of the sensory and enteric nervous systems.\textsuperscript{177-182} Activation of 5-HT\textsubscript{3} receptors triggers a rapid depolarization.\textsuperscript{182-184} In the CNS, 5-HT\textsubscript{3} antagonists profoundly influence animal behavior, implicating a role for 5-HT\textsubscript{3} receptors in psychosis, anxiety, cognition and the rewarding and withdrawal effects from drugs of abuse and eating disorders.\textsuperscript{185, 186}

The 5-HT\textsubscript{4} receptor was first described in 1988.\textsuperscript{187} Effects of 5-HT mediated by 5-HT\textsubscript{4} receptors have been described in heart, adrenal, bladder and alimentary canal, which implies a role of this receptor subtype in diseases of the gastrointestinal tract, as well as in cardiac, urinary and endocrine functions.\textsuperscript{188} In the brain, high densities of 5-HT\textsubscript{4} receptors are found in several limbic areas, such as olfactory tubercles and nucleus accumbens, corpus striatum, globus pallidus and substantia nigra. This suggests a possible role in affective disorders, psychoses, motor coordination, arousal and visual perception, in addition to learning and memory.\textsuperscript{189-191}

At present, there are no functional correlates or transductional characteristics known of the recombinant 5-h\textsubscript{5A} and 5-h\textsubscript{5B} receptors. Because of their high affinity to LSD (D-lysergic acid diethylamine, 1.57) and ergot derivatives, they are postulated to mediate some of the effects of these drugs. The chromosomal localization of the 5-h\textsubscript{5A} receptors corresponds to regions in which mutations lead to abnormal brain development, which suggests a possible role for this receptor subtype in brain development. Both receptor genes show a strong sequence similarity (77%), but similarities to other 5-HT receptor subtypes are low.\textsuperscript{192-195}
There is increasing circumstantial evidence to suggest that the putative 5-ht₆ receptor is expressed endogenously in neuronal tissue. 5-ht₆ receptors exhibit a high affinity for tricyclic antipsychotic drugs, like clozapine, amitriptyline, mianserin and ritanserin (1.58), and because 5-ht₆ receptor transcripts have been localized in limbic and cortical regions of the brain (striatum, olfactory tubercles, cerebral cortex, hippocampus) it has been suggested that the 5-ht₆ receptor may play a role in several neuropsychiatric disorders that involve serotonergic systems.¹⁹⁶, ¹⁹⁷ There is no evidence for the presence of the 5-ht₆ receptor in peripheral tissues. In ‘knock-out’ rats, a specific behavioral syndrome of yawning, stretching and chewing is revealed.¹⁹⁸ Because atropine (1.59) dose-dependently antagonizes this behavioral syndrome, the 5-ht₆ receptors may be involved in the control of cholinergic transmission.

[Chemical structures] Chart 1.6 Chemical structure of LSD (1.57), ritanserin (1.58) and atropine (1.59).

In 1997, the 5-HT₇ receptor was upgraded from putative serotonin orphan receptor to 5-HT₇ receptor.¹⁹⁹ The 5-HT₇ receptor has been cloned from rat, mouse, guinea pig and human cDNA.²⁰⁰-²⁰⁵ It exhibits a high degree of interspecies homology (95%), but a low sequence homology with other 5-HT receptors (<40%). In the brain, the 5-HT₇ receptor is located in the thalamus, hypothalamus and several limbic and cortical regions.²⁰⁴, ²⁰⁶, ²⁰⁷ Furthermore, there are high densities of 5-HT₇ receptors in certain peripheral nervous structures, such as lumbar dorsal root ganglia, and in vascular and non-vascular smooth muscle, including the external carotid vascular bed, the coronary artery and several regions of the gastrointestinal tract (stomach, descending colon, ileum).²⁰⁵, ²⁰⁸, ²⁰⁹ The localization of 5-HT₇ receptors in these tissues implies a role for these subtypes in depression, regulation of circadian rhythm, sensory processing, sleep disorders, schizophrenia, migraine, modulation of sympathetic afferent pathways and hypertension.
**Table 2** 1996 NC-IUPHAR classification and nomenclature for 5-HT receptors and some of their characteristics

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Previous name</th>
<th>Location</th>
<th>Agonist</th>
<th>Antagonist</th>
<th>Effector/Response</th>
<th>Structural information</th>
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<td>Hipp, RNu</td>
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<td>h 421aa 7TM</td>
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<tr>
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<td>5-HT1Dβ*</td>
<td>Str, Hipp, OT</td>
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<td>1.70</td>
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<td>1.71</td>
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<td>—</td>
<td>SN, GP</td>
<td>—</td>
<td>—</td>
<td>Gi/o cAMP↓</td>
<td>h 365aa 7TM</td>
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<td>—</td>
<td>Gi/o cAMP↓</td>
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<td>1.73</td>
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<td>Hipp, Hyp</td>
<td>—</td>
<td>—</td>
<td>Gs?</td>
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<td>1.77</td>
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<td>h 445aa, 7TM</td>
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</tbody>
</table>

* Only the non-rodent form of the receptor was previously called 5-HT1D; † No human equivalent has been identified; Abbreviations: Hipp, hippocampus; RNu, raphé nuclei; SN, substantia nigra; TgN, trigeminal nucleus; GP, globus pallidus; OT, olfactory tuberue; CP, choroid plexus; FC, frontal cortex; Hyp, hypothalamus; Str, striatum; Thal, thalamus; cAMP, cyclic adenosine monophosphate; IP3, inositol triphosphate; DAG, diacyl glycerol.
Chart 1.7 Chemical structure of 5-HT agonists 8-OH-DPAT (1.60), CP931259589 (1.61), L-694,247 (1.62), (R)-DOI (1.63), BW 723C86 (1.64), Ro 60-0175 (1.65), m-CPBG (1.66), cisapride (1.67) and 5-CT (1.68).

Chart 1.8 Chemical structure of 5-HT antagonists WAY100635 (1.69), SB224289 (1.70), GR127935 (1.71), ketanserin (1.72), SB204741 (1.73), SB242084 (1.74), MDL72222 (1.75), GR113808 (1.76) and SB258719 (1.77).
1.5 ANIMAL MODELS IN ANTI-DEPRESSANT RESEARCH

There are several different strategies in the antidepressant research. The first strategy focuses on a known property of existing anti-depressants that is held responsible for the therapeutic effect of these drugs. The goal of this strategy is to maximize this property, while others - that are believed to induce the unwanted side-effects – are minimized. An example of this approach are e.g. the SSRIs, in which the 5-HT-reuptake-inhibition property is maximized, while the noradrenergic and acetyl cholinergic properties are minimized. A second strategy focuses on targeting the neurochemical effects that are identified as common to a variety of antidepressants. Examples of this approach are the 5-HT autoreceptor down-regulation or the NMDA receptor modulation. A third possible strategy would be the screening of novel compounds in behavioral tests that are predictive of antidepressant activity. Of course, in practice, in vivo models are used primarily to follow-up on leads that have emerged from neurochemical strategies. In theory, however, this approach would require no preconceptions as to mechanisms of action and could generate novel neurochemical hypotheses for antidepressant drugs.

1.5.1 ACUTE MODELS FOR DEPRESSION

For the screening of anti-depressant action of novel compounds, several acute, non-invasive models for depression have been developed. In 1977, Porsolt’s forced-swim test or behavioral despair test was first described.210 Rats are forced to swim in a cylinder of water and, after an initial period of active escape attempts, they adopt an immobile posture. On a second exposure, the immobile posture is entered more rapidly. Antidepressants delay the onset of immobility in this second test. Important false negatives are the SSRI’s. 5-HT1A agonists and NMDA antagonists, on the other hand, possess antidepressant-like activity according to this procedure.211,212 The learned helplessness test, although already described in dogs in 1967, was not applied in psychopharmacology until 1979.213 Learned helplessness refers to the incapacity of animals (rats or dogs), pre-exposed to a series of inescapable shocks, to subsequently learn to escape from shock, for example by pressing a lever in a Skinner Box or crossing to the other side of a shuttle box. The test derives its name from the assumption that the exposure to situations over which the animal has no control, induces feelings of helplessness and signs of cognitive incapacity. The procedure is time-consuming and difficult to reproduce in rats. However, learned helplessness is sensitive to a wide range of antidepressants, including SSRIs, 5-HT1A agonists and NMDA antagonists.214 The tail suspension test or restraint-induced depression was originally conceived as a dry version of the forced-swim test, in which simple suspension by the tail of the animal (rats or mice) induced immobility. The test shows similar sensitivity to the behavioral despair test, but differs in that it is sensitive to SSRIs, but not to 5-HT1A agonists (see table 1.3).212, 215
Table 1.3 Hits and misses of the acute models for antidepressant detection

<table>
<thead>
<tr>
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<th>Forced-swim test</th>
<th>Learned helplessness</th>
<th>Tail suspension</th>
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<tbody>
<tr>
<td>Hits</td>
<td>TCAs; MAOIs; RIMAs; Sertraline; Zimeldine; Fluoxetine; Atypicals; 5-HT1A agonists; NMDA antagonists; Ca2+ channel antagonists</td>
<td>TCAs; MAOIs; RIMAs; SSRIs; Atypicals; 5-HT1A agonists; NMDA antagonists; Ca2+ channel antagonists</td>
<td>TCAs; MAOIs; RIMAs; SSRIs; Atypicals; NMDA antagonists</td>
</tr>
<tr>
<td>Misses</td>
<td>Clomipramine; Fluvoxamine</td>
<td>?</td>
<td>5-HT1A agonists</td>
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1.5.2 CHRONIC MODELS FOR DEPRESSION

The major disadvantage of the acute models for depression, is that the antidepressant activity is detected following acute administration, whereas in clinical depression, several weeks elapse before a therapeutic effect is observed. This consideration argues strongly for the use of chronic models of depression. These models should not only be of demonstrable validity, but should also maintain an abnormal state for a prolonged period, during which therapy may be administered. Models that meet these criteria are of value not only for drug development, but also as experimental tools for investigating the physiological mechanisms underlying the depression-like behavior and the therapeutic action of antidepressant drugs. Because these models would simulate depression in a relatively realistic and valid manner, the conclusions of such studies are likely to generalize to the clinic. And while the traditional screening tests are limited in that they only detect antidepressant action, the chronic models can also be used to discover new antidepressants with a shorter onset of action.

CHRONIC MILD STRESS

In the chronic mild stress (CMS) model, rats or mice are exposed sequentially to a variety of extremely mild stressors (e.g. overnight illumination, cage tilt, change of cage mate), which change every few hours over a period of weeks or months. This procedure causes a decrease in sensitivity to rewards.216-218 It should be noted that the variety in stressors is essential.219 CMS-induced behavioral deficits may be maintained for several months. However, chronic treatment with tricyclic or atypical antidepressants during continued application of CMS, will restore normal behavior. A variety of neurochemical systems have been examined as a potential basis for the behavioral changes following CMS. Although the 5-HT and NA systems are traditional starting points for thinking about antidepressants, it seems logical to focus
also on the mesolimbic DA system, because it has been the major focus for studies concerning the responsiveness to rewards. Both pre- and postsynaptic markers indicate a decrease in DA transmission in animals exposed to CMS, specific to the nucleus accumbens. In animals successfully treated with antidepressants, behavioral recovery is reversed by acute administration of D2/D3 receptor antagonists. It has been suggested that sensitization of D2/D3 receptors may be responsible for the therapeutic action of antidepressants in this model. Hence, the mesolimbic DA system might be a potential target for antidepressant drug development. DA reuptake inhibition is actually a feature of several recent antidepressants, like nomifensine, buproprion, amineptine and minaprine. However, these drugs have not yet been tested in the CMS model. Another antidepressant candidate, tolcapone - which raises synaptic levels of DA by inhibition of the catabolic enzyme COMT - does show antidepressant-like activity in the CMS model. The novel D2/D3 receptor agonist pramipaxole also reversed the effects of CMS, exactly as observed with conventional antidepressants. Pramipaxole is currently evaluated in Phase 3 trials as an antidepressant.

Besides the effects of CMS on the mesolimbic DA system, several interactions with the 5-HT system have been described. It has been found that CMS increases tissue levels of 5-HT and its metabolite 5-HIAA in the nucleus accumbens, but not in the dorsal striatum. Receptor binding studies revealed that CMS increased the density of both 5-HT1A and 5-HT2 receptors in the cerebral cortex. Chronic treatment with imipramine decreased the density of the 5-HT2 receptors in control animals and normalized the 5-HT2 receptor density in animals exposed to CMS. However, in the case of the 5-HT1A receptor, imipramine failed to normalize the effect of CMS. The full 5-HT1A receptor agonist 8-OH-DPAT and partial 5-HT1A receptor agonist ipsapirone, also did not reverse the CMS-induced adhedonia. However, the 5-HT1A receptor antagonist WAY100635 shows antidepressant-like activity in the CMS model.

Studies of the interactions between the NA system and CMS have been less extensive than those of the DA and 5-HT system. It has been shown that CMS increases the density of -adrenergic receptors in the cerebral cortex and also that the cAMP response to NA in cortical slices is increased. Chronic treatment with imipramine reversed these effects of CMS.

**Olfactory bulbectomy**

A second chronic animal model for depression is the olfactory bulbectomized (OB) rat. This model differs from the acute and CMS models for depression, in that it is not based on stress. In the OB rat model, both main olfactory bulbs are removed, without disturbing the anterior olfactory nucleus. The OB rat model is capable of detecting antidepressant activity following chronic, but not acute treatment with antidepressants. Following bilateral olfactory bulbectomy, several behavioral
changes have been observed, e.g. hyperactivity in the open-field test, enhanced nocturnal activity, deficits in memory and changes in food motivated and conditioned taste aversion behavior. Removal of both olfactory bulbs also leads to alterations in noradrenergic, serotonergic, cholinergic, GABA-ergic and glutamatergic neurotransmitter systems. Immune changes in the OB rat model include reduced neutrophil phagocytosis, lymphocyte proliferation, thymus and spleen weight and increased monocyte proliferation, neutrophils and α1-acid glycoprotein levels. The most commonly employed indicator of antidepressant activity is attenuation of the OB-related hyperactivity in the open-field.

TCAs (amitriptyline, desipramine), atypical antidepressants (mianserin, mirtazapine, nomifensine, trazodone), SSRIs (paroxetine, sertraline, fluvoxamine, but not fluoxetine), RIMAs (moclobemide), as well as putative antidepressants such as 5-HT1A agonists (ipsapirone, 8-OH-DPAT), noncompetitive NMDA antagonists (1.84, MK-801) and 5-HT2 antagonists (ketanserin) have showed antidepressant activity in the OB rat model.

Many of the changes observed in the OB rat are qualitatively similar to those observed in depressed patients. Hence, the OB rat model is not only an animal model for detecting antidepressant activity, but also a model for exploring the links that exist between the neurotransmitter, behavioral and endocrine and immune systems. The OB rat model might also be of use in detecting antidepressant therapies with early onset of action, since it is only capable of detecting antidepressant action following chronic administration of the current (putative) antidepressants.

Figure 1.8 Brief outline of the connections of the main and accessory olfactory bulbs. MOB, main olfactory bulb; AOB, accessory olfactory bulb; AON, anterior olfactory nucleus; APir, anterior pyriform cortex; Pir, pyriform cortex; DOT, dorsal olfactory tract; Ent, entorhinal cortex; me, medial nucleus of the amygdala; PLCo, posterolateral cortical amygdaloid nucleus; ACo, anterior cortical amygdaloid nucleus; BNST, bed nucleus of the stria terminialis; PmCo, periamygdaloid cortex; VNO, vomeronasal organ.
Chart 1.9 Chemical structure of nomifensine (1.78), amineptine (1.79), minaprine (1.80), tolcapone (1.81), pramipaxole (1.82), ipsapirone (1.83), MK-801 (1.84), methiothepin (1.85).

1.6 DEVELOPMENT OF A THIRD GENERATION OF ANTIDEPRESSANTS; FUTURE PHARMACOLOGICAL APPROACHES

The principal advantage of the second generation of antidepressants is an improved side-effect profile compared to the classical tricyclic antidepressants and MAOIs. In order to achieve a clear improvement over current antidepressants, however, two major therapeutic objectives must be met. First, compared to other treatments for neuropsychiatric disorders, the efficacy of currently available antidepressant drugs is relatively low. There are no antidepressants that are effective in all depressed patients. In general, only ~70% of patients exhibit some response to any given antidepressant, with merely one-half of these experiencing a full response. Combination therapies administering, for instance, TCAs with non-selective inhibitors of MAO have been used to improve antidepressant efficacy, but controlled studies indicate that there is no significant therapeutic advantage over treatments with a single antidepressant drug. Second, a reduction (and in the ideal, elimination) of the ‘therapeutic lag’ encountered with the traditional antidepressants. The time between the start of medication and the clinical onset-of-action remains one of the great enigmas of antidepressant therapy. Since it has been estimated that, world wide, about 340 million people suffer from depression and that the current cost (from absenteeism, lost productivity, lost earnings, treatment and rehabilitation) exceeds $40 billion yearly in the United States alone, this objective is both worth and timely. Thus there is a need for a new generation of antidepressants that have higher efficacy and that retain the gains...
already achieved in terms of fewer side effects with the second generation of antidepressant drugs.

### 1.6.1 Reuptake Inhibition Combined with 5-HT Autoreceptor Blockade

Given the ability of SSRIs to selectively inhibit the 5-HT transporter thereby increasing the synaptic concentration of 5-HT, their clinical actions can be attributed to an enhanced activation of one or several postsynaptic 5-HT receptors. This hypothesis is supported by clinical data showing that the administration of tryptophan-free amino acid mixtures to recovered major depressive patients receiving either SSRIs or MAOIs transiently abolishes their antidepressant effect. In rats that are chronically treated with the SSRI fluvoxamine, this procedure causes a very marked reduction of neuronal 5-HT release, thus supporting the association between recovery from depression and enhancement of 5-HT activity. There is evidence that hippocampal postsynaptic 5-HT$_{1A}$ receptors participate in the action of several types of antidepressant drugs. However, given the abundance of symptoms exhibited by depressed patients, it is likely that the effects of antidepressants involve the activation of receptors in more than one brain structure. Despite the ability of the SSRIs to block the 5-HT transporter soon after their administration, significant clinical improvement of depressed patients requires prolonged administration. This suggests the existence of neurobiological adaptive mechanisms responsible for their clinical action. This delay cannot be attributed to a downregulation of the cortical -adrenoceptor-coupled cAMP generating system, because most SSRIs do not induce such effects after chronic treatment. More likely, the slow onset of clinical action and the limited efficacy of antidepressant drugs (less than two-thirds of patients usually respond to the first drug administered) may be partly ascribed to the inhibition of 5-HT release by forebrain serotonergic nerve terminals after the administration of drugs that inhibit 5-HT uptake or MAO activity. This negative feedback involves stimulation of the 5-HT$_{1A}$ and 5-HT$_{1B}$ autoreceptors on the serotonergic neurons. Early in the course of treatment, SSRIs fail to elevate 5-HT release in the forebrain. However, as treatment continues, cell body autoreceptors desensitize, firing rate increases, and 5-HT release in the forebrain normalizes; in these circumstances, blockade of reuptake by an SSRI now causes the expected increase in levels of extracellular 5-HT. Thus, the slow onset of action of SSRIs may reflect the time necessary to cause a desensitization of 5-HT autoreceptors (see figure 1.9). Activation of the somatodendritic 5-HT$_{1A}$ receptors is also responsible for the attenuation of cell firing observed after a single administration of antidepressant drugs. Further attempts to reveal the desensitization of somatodendritic 5-HT$_{1A}$ autoreceptors have yielded somewhat contradictory results, although the most recent data give additional support to this hypothesis. The loss in efficacy of the 5-HT$_{1A}$ autoreceptors does not appear to be accounted for by a reduction in their number. Hence, the changes in the sensitivity of 5-HT$_{1A}$ (auto)receptors - revealed
by behavioral, neurochemical and electrophysiological means - must be accounted for by other factors, for example, variations in the efficacy of the receptor-effector coupling.

**Figure 1.9** Schematic representation of the effects of 5-HT uptake inhibitors on 5-HT neurons. The blockade of the 5-HT transporter at the level of the raphé nucleus leads to activation of the somatodendritic 5-HT$_{1A}$ receptors. This causes a reduction in 5-HT release by axon terminals, the opening of K-channels and a subsequent reduction in the firing rate of the 5-HT neuron. The terminal 5-HT$_{1B}$ receptors exert a local control of 5-HT synthesis and release. Activation of these autoreceptors, upon the administration of SSRIs, reduces the efficiency of released 5-HT on postsynaptic receptor sites. Heteroregulation of the activity of serotonergic neurons is exerted by afferents at somatodendritic and terminal levels.

In view of the above observations, it has been put forward that the treatment with SSRIs (or MAOIs) combined with a 5-HT autoreceptor antagonists would accelerate and enhance the antidepressant effects of the former.\textsuperscript{266} The normalization of cell firing and release produced by 5-HT$_{1A}$ (auto)receptors antagonists would enable SSRIs to increase the 5-HT levels to a greater extent than when administered alone. The accumulated experimental evidence fully supports this working hypothesis. Early experiments indicated that in the dorsal raphé nucleus of the nonselective 5-HT$_1$ antagonist methiothepin (1.85) enabled a low dose of citalopram to significantly increase the release of 5-HT in the frontal cortex.\textsuperscript{280} Further reports using selective 5-HT$_{1A}$ antagonists, such as WAY100635, or a 5-HT$_{1B/1D}$ partial agonist, such as GR127935, or a 5-HT$_{1B}$ inverse agonist, such as SB224289, have documented greater increments of 5-HT release when concurrently administered with SSRIs.\textsuperscript{281,282} The further enhancement in 5-HT release after the combination of the SSRI citalopram and the antagonist GR127935, e.g., is accounted for by the simultaneous blockade of both reuptake sites and autoreceptors of the 5-HT neuron (see figure 1.10). These greater effects on 5-HT release of antidepressants in combination with a 5-HT autoreceptor antagonist, demonstrate the importance that self-inhibition of 5-HT neurons have in their mechanism of action.
Figure 1.10 Effects of GR127935 (1 µM/kg sc.), citalopram (10 µM/kg sc.) and the co-administration of GR127935 with citalopram (1 + 10 µM/kg sc.) on 5-HT release in the ventral hippocampus of the freely moving rat (mean ± SEM, n=5, *p < 0.05 vs. control).

If the hypothesis that enhancement of serotonergic activity leads to clinical improvement in major depression is correct, pharmacological interventions increasing terminal 5-HT release are advisable. It is therefore important to obtain a deeper understanding of the mechanisms that determine extracellular 5-HT concentrations at the somatodendritic and terminal levels, encompassing both self-control mechanisms (i.e. transporter and autoreceptors) and heterocontrol mechanisms exerted by excitatory and inhibitory afferents at somatodendritic and terminal levels.

Another aspect that deserves attention are the postsynaptic changes elicited by enhanced 5-HT levels after chronic SSRI treatment, and the identification of the receptors involved. The existence of adaptive changes in second and third messengers by chronic antidepressant treatments in target neurons has been documented. 283-285 However, it is uncertain if the clinical improvement is the immediate consequence of an enhanced activation of certain 5-HT receptor subtypes, or whether postsynaptic adaptive changes are required. In the first case, only the presynaptic component would be responsible for the delayed onset of action.

1.6.2 Other strategies

5-HT-Moduline

The existence of an endogenous ligand able to specifically interact with 5-HT$_1$ receptors was suspected for a long time. 286-288 Purification from brain tissue and protein microsequencing resulted in the characterization of a short neuropeptide, 5-HT-Moduline, consisting of four amino acids: Leu-Ser-Ala-Leu. The principal
property of this peptide is its high affinity for 5-HT\textsubscript{1B} receptors (IC\textsubscript{50} \(\sim\) 10\textsuperscript{-10} M) and its capacity to effectively antagonize the binding of \[^3\text{H}\]-5-HT to 5-HT\textsubscript{1B} receptors.\textsuperscript{289} 5-HT-Moduline appears to regulate 5-HT activity, particularly its release. Interaction of this peptide with the 5-HT\textsubscript{1B} autoreceptor leads to an increase in 5-HT activity. This effect is shared with a variety of antidepressant drugs, in particular SSRIs. Hence, compounds that could interfere with the 5-HT-Moduline/5-HT\textsubscript{1B} receptor interactions, may have interesting therapeutic properties.

**CALCIUM CHANNEL ANTAGONISTS**

Calcium channel antagonists are similar to lithium in their pharmacodynamic profile of psychotropic activity, but differ in their mechanism of action at the cellular level. They first emerged as a therapeutic group in the 1970s. Calcium channel antagonists share a common mechanism of action, but are chemically very heterogeneous. In general, three main classes can be distinguished: 1,4-dihydropyridines, phenylalkylamines and benzothiazepines. The prototypes of these classes are nifedipine (1.86), verapamil (1.87) and diltiazem (1.88), respectively. The first two were developed as coronary vasodilators.\textsuperscript{290-292} Diltiazem, however was developed as a potential psychotropic agent, with an antidepressant/anxiolytic profile. Only later studies revealed its marked cardiovascular activity and calcium channel blocking properties.\textsuperscript{293}

The most consisting animal data support potential antidepressant and antimanic activity of calcium channel antagonists.\textsuperscript{55, 56} On a clinical level, the evidence for a potential use of these drugs in mood disorders is not conclusive. The data obtained in bipolar disorder, however, may be viewed as encouraging.\textsuperscript{57} TCAs have calmodulin-blocking properties and appear to inhibit voltage-activated calcium and calcium-activated potassium channels at clinically relevant concentrations.\textsuperscript{58-60} ECT inhibits calcium transport form cerebrospinal fluid to the brain, decreases protein kinase C activity and, depending on the brain area, either decreases or increases the density of dihydropyridine recognition sites.\textsuperscript{61-65} Chronic treatment with the SSRI citalopram, leads to an increase in receptor density (\(B_{\text{max}}\)) of dihydropyridine recognition sites.\textsuperscript{66}

The evidence that calcium channel antagonists score positive in several animal models of depression as well as in screening tests for antidepressant-like properties (\textit{e.g.}, the forced-swim test) and that current antidepressant therapies influence cellular calcium trafficking, together with the important role of intracellular calcium in neuronal processes and the promising results from preliminary clinical studies give reason to scrutinize more carefully the possible role of (a new generation) of calcium channel antagonists in the treatment of depression and bipolar mood disorders.
NMDA-ANTAGONISTS

The NMDA receptor is a fast acting, use-dependent, ligand-gated ion channel that mediates glutamatergic neurotransmission under conditions of strong depolarization. The influx of calcium through the NMDA subtype of glutamate receptors initiates long-term modifications of synaptic and cellular responses that regulate neural plasticity. The competitive NMDA antagonist 2-amino-7-phosphonoheptanoic acid (AP-7), the NMDA receptor-coupled channel blocker MK-801 and the partial agonist at the strychnine-insensitive glycine modulatory site of the NMDA receptor 1-aminocyclopropanecarboxylic acid (ACPC) all reduced the duration of immobility in the forced-swim test with efficacies comparable to TCAs. These results support the hypothesis that NMDA receptor activation is involved in the behavioral deficits observed after inescapable stressors. Although the pharmacological side-effect profile of competitive NMDA antagonists restricts their potential clinical use, partial agonists and antagonists at the glycine site of the NMDA receptor appear to be devoid of neurotoxic, ataxic, amnesic and psychomimetic side effects. While the potential absence of undesirable side effects by glycine/NMDA antagonists predicted in animal models needs to be substantiated by clinical experience, there is already evidence from Phase 1 clinical trials indicating that ACPC lacks psychomimetic-like actions. In addition, ACPC shows good oral efficacy and a high therapeutic index. Thus, functional NMDA antagonists at the glycine site may constitute a novel class of antidepressants.

SIGMA RECEPTORS

Sigma (σ) receptors have been implicated in several behavioral and biochemical effects. For example, it has been shown that the σ-receptors are involved in the motor side effects of neuroleptics, have been implicated in psychotic disorders and may play a role in neuroprotective mechanisms. Based on specific ligand and biochemical studies, the σ-receptors have been classified in two main types, σ1 and σ2. The σ-receptors are unique in that drugs form different therapeutic groups show high affinity for this receptor. For example, the antipsychotics haloperidol (1.91) and chlorpromazine bind to σ-receptors. However, this is not a common property of most neuroleptics. In addition, some antidepressants, including the MAOIs bind to σ-receptors. Recent experimental studies have shown that NPY - an endogenous ligand for the σ-receptors - may play a role in the mechanism of action of antidepressant drugs and in the pathology of mood disorders. Several clinical studies have implicated a dysfunctional NPY system in the etiology of depression. The functional modulation of σ-receptors by NPY suggests that these receptors may play an important role in the pathogenesis of depression.
Using electrophysiological studies in rats, it has been shown that the administration of the SSRI sertraline selectively potentiates the effect of NMDA on pyramidal neurons in the CA3 region in the hippocampus. This potentiation was reversed by the \( \sigma \)-receptor ligands haloperidol, pentazone (1.82) and N-allyl-normethazocine (1.93).\(^{317, 318} \) The modulation of the glutamate-NMDA receptor complex by antidepressant action on \( \sigma_1 \) receptors may open up new possibilities to the understanding of the mechanism of action of antidepressants and the development of novel therapeutic agents.

![Chemical structures of various compounds](chart11.png)

**Chart 1.11** Chemical structure of nifedipine (1.86), verapamil (1.87), diltiazem (1.88), AP-7 (1.89), ACPC (1.90), haloperidol (1.91), pentazone (1.92) and N-allyl-normethazocine (1.93).

1.7 **Scope of the thesis**

As described in this chapter, the 5-HT system is believed to play an important role in the pathogenesis and treatment of depression. Today, the antidepressants form the 3rd largest therapeutic area, with a current annual growth of 20%. Two-thirds of the prescribed antidepressants are reuptake inhibitors. Today’s’ antidepressants suffer from poor response, late clinical onset-of-action and side effects - nausea and sexual dysfunction - that seriously affect patience compliance. Most antidepressants (TCAs, MAOIs and SSRIs) elevate synaptic levels of 5-HT and their clinical action may very well be attributed to the enhanced activation of postsynaptic 5-HT receptors that modulate GABA, glutamate and ACh release.
It has been postulated that the 5-HT autoreceptors are an important factor in the delay in time-of-onset of therapeutic action of the first and second generation of antidepressants.

**Chapter 2** focuses on the 5-HT$_{1A}$ antagonist WAY100635 (1.69). The synthesis of several $O$-substituted phenylpiperazine, $N$-substituted 4-$N$-(o-methoxyphenyl)-aminopiperidine and benzamide analogues of WAY100635 is described, together with their in vitro and in vivo profile at both pre and postsynaptic 5-HT$_{1A}$ receptors.

In **Chapter 3**, the in vitro and in vivo profile of WAY100635 and the para-substituted benzamide analogues both pre- and postsynaptic 5-HT$_{1A}$ receptors is studied in more detail. Dose-response curves vs 8-OH-DPAT (0.2 mg/kg sc) are recorded, measuring the antagonism of induced hypothermia and the depressed 5-HT release (microdialysis in ventral hippocampus, presynaptic model).

**Chapter 4** focuses on the 5-HT$_{1B/1D}$ antagonist GR127935 (1.71) and the 5-HT$_{1B}$ inverse agonist SB224289 (1.70). Several close analogues are evaluated in vitro for their affinity for the 5-HT autoreceptors and the 5-HT transporter. Of these, the in vitro most potent ligands are evaluated in vivo for their potential to elevate 5-HT release alone and upon co-administration with the SSRI citalopram.

**Chapter 5** and **6** cover the in vitro and in vivo profile of 6-methoxymianserin. In chapter 4, the tetracyclic azepin is used to replace the 2-methoxyphenyl piperazine moiety of GR127935. The fact that this azepin is structurally very close to the atypical antidepressants mianserin (1.32) and mirtazapine (1.33) justifies an elaborate study of this new tetracyclic compound itself. In **Chapter 5**, the synthesis and in vitro binding profile of several analogues of 6-methoxymianserin is described, including the two probable metabolites 6-hydroxymianserin and $N$-desmethyl-6-methoxymianserin. The ability of 6-methoxymianserin to influence the release of NA and 5-HT in the ventral hippocampus at dosages similar as used for mianserin and mirtazapine, is studied.

**Chapter 6** deals with the separation of the enantiomers of 6-methoxymianserin on chiral HPLC and the determination of the absolute configuration of the enantiomers. The in vitro binding profiles of the enantiomers are assessed and they are both evaluated in vivo for their effects on the release of NA, DOPAC, 5-HT and 5-HIAA.

To summarize, this thesis deals with the synthesis and preliminary in vitro and in vivo evaluation of new chemical entities that are of possible use in the treatment and/or the study of the pathogenesis of depression. Further evaluation in animal models of depression and farmacodynamic, farmacokinetic and toxicology studies are required to determine the therapeutic potential of these new compounds.
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* Arvid Carlsson received the Nobel prize in 2000 for his life-long contribution in the field of dopamine research


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