CHAPTER 1

General introduction


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Chapter 1

1 Introduction

The term depression is used for a variety of affective disorders sharing feelings of depressed mood but which otherwise consist of a highly variable set of symptoms. This heterogeneity in combination with a high co-morbidity of other psychiatric disorders complicates diagnosis and treatment considerably, but is also a confounding factor when investigating the pathophysiology of depression.

Research into the pathophysiology and pharmacotherapy of depression more or less started by serendipity in the late 1950s when an attentive physician noted that the antitubercular drug isoniazide also improved mood in some of the patients. It appeared that isoniazide interfered with the metabolism of monoaminergic neurotransmitters through inhibition of the enzyme monoamine oxidase (MAO). The idea that depression is caused by a monoamine deficiency in the brain is a key element of the monoamine hypothesis (Schildkraut, 1965), and still forms the basis of (rational) antidepressant drug development. Although far from offering a satisfactory explanation of the neurobiological origin of depression the monoamine hypothesis is still used in drug development, simply because psychiatric research did not come up with better alternatives.

The improvement of monoaminergic transmission is also the leading theme in the present thesis. It is generally believed that the therapeutic effect of a majority of currently prescribed antidepressants is related to the enhancement of serotonin levels in the brain. How this increase exactly leads to an antidepressant response is still far from clear, but as detailed in the following section, it can be used as a conceptual framework for further research. The need for improved efficacy is emphasized by the moderate effectiveness of antidepressants (Kirsch et al., 2002) compared to placebo, the considerable non-response rate and the late onset of action. In terms of pharmacology, the enhanced therapeutic effect might be realized through an additional increase, or augmentation, of the neurotransmitter serotonin. Although it is a rather crude approach for a complex disease like depression, this offers a rational starting point to improve antidepressant treatment.
2 Neurobiology/pathophysiology of depression

2.1 Monoamine hypothesis of depression

The monoamine hypothesis of depression does not only propose the crucial involvement of monoamines in the therapeutic effects of antidepressant drugs but also suggests that depression is directly related to decreased monoaminergic transmission. It is based on the observations that depletion of monoamine stores in the brain by reserpine induced depressive symptoms, while increasing extracellular monoamine levels in the brain appeared to be effective in several forms of depression (Schildkraut, 1965).

The monoamine hypothesis has not been without criticism, but there are new data that could fit in. Many of them come from positron emission tomography (PET) studies. By using selective radioligands evidence was found for reduced pre- and postsynaptic 5-HT$_{1A}$ receptor binding in depression. Drevets et al. (1999) demonstrated that the mean 5-HT$_{1A}$ receptor binding potential (BP) was reduced in unmedicated depressed patients relative to healthy controls using [$^{11}$C]WAY-100635. These data are consistent with those of Sargent et al. (2000). However, a subgroup of the subjects were scanned both pre- and post-paroxetine treatment, and 5-HT$_{1A}$ receptor BP did not significantly change in any area. A recent PET study with [$^{11}$C]DASB, a selective radioligand for the 5-HT transporter (5-HTT), in patients suffering from major depressive episodes (MDE) also investigated the contribution of another factor associated with depressed mood, namely the presence of dysfunctional attitudes and the relationship thereof with the 5-HTT binding potential (Hervas et al., 2001). Dysfunctional attitudes are negatively biased assumptions and judgements about the world and oneself and constitute a negative cognitive interpretative bias of the future. Most studies have investigated the relationship with depression as a syndrome and ignored the presence of dysfunctional attitudes. Interestingly, no differences in 5-HTT binding potential (BP) were found among the entire sample of depressed patients compared to healthy controls. However, depressed patients with high regional 5-HTT BP (up to 21%) had higher levels of dysfunctional attitudes. It has been suggested that an increased density of the 5-HT transporter (5-HTT) may lead to increased 5-HT clearance from the synapse, thus leading to a reduced availability of synaptic 5-HT. A PET study using the 5-HTT ligand [$^{11}$C](+)-McN5652 has demonstrated that the distribution volume ratio of this PET ligand was larger in left prefrontal cortex and right cingulate cortex of depressed patients compared to controls (Reivich et al., 2004). These findings suggest that 5-HTT sites may be increased in frontal and cingulate cortical areas of patients suffering from major depression. Such changes might not lead to depression per se, but to a negative cognitive bias, which could pave the way
for the development of personality disorders and other psychiatric syndromes. According to these neuroimaging studies serotonin is likely to play a role in the neurobiology of depression in at least a subgroup of patients.

Another approach that supports the monoamine hypothesis is depletion of the serotonin precursor tryptophan. Studies using this paradigm support the role of serotonin in the modulation of mood, as witnessed by the ability of tryptophan depletion to lower mood (Delgado et al., 1990; Miller et al., 1992). Smith et al. (1999) studied the effect of relapse, following tryptophan depletion, on cognitive function of depressed patients. They reported an attenuation of the task (verbal fluency) related activation in the anterior cingulate during relapse, which was correlated to an increase in depressive symptoms. These results are in agreement with previous studies using a similar paradigm. For instance, inhibition of 5-HT synthesis by p-chlorophenylalanine (Shopsin et al., 1975; Shopsin et al., 1976) or L-tryptophan depletion (Delgado et al., 1990; Miller et al., 1992) caused a relapse of symptoms in depressed patients that were successfully treated with SSRIs (Bell et al., 2001; Reilly et al., 1997). These studies clearly demonstrate that serotonin plays a crucial role in the therapeutic effect of SSRIs.

Studies into polymorphisms of the genes that code for tryptophan hydroxylase (TPH), the rate limiting enzyme in the synthesis of serotonin, have lead to mixed results. A polymorphism of the tryptophan hydroxylase (TPH1) gene, which is expressed both peripherally and centrally, was found to be associated with suicidal behavior and not with depression per se (Bellivier et al., 2004). More encouraging results were reported for two different centrally expressed TPH2 gene polymorphisms, both displaying a significant association with major depression (Zhang et al., 2005; Zill et al., 2004a) and in one case also suicidal behavior (Zill et al., 2004b).

The studies cited above clearly support the monoamine theory of depression, but evidence is also accumulating against a direct relation between depression and a monoamine deficiency (Delgado, 2000; Hirschfeld et al., 2000). For example, current evidence concerning serotonin does not imply depression but rather aggressiveness, failing impulse control and violent suicide as directly related to impaired brain serotonergic function (Russo et al., 2003). It is also worthy to note that tianeptine, a reuptake enhancer, also has antidepressant effects. Moreover, other reuptake inhibitors such as cocaine do not possess antidepressant activity.

Notwithstanding the arguments raised against a direct relation between depression and a monoamine deficiency, it is concluded that there is sufficient evidence to support the use of the monoamine hypothesis as conceptual framework, in particular for pharmacotherapy.
3 Serotonergic system

Since it was discovered that serotonin has a function as neurotransmitter in the central nervous system, the serotonergic system has been the target of scientific research, which has resulted in a vast amount of information regarding its anatomy, physiology and pharmacology.

3.1 Anatomy

Serotonergic neurons are present throughout the brain, but most cell bodies are clustered in nine serotonergic nuclei located in the brain stem. The majority of serotonergic innervations are derived from the two largest nuclei, the dorsal raphe nucleus (DRN) and the median raphe nucleus (MRN) (Dahlstrom and Fuxe, 1964), which innervate important brain structures such as the cortex, hippocampus and hypothalamus. These brain areas are part of the limbic system, which is involved in emotional behavior and its dysfunction is thought to underlie the pathology of several affective disorders.

Most brain areas are jointly innervated by both nuclei, but exceptions are the prefrontal cortex and the dorsal hippocampus, which are predominantly controlled by the DRN and MRN, respectively (McQuade and Sharp, 1997). In addition to the classical view of nuclei controlling terminal release, projections from terminal areas also appear to exert some control on activity of the raphe nuclei, creating a subtle balance between the brain stem nuclei and the brain areas they project to (Bosker et al., 2001).

![Diagram of serotonin pathways](image)

**Fig. 1.** Overview of the main serotonin-containing pathways in the rat central nervous system.
3.2 Physiology

Serotonin is present both centrally and peripherally. Because serotonin is unable to pass the blood brain barrier under normal conditions, it has to be synthesized from the precursor tryptophan, which can only be derived from the diet. Plasma tryptophan is actively transported by a non-selective carrier mechanism and has to compete with other large neutral amino acids such as tyrosine, phenylalanin, (iso)leucine and valine. Consequently, the amount of tryptophan transported into the brain does not only depend on blood tryptophan levels but also on the levels of other circulating amino acids (Dahlstrom and Fuxe, 1964).

After transport over the blood brain barrier, tryptophan is converted into 5-hydroxy tryptophan (5-HTP) by the specific enzyme tryptophan hydroxylase, which is located both in the cell body and projection area. Finally, serotonin is synthesized from 5-HTP by the non-specific enzyme aromatic amino acid decarboxylase (AADC), which is localized in the axon terminals of all monoaminergic neurons. Normally, tryptophan hydroxylase is unsaturated and hence, the amount of serotonin synthesized solely depends on the levels of tryptophan in the brain. But if tryptophan levels rise, the enzyme gets saturated and will become the rate-limiting factor (Carlsson and Lindqvist, 1978; Westerink and Devries, 1991).

When released from the neuron, the action of serotonin can be terminated by metabolism or reuptake into the presynaptic neuron. Serotonin is metabolized by monoamine oxidase into 5-hydroxyindolacetaldehyde, which subsequently can be oxidized by aldehyde dehydrogenase into 5-hydroxy indolacetic acid (5-HIAA), or reduced into 5-hydroxytryptophol. Oxidation is the main metabolic route, which makes 5-HIAA the predominant metabolite of serotonin. 5-HIAA itself is actively transported out of the brain, so central levels of 5-HIAA can be used as a marker of serotonergic metabolism. Once released, serotonin can be transported back into the presynaptic neuron by a specific carrier protein, which also is the main target of selective serotonin reuptake inhibitors (SSRIs).

3.3 Pharmacology

Until now, 14 different receptor subtypes have been discovered, which are classified in 7 different families. By increasing extracellular serotonin, antidepressants act as indirect agonists on all 14 receptors, of which some are known to control serotonergic activity through an inhibitory feedback mechanism. Antagonists of such autoreceptors are able to augment the serotonergic response of antidepressants, demonstrating the role of these receptors in the pharmacology of antidepressants.
3.3.1 5-HT\textsubscript{1A} receptors
When activated, the G-protein linked 5-HT\textsubscript{1A} receptor stimulates the opening of potassium channels, thereby decreasing the ability of serotonergic neurons to depolarize. This causes a reduction of neuronal activity and as a result, serotonergic release is also diminished.
5-HT\textsubscript{1A} autoreceptors are localized on the cell body, effectively controlling firing rate, serotonin release and synthesis (Hutson et al., 1989; Sharp et al., 1989; Sprouse and Aghajanian, 1987).
Electrophysiology studies have demonstrated a strong reduction of the firing rate after administration by 5-HT\textsubscript{1A} receptor agonists, including the endogenous ligand serotonin (Sprouse and Aghajanian, 1987). Similarly, microdialysis studies have shown a diminished release of serotonin following the administration of 5-HT\textsubscript{1A} receptor agonists (Hutson et al., 1989). The effects were completely blocked by 5-HT\textsubscript{1A} receptor antagonists, indicating the involvement of 5-HT\textsubscript{1A} receptors.

3.3.2 5-HT\textsubscript{1B/1D} receptors
Species differences in pharmacological profile of 5-HT\textsubscript{1B} receptors have been the reason for confusion with respect to their nomenclature. Receptors could be assigned both 1B and 1D, depending on the property observed. Nowadays, a unifying nomenclature exists which is used by most scientists. In order to avoid any ambiguity, both receptor subtypes will be described comprehensively.
The 5-HT\textsubscript{1B} receptors of human, guinea pig and most other species are very similar with respect to their structure and pharmacological profile. In contrast, rat and mice 5-HT\textsubscript{1B} receptors, in spite of having a comparable primary structure, display a substantially different pharmacological profile. With respect to their function in the brain, these receptors are all thought to be species homologues. 5-HT\textsubscript{1B} receptors are localized on the axon terminals, directly controlling serotonin synthesis and release. Administration of 5-HT\textsubscript{1B} receptor agonists decreases serotonin release in terminal regions, which can be blocked by 5-HT\textsubscript{1B} antagonists (Bosker et al., 1995; Hjorth and Tao, 1991).
Using the new nomenclature, 5-HT\textsubscript{1D} receptors are species homologues with respect to their structure, functionality as well as their pharmacological profile. However, the amount of 5-HT\textsubscript{1D} receptors in the brain does differ between species; the rat for example has a relatively low density of these receptors. The 5-HT\textsubscript{1D} receptor is also a G protein coupled receptor, which upon activation exerts its inhibitory action by a decrease of the second messenger cAMP. In contrast with 5-HT\textsubscript{1B} receptors, 5-HT\textsubscript{1D} receptors are localized in both terminal regions and on the cell bodies in the raphe nuclei. Although this receptor is scarcely involved in control of serotonergic
release in terminal regions, it does seem to have an autoreceptor function in the raphe regions (Sprouse et al., 1997; Starkey and Skingle, 1994).

### 3.3.3 5-HT₂ receptors

The 5-HT₂ receptor family consists of three subtypes, using the same second messenger system, the inositol triphosphate (IP₃) pathway, to activate protein kinase C. Moreover, all 5-HT₂ receptor subtypes have an inhibitory role. Whereas the 5-HT₂B receptor is merely found in the periphery, 5-HT₂A and 5-HT₂C receptors are predominantly located in the brain (Barnes and Sharp, 1999; Hoyer et al., 1994). Although both 5-HT₂A and 2C receptors are thought to be localized in terminal regions, their agonists not only decreased terminal serotonin release, but also diminished the firing rate of the serotonergic neurons. The effects on release and firing rate could, however, not be blocked with receptor antagonists, indicating that 5-HT₂ receptors are not directly involved in the release of serotonin (Moret and Briley, 1997).

### 4 Neuropharmacology of antidepressants

#### 4.1 A short history

The antidepressant effect of the antitubercular drug isoniazide was attributed to its ability to increase monoamine levels in the brain through inhibition of the enzyme monoamine oxidase. Monoamine oxidase inhibitors (MAOis) appeared to be effective antidepressants, but they had serious side effects not in the least because they also inhibited monoamine oxidase-A in the liver. Incidentally this has led to hypertensive crisis (tyramine effect), which could be fatal especially for patients of advanced age.

The next generation of antidepressants, the tri- and tetracyclics (TCAs) also increased monoamine levels in the brain albeit via a different mechanism. Originally developed to treat schizophrenia, TCAs appeared to block the reuptake of monoamines, which also resulted in increased monoamine levels in the brain. Unfortunately, this generation of antidepressants also had severe side effects, which was attributed to their lack of selectivity. This has led to the development of selective serotonin reuptake inhibitors (SSRIs). Their side effect profile appeared relatively benign in comparison with TCAs and MAOis. The image of efficacious and save drugs has strongly contributed to their current status in antidepressant pharmacotherapy.
4.2 Desensitization hypothesis

A large body of evidence indicates that SSRIs increase extracellular levels of serotonin by preventing this neurotransmitter to be taken up again after being released (Fuller, 1994). Although this pharmacological effect occurs immediately, the therapeutic response is typically delayed for several weeks, suggesting that adaptive changes at the cellular level are required to attain the full antidepressant effect. This observation is the basis of the desensitization hypothesis raised by Blier et al. (1987a). Using single cell recordings in rats it was found that acute administration of antidepressants decreased serotonergic cell firing through the activation of 5-HT autoreceptors, but tolerance developed upon chronic antidepressant treatment, which was attributed to desensitization of 5-HT autoreceptors (Chaput et al., 1986). Because of the striking similarity between onset of antidepressant action and the time required for desensitization of the autoreceptors, it was proposed that these effects were connected. In addition to the electrophysiology experiments, microdialysis studies have shown that chronic treatment also reduced the inhibitory effect of 5-HT agonists on serotonin release, further supporting the desensitization hypothesis (Chaput et al., 1986; Invernizzi et al., 1994; Kreiss and Lucki, 1995).

As a consequence of 5-HT reuptake inhibition, SSRIs increase extracellular 5-HT levels and one would expect this effect to become more prominent following chronic treatment, due to the restoration of 5-HT firing rate. This is supported by microdialysis studies, combining SSRIs with specific antagonists of 5-HT autoreceptors in order to mimic desensitization of 5-HT autoreceptors, which indeed resulted in further increases of extracellular 5-HT levels (Hjorth, 1996; Invernizzi et al., 1997).

Although Blier’s desensitization hypothesis is now broadly accepted, some marginal notes have to be made. Desensitization has been postulated as a general process, but in fact this phenomenon has only been truly established for the presynaptic 5-HT$_{1A}$ autoreceptors in the raphe nuclei. Evidence for the desensitization of postsynaptic 5-HT$_{1A}$ receptors and other 5-HT receptor subtypes appeared to be far less convincing, reducing the general validity of the desensitization hypothesis. In addition, not all antidepressants diminish the function of 5-HT$_{1A}$ autoreceptors following chronic treatment, suggesting that desensitization is not essential in order to achieve the antidepressant effect.

4.3 Antidepressant drug targets

SSRIs inhibit the reuptake of serotonin throughout the brain, but the effect varies considerably between brain areas (Fuller, 1994). Consequently, investigating the regional effects of antidepressants might give an impression which brain areas are involved in depression and in the
antidepressant effect of SSRIs. Neuroimaging techniques have allowed us to investigate the human brain in a non-invasive manner, thereby greatly expanding our knowledge of the neuronal networks involved in both pathophysiology and pharmacotherapy of psychiatric disorders. Affective behavior and control of emotions is generally related to the limbic system, which consists of various brain areas including the amygdala, prefrontal cortex, hippocampus and hypothalamus. Problems with control of emotional behavior associated with affective disorders such as depression have been attributed to dysfunctions of the limbic system. Evaluation of the neuropharmacological effects of psychoactive drugs did, however, not lead to unequivocal results, with varying outcomes depending on the imaging technique used. Microdialysis is an excellent technique to study regional effects of antidepressants on extracellular serotonin levels, but information is restricted to the brain region where the dialysis probe is located. The changes of extracellular serotonin levels might have different postsynaptic consequences depending on the brain region being investigated. By studying such effects in several brain areas simultaneously, cellular markers of neuronal activity, for instance the immediate early gene c-fos, might provide a useful extension of the (presynaptic) microdialysis data.

4.3.1. Neuroimaging

4.3.1.1 Depression

Most brain imaging studies conducted in patients with major depression episodes (MDE) have been able to identify abnormalities associated with MDE, e.g. (Dolan et al., 1992; Drevets, 2000). In the vast majority of studies conducted in the eighties and early nineties, correlates were sought of the syndrome of major depression, without paying much attention to specific brain regions related to symptom components of MDE. There is, however, a large inter-individual variability in severity and psychopathological features associated with MDE. A more fruitful approach would be to search correlates between processes in the brain and subcomponents of MDE, such as motivation, depressed mood, dysfunctional attitudes and sleep disturbances, instead of trying to find neuronal correlates for depression as a syndrome. Several studies found that severity of depression is positively correlated with regional cerebral glucose uptake, as measured with $[^{18}F]$-fluorodeoxyglucose positron emission tomography ([18]-FDG PET). These studies reported positive correlations between depression-severity and brain regions such as the bilateral medial frontal cortex, anterior cingulate, right dorsolateral hippocampus, cingulate and other paralimbic areas (Osuch et al., 2000; Videbech et al., 2002).
There is more controversy with respect to studies that report negative correlations between depression severity and regional brain activity (for references, see Milak et al. (2005). A possible explanation could be that the diversity of clinical manifestations of MDE may obscure associations with specific brain regions. In a recent study, Milak and colleagues (2005) have investigated the association between different psychopathological clusters of the Hamilton Depression Rating Scale (HDRS) and resting glucose metabolism using [$^{18}$F]-FDG PET. They found distinct correlations between three HDRS factors and regional glucose metabolism. The first factor, psychic depression showed a positive correlation with metabolism in the basal ganglia, thalamus and cingulate cortex. The second factor, sleep disturbance, showed a positive correlation with metabolism in limbic structures and basal ganglia, and the third factor (loss of motivation) was negatively correlated with parietal and superior frontal cortical areas. Interestingly, this study shows that positive correlations with aspects of depression severity are subcortical ventral, ventral prefrontal and limbic structures, whereas negative correlations are found in dorsal cortical areas (Milak et al., 2005).

4.3.1.2 Antidepressants

Studies in healthy volunteers using acute serotonergic challenges such as fenfluramine or SSRIs have shown changes in a discrete network of brain regions. Most studies have found relative decreases in activation in the thalamus and (medial) temporal areas, including hippocampus and amygdala, and increases in frontal areas, including the anterior cingulate (Cook, Jr. et al., 1994; Geday et al., 2005; Kapur et al., 1994; Mann et al., 1996; Meyer et al., 1996; Smith et al., 2002b). These areas show abnormal activation patterns in depressed patients, and in most studies a reversal of pretreatment abnormalities in brain activity is seen after SSRI treatment. These studies also found evidence for a role of the anterior cingulate in the pathology and treatment of depression (Brody et al., 1999; Kegeles et al., 2003; Mayberg et al., 2000; Oquendo et al., 2003; Smith et al., 2002a). Studies in patients with impulsive-aggressive personality disorder (Siever et al., 1999) and panic disorder (Meyer et al., 2000) reported abnormal fenfluramine-induced changes in frontal regions and the parietal-superior temporal cortex, respectively. In patients with obsessive-compulsive disorder (OCD) increased baseline activity is found in the cingulate cortex, orbitofrontal cortex, caudate nuclei and thalamus when compared to healthy controls, and SSRI-related clinical improvement appeared to correlate with a decrease in these regions (Perani et al., 1995; Saxena et al., 1998; Saxena et al., 1999; Saxena et al., 2002).
4.3.2 Expression of immediate early genes

Expression of immediate early genes, assessed either via mRNA or corresponding protein, has been used as index for the postsynaptic effects of antidepressants. Although far from being a selective measure for antidepressant effects it may give some idea which neuronal networks in the brain are initially activated by antidepressants. Assessment of the chronic effects is probably more difficult because tolerance to this type of gene expression easily develops (Veening et al., 1998). A study by Miyata et al. (2005) showed that fluoxetine and reboxetine similarly increased Fos-immunoreactivity (Fos-ir) in the shell of the nucleus accumbens (Nacc), however activation patterns were quite different in cortical areas and central nucleus of the amygdala (CeA). The authors note that activation of the “limbic” nucleus accumbens by both fluoxetine and reboxetine is in accordance with the compounds’ comparable efficacies in treating symptoms of anhedonia. Conversely, their differential efficacy regarding other symptoms of depression may relate to the different activation pattern seen in the other areas. A tendency to increase Fos-ir in NAcc and CeA was also seen for citalopram, which became significant when the SSRI was co-administered with the 5-HT₁A receptor antagonist WAY 100.635 (Jongsma et al., 2002). Augmentation was also seen in paraventricular nucleus of the hypothalamus (PVN), ventromedial hypothalamus and dorsolateral striatum. It has been hypothesized that the mood-stabilizing effect of antidepressants is achieved by their action on the limbic-hypothalamic-pituitary-adrenocortical system (Barden et al., 1995). Given the strong augmentation seen in Nacc, CeA and PVN, co-administration of a 5-HT₁A receptor antagonist with an SSRI seems an attractive proposition. On the other hand co-administration of WAY 100.635 may exacerbate SSRI induced sexual dysfunction (de Jong et al., 2005). Brain areas in which no Fos-ir was found in both control and any of the treated animals included the median raphe nucleus and the hippocampus. The latter is in accordance with a study by Tordera et al. (2003), which has assessed the expression of two early genes following combinations of paroxetine with different selective 5-HT₁A receptor antagonists. Augmentation was seen in several cortical areas and caudate putamen. However, differences in activation pattern were also noted between the antagonists, which had not been apparent when measuring extracellular 5-HT levels.

In conclusion, although many questions are left unanswered and some discrepancies have been noted, literature data from both neuro-imaging and early gene expression converge to the involvement of both cortical (anterior cingulate) and limbic areas (CeA, Nacc, dorsal hippocampus).
4.4. **Pre- vs. postsynaptic effects**

It has always been assumed that serotonergic release in the projection areas is mainly controlled by autoreceptors located presynaptically. Consequently, the therapeutic effect of antidepressants is generally attributed to desensitization of these receptors. However, evidence is accumulating that postsynaptic 5-HT\textsubscript{1A} receptors are also involved in the control of firing rate and release of serotonergic neurons, and that their function might also change following chronic antidepressant treatment. Besides, SSRIs may be considered as indirect agonists at all fourteen 5-HT receptor subtypes. Most of these are located postsynaptically and display different distribution patterns within the brain. Therefore, it is important to have some idea as to the brain areas and (postsynaptic) 5-HT receptors involved in the neurobiology of depression and the effects of SSRIs.

4.4.1 **Postsynaptic 5-HT receptors and (side) effects of SSRIs**

Most microdialysis studies have assessed the effect of SSRIs on extracellular 5-HT levels, which confines retrieval of information to the presynaptic element of the 5-HT neuron. It is conceivable, however, that 5-HT receptor subtypes differentially distribute the 5-HT signals over the various brain areas. Several attempts have been made to identify the postsynaptic 5-HT receptors that mediate the therapeutic effect and side effects of antidepressants. For instance, the effect of the SSRI fluvoxamine on forced-swimming–induced immobility in mice, a model for depression, has been reported not to involve the 5-HT\textsubscript{1A} and 5-HT\textsubscript{2} receptor subtypes (Egawa et al., 1995). Similarly de Vry et al. (De Vry et al., 1997) hypothesized that 5-HT\textsubscript{1B/1D}, 5-HT\textsubscript{2C}, 5-HT\textsubscript{3} and 5-HT\textsubscript{4} receptors may not critically be involved in the therapeutic effects of SSRIs. Conversely, blockade of 5-HT\textsubscript{2} and 5-HT\textsubscript{1} receptors (Berk et al., 2000; Boyarsky et al., 1999), but not 5-HT\textsubscript{1B/1D} receptors (Jongsma et al., 2005) has been shown to reduce sexual dysfunction, suggesting that this side effect of SSRIs is mediated by 5-HT\textsubscript{2} and 5-HT\textsubscript{3} receptors. Moreover, 5-HT\textsubscript{2C} antagonists, but not 5-HT\textsubscript{2A}, 5-HT\textsubscript{1A} or 5-HT\textsubscript{3} antagonists, reduce the anxiogenic-like effect of acutely administered citalopram, as demonstrated by the social interaction test in rats (Dekeyne et al., 2000). Interestingly, the discriminative stimulus (DS) properties of citalopram are mediated by 5-HT\textsubscript{2C} receptors. The antidepressants mianserin and mirtazapine, both being 5-HT\textsubscript{2C} antagonists, dose-dependently blocked DS properties of citalopram, suggesting that DS effects are not related to the antidepressant effect of SSRIs per se (Dekeyne et al., 2001). Recently it was reported that antidepressants are functional antagonists at 5-HT\textsubscript{3} receptors, suggesting a hitherto unknown pharmacological mechanism (Eisensamer et al., 2003).
4.4.2 Postsynaptic 5-HT\textsubscript{1A} receptor mediated long loop type of feedback

Firstly proposed by Blier and coworkers (Blier et al., 1987b; Blier and de Montigny, 1987) postsynaptic 5-HT\textsubscript{1A} receptors may also play a role in the regulation of 5-HT release. Since then several studies on this item have been published (Bosker et al., 1997; Bosker et al., 2001; Casanovas et al., 1999; Ceci et al., 1994; Celada et al., 2001; Romero et al., 1994). Interestingly, chronic treatment with citalopram via osmotic minipumps reduced postsynaptic 5-HT\textsubscript{1A} receptor-mediated feedback in the amygdala (Bosker et al., 2001). The notion that both pre-and postsynaptic 5-HT\textsubscript{1A} receptors may desensitize following chronic SSRI treatment makes augmentation strategies based on blockade of this receptor subtype even more interesting. Infusion of the 5-HT\textsubscript{1A} receptor antagonist WAY 100.635 into the amygdala via retrograde microdialysis also markedly increased the effect of citalopram on local extracellular 5-HT levels, indicating that 5-HT\textsubscript{1A} receptor-mediated long loop type of feedback is particular strong in this area.

Chronic treatment with citalopram via osmotic minipumps completely abolished augmentation with WAY 100.635. Clearly this type of feedback must also be taken into account when assessing the chronic effects of SSRIs. In this respect the prefrontal cortex is particularly interesting. Previous studies had already shown that postsynaptic 5-HT\textsubscript{1A} receptor-mediated long loop type of feedback also exists within the prefrontal cortex (Celada et al., 2001). However, chronic treatment with citalopram did not lead to desensitisation of such postsynaptic 5-HT\textsubscript{1A} receptors, in fact quite the opposite occurred. Since 5-HTT and 5-HT\textsubscript{1A} receptor binding had not changed (see chapter 4), other mechanisms such as changes in receptor-G protein interaction (Castro et al., 2003) or effects on processes more downstream should be considered (Hensler, 2002). An increase of sensitivity was also observed when a 5-HT\textsubscript{1A} receptor antagonist was co-administered systemically (Gundlah et al., 1997; Hjorth and Auerbach, 1999). It is also noteworthy that chronic antidepressant treatment markedly increased the effect of 5-HT\textsubscript{1A} receptor antagonism on the firing activity of hippocampal CA3 pyramidal neurons, which also suggests an increased sensitivity of local postsynaptic 5-HT\textsubscript{1A} receptors (Haddjeri et al., 1998). Interestingly, opposite effects on pre and postsynaptic 5-HT\textsubscript{1A} receptors following chronic antidepressant treatment have been reported by a recent study wherein an increased and decreased agonist stimulated GTP\textsubscript{γ}S binding was found in hippocampus and raphe nucleus, respectively (Castro et al., 2003). Such opposite effects on pre and postsynaptic 5-HT\textsubscript{1A} receptor-mediated feedback would imply a shift in control of terminal 5-HT release from the autoreceptors to their postsynaptic counterparts, which could be a factor in the clinical efficacy of antidepressants.
5 Augmentation strategies

5.1 Rationale of augmentation strategies
According to the World Health Organization major depression (MD) will become the number one disabling disease in the next decade. This prognosis is even more alarming considering the outcome of a meta-analysis of clinical studies involving the six most widely prescribed antidepressants approved between 1987 and 1999 by the FDA, which suggested that antidepressant treatment is only marginally more effective than placebo (Kirsch et al., 2002). Other worrying aspects of antidepressant treatments are the considerable nonresponse rates (30-40%) and the late onset of action (2-5 weeks). There is thus a need for improved antidepressant treatment and one approach is augmentation strategies. Price (1998) defines augmentation as an increase of therapeutic efficacy of an antidepressant by an additional drug. The latter drug may be devoid of antidepressant properties (Joffe et al., 1996; Price, 1998). Depending on one’s position the concept “augmentation strategy” may be interpreted quite differently. Health professionals have combined antidepressants with other drugs in order to improve the treatment of their patients, and may be more inclined to see it as a way to reduce depressive and comorbid symptoms. Neurochemists, neurobiologists and medicinal chemists are more concerned with drugs effects at the molecular level, and how these effects will influence the function of the brain circuits putatively involved in the pathology of depression. They may be more inclined to view “augmentation strategy” as a means to optimize these drug effects. Without direct access to patients they have to rely on animal models and a conceptual framework to predict the therapeutic consequences, both in terms of efficacy and side effects.

In the present thesis, augmentation of the antidepressant response will be approached according to the latter view.

5.2 Limitations and risks of augmentation
Several homeostatic processes are known to counteract the effect of SSRIs on extracellular 5-HT levels; some make use of 5-HT1A and 5-HT1B autoreceptors, while others act via postsynaptic 5-HT1A and 5-HT2C receptors. Another factor that might limit the effect of SSRIs on extracellular 5-HT levels is the availability of the serotonin precursor and essential amino acid tryptophan. Interfering with such homeostatic processes offers the opportunity to further increase the effect of SSRIs on 5-HT levels.

However, the concept of further increasing 5-HT levels has its weaknesses. For instance, fenfluramine is extremely effective in boosting extracellular 5-HT levels and yet it is not the
ideal antidepressant. The compound is both a 5-HT releaser and reuptake inhibitor, which is likely to have differential intra- and extrasynaptic consequences, but this example clearly indicates that solely aiming at increased 5-HT levels may be too crude a measure to rely upon. Attention should also be paid to unwanted side effects associated with too large increases of central serotonin levels such as the serotonergic syndrome. The syndrome is characterized by restlessness, agitation and confusion and can be fatal on occasion. Although this side effect has been reported in only few case studies, it is clear that there is a limit in increasing extracellular serotonin levels. One may ask to which extent serotonin levels should be increased to improve the antidepressant effect without inducing these side effects.

By using competition studies with PET tracers, changes of dopamine levels in the human brain can readily be assessed. Unfortunately, this does not apply for serotonin (De Haes et al., 2002) making it as yet impossible to connect antidepressant effect and serotonin levels in humans.

5.3 Augmentation with 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor antagonists

It has been argued that the loss of 5-HT autoreceptor function in consequence of chronic antidepressant treatment could be mimicked instantaneously by blocking these receptors with an antagonist. Such diminished function of 5-HT autoreceptors could indeed be demonstrated by microdialysis studies, wherein the increase of extracellular 5-HT elicited by a single dose of an SSRI was augmented by co-administration of a 5-HT$_{1A}$ receptor antagonist (Cremers et al., 2000; Gundlah et al., 1997; Hjorth, 1993; Hjorth et al., 1996; Invernizzi et al., 1992). In addition to the somatodendritic 5-HT$_{1A}$ autoreceptor-mediated feedback, 5-HT release is also controlled by terminal 5-HT$_{1B}$ receptors. Accordingly, simultaneous administration of the putative 5-HT$_{1B}$ receptor antagonist GR 127935 and an SSRI has been shown to augment the effect of the latter on extracellular 5-HT levels (Gobert et al., 1997; Rollema et al., 1996; Sharp et al., 1997).

5.3.1 Clinical studies

Based on solid preclinical research by his group and others, Artigas (Artigas, 1993) has proposed to improve antidepressant efficacy and onset of action by co-administering SSRIs with a 5-HT$_{1A}$ receptor antagonist. Since selective 5-HT$_{1A}$ receptor antagonists were not available for use in humans the combined β-adrenergic/5-HT$_{1A}$ receptor antagonist pindolol was chosen, but for safety reasons the dose had to be based on the compound’s much higher potency for β-adrenoceptors. A preliminary study with previously untreated depressed patients suggested indeed an improvement in both latency and efficacy by combined treatment with paroxetine and pindolol (Artigas et al., 1994). Since then many open label and controlled studies with pindolol have
followed, albeit with variable success (for meta-analysis see (McAskill et al., 1998)). Soon it became evident that the observed clinical effects of pindolol co-administration could not readily be explained by complete antagonism of somatodendritic 5-HT$_{1A}$ receptors. Several PET scan studies have been published on pindolol binding in the human brain (Andree et al., 1999; Martinez et al., 2000a; Martinez et al., 2000b; Rabiner et al., 2000a; Rabiner et al., 2000b). The studies agree that pindolol binds to somatodendritic 5-HT$_{1A}$ autoreceptors at the doses used in clinical studies, however receptor occupancy is moderate and highly variable. A microdialysis study in guinea pigs indicated that the dose of pindolol in clinical studies had been far too low to reasonably expect augmentation of extracellular 5-HT levels in humans (Cremers et al., 2001). Moreover preclinical data also indicated that pindolol has agonistic properties at 5-HT$_{1A}$ receptors in the raphe nuclei in vivo (Fornal et al., 1999a; Fornal et al., 1999b; Sprouse et al., 1998; Sprouse et al., 2000). A recent meta-analysis indicated, however, that pindolol was able to significantly accelerate the therapeutic effect of an antidepressant in the first weeks of treatment (Ballesteros and Callado, 2004). Although it is tempting to use the latter as support for Artigas’ concept, the evidence from animal and PET studies cannot be denied. Alternative explanations such as a rapid partial desensitization of the 5-HT$_{1A}$ autoreceptors by the “agonist” pindolol and/or antagonism of β-adrenergic receptors seem therefore more likely.

5.4 Augmentation with 5-HT$_{2C}$ receptor antagonists

Recently evidence was presented for a novel augmentation strategy based on 5-HT$_{2C}$ receptor antagonism (Cremers et al., 2004). Augmentation of extracellular 5-HT was observed in rat hippocampus and cortex with citalopram, sertraline and fluoxetine. The effect was at least of a similar magnitude as that seen with 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor antagonists (Cremers et al., 2000). Genetic elimination of these receptors in mice (5-HT$_{2C}$-knock out mice) also augmented the effects of SSRIs on extracellular serotonin levels in the brain. Disabling the 5-HT$_{2C}$ receptors also resulted in a significantly increased antidepressant-like effect of SSRIs in the tail suspension test. In the schedule induced polydipsia test, an animal model for obsessive-compulsive disorder with predictive value for the onset of action of antidepressants, the selective 5-HT$_{2C}$ antagonist RS 102221 dramatically decreased latency time of paroxetine (Cremers et al., 2002), indicating potential to hasten antidepressant response. Microdialysis experiments using the selective 5-HT$_{2C}$ receptor antagonist SB 242084 did not show tolerance following chronic paroxetine treatment (Cremers et al., 2002). However, several behavioral studies suggest that 5-HT$_{2C}$ receptors desensitize following chronic antidepressant treatment ((Bristow et al., 2000) and references therein). The reason for this discrepancy is unknown. Apparently, adaptation of 5-HT$_{2C}$ receptors critically depends on their location and/or function. Recent microdialysis experiments indicated
that the mechanism underlying the augmentation of SSRIs by 5-HT$_{2C}$ receptor antagonists is rather complex with GABA$_B$ receptors involved (Jongsma et al., 2004) and possibly also $\alpha_1$ adrenoceptors.

### 5.4.1 Clinical studies

Several clinical studies have investigated combinations of SSRIs with atypical antidepressants such as mianserin (Ferreri et al., 2001; Maes et al., 1999) or with antipsychotics such as olanzapine (Shelton et al., 2001). For the first combination the goal was a faster onset of action and/or treatment of refractory depression, but the latter combination was aimed at depression with comorbid psychotic features.

Mirtazapine (Remeron®) is a very successful antidepressant, and it is under investigation for its ability to augment the clinical efficacy of SSRIs (Besson et al., 2000). The latter could easily be explained in terms of synergy between the antidepressant effects of mirtazapine and the SSRI. However, mirtazapine and mianserin are potent 5-HT$_{2C}$ receptor antagonists, and when co-administered with citalopram both markedly augmented the effect of the SSRI on extracellular 5-HT levels (Cremers et al., 2002). It can be speculated that the synergy between these atypical antidepressants and SSRIs connects to this augmentation, however the final word is to the clinical studies with selective 5-HT$_{2C}$ receptor antagonists yet to come.

Olanzapine has also been shown to augment the effects of fluoxetine in the clinic (Shelton et al., 2001). Microdialysis studies have shown that combined administration of fluoxetine and olanzepine enhances extracellular brain levels of dopamine and noradrenaline more than fluoxetine does alone (Zhang et al., 2000). This may be attributed to the prominent 5-HT$_{2C}$ receptor antagonistic properties of olanzapine, since blockade of this 5-HT receptor subtype has been reported to increase extracellular dopamine and norepinephrine in the brain (Millan et al., 2003; Zhang et al., 2000). Recently it was shown that 5-HT$_{2C}$ receptor antagonists also augment the effects of SSRIs on extracellular serotonin (Cremers et al., 2004). Notably, olanzapine did not augment fluoxetine-induced increases of extracellular serotonin, which may be due to the concurrent blockade of $\alpha_1$-adrenoceptors in the raphe nuclei by olanzapine. This is supported by the notion that augmentation by the specific 5-HT$_{2C}$ receptor antagonist SB 242084 was completely abolished by the $\alpha_1$-adrenoceptor antagonist prazosine.

### 5.5 Augmentation with tryptophan

The rate of synthesis of cerebral serotonin depends on the availability of its precursor tryptophan, which might limit the therapeutic efficacy of antidepressants if insufficiently present. Levels of
circulating tryptophan are to a large extent determined by dietary intake and catabolism. Persistently low tryptophan levels may form a risk to develop psychopathologies, including depression, aggressive behavior and impaired impulse control. Following acute depletion of tryptophan similar symptoms may emerge. Depressed patients treated successfully with SSRIs have been reported to suffer from a short-lasting relapse, concomitant with an acute and transient depletion of tryptophan (Delgado et al., 1990) (for review see (Bell et al., 2001; Reilly et al., 1997)), emphasizing that the antidepressant response is dependent on the continuous availability of the 5-HT precursor.

Rodent studies have shown that, in addition to autoreceptor control, 5-HT release strongly depends on precursor availability (Schaechter and Wurtman, 1989; Westerink and Devries, 1991). Tryptophan depletion by either a tryptophan free diet or administration of a tryptophan free amino acid drink resulted in decreased central 5-HT levels in rodents (Fadda et al., 2000; Lieben et al., 2004). Conversely, increased levels of circulating tryptophan resulted in a higher basal 5-HT release and an increased SSRI induced 5-HT response (Gartside et al., 1992; Perry and Fuller, 1993), emphasizing the need for exploring the use of tryptophan in antidepressant therapy. Because the release of serotonin depends on both autoreceptor control and synthesis, tryptophan may also have merit in augmentation strategies.

5.5.1 Clinical studies

There is increasing evidence that patients treated with antidepressants may suffer from tryptophan depletion. Moreover several studies indicated that the therapeutic effect of an SSRI is critically linked to the availability of tryptophan (Bremner et al., 1997; Leyton et al., 2000; Moreno et al., 1999; Morris et al., 1999; Neumeister et al., 2004). A subgroup of patients suffering from major depression has lower blood tryptophan values and lower levels of 5-hydroxy-indole-aceticacid (5-HIAA) in the cerebrospinal fluid (Asberg et al., 1976; Asberg and Traskman, 1981; Oreland et al., 1981; Traskman et al., 1981). Microdialysis studies in laboratory animals have shown increased extracellular 5-HT levels with tryptophan loading (van der Stelt et al., 2004; Westerink and Devries, 1991). Tryptophan may therefore have some antidepressant potential, and since the late eighties the compound can be obtained over-the-counter as a dietary supplement. However, several cases of eosinophilia-myalgia syndrome have been reported caused by the intake of contaminated tryptophan, which has harmed its image as a relatively safe antidepressant. A recent meta-analysis of clinical trials with tryptophan and 5-hydroxy-tryptophan suggested modest antidepressant efficacy of these serotonin precursors, but their clinical usefulness was questioned since safe and more effective alternatives are available (Shaw et al., 2002).
6 Scope of this thesis

To augment or not to augment, that is the question. The pros and cons of SSRI augmentation strategies have been detailed extensively in chapter one. SSRI augmentation strategies have their limitations and they are certainly not without risk, but they may be the only viable option to improve antidepressant treatment in the short term. Convincing clinical evidence in support of SSRI augmentation strategies is very limited, mainly because potent and selective 5-HT receptor antagonists are not yet available for use in humans. However, this does not apply for studies in laboratory animals. The aim of the present thesis is to further explore SSRI augmentation by addressing a number of important questions that can be raised with this approach. For instance, does SSRI augmentation lead to increased neuronal activity in brain areas that have been associated with major depression? Expression of immediate early genes, assessed either via mRNA or corresponding protein, has been used as index for the postsynaptic effects of antidepressants. Although far from being a selective measure for antidepressant effects it may give some idea which neuronal networks in the brain are initially activated by antidepressants. In chapter two the expression of the immediate early gene c-fos is used to assess the neuronal activation pattern elicited by a single dose of the SSRI citalopram both in absence and presence of the 5-HT$_{1A}$ receptor antagonist WAY 100635. The results are discussed in the context of the available preclinical and clinical literature regarding the brain areas putatively involved in the neurobiology and pharmacotherapy of affective disorders, including major depression.

The concept of SSRI augmentation with a 5-HT$_{1A}$ receptor antagonist is strongly based on Blier’s desensitization hypothesis. There is indeed a large body of evidence that supports the desensitization of 5-HT$_{1A}$ autoreceptors following chronic antidepressant treatment. The question is whether this also applies for 5-HT$_{1B}$ autoreceptors and postsynaptic 5-HT$_{1A}$ receptors involved in long loop type of feedback. In chapter three intracerebral microdialysis in conscious rats is used to assess the effect of chronic treatment with the SSRI citalopram on the sensitivity of 5-HT$_{1B}$ receptors. Importantly, measurements were performed while the animals were still on the drug to avoid rapid desensitization of 5-HT$_{1B}$ receptors during washout. The effects of chronic SSRI treatment on stress (e.g. HPA-axis activity) are well documented, both in humans and animals. Accordingly, several peripheral stress markers were also measured in the study. Previously, it was demonstrated that postsynaptic 5-HT$_{1A}$ receptors in the amygdala, involved in long loop type of feedback, desensitize following chronic treatment with an SSRI. In chapter four it is investigated whether this also applies for such receptors in the prefrontal cortex, an area that has been strongly implicated in major depression.
Another important question concerns the availability of the serotonin precursor tryptophan. Serotonin does not enter the brain, and 5-HT neurons have to synthesize the transmitter from tryptophan, which is actively transported into the brain. It is conceivable that the effect of an SSRI on extracellular 5-HT levels and in particular its augmentation with 5-HT receptor antagonists is restricted by the availability of tryptophan. This question is addressed in chapter five using two different approaches to manipulate serotonin synthesis viz. oral tryptophan supplementation and inhibition of serotonin synthesis by retrograde microdialysis of NSD 1015. The latter compound inhibits the enzyme aromatic aminoacid decarboxylase, the enzyme responsible for the conversion of 5-hydroxytryptophan into serotonin. Chapter six involves the effect of chronic SSRI treatment on total serotonin content, synthesis and metabolism. Intracellular serotonin stores depend on both synthesis and reuptake of previously released serotonin. It is conceivable that prolonged reuptake inhibition will deplete these stores. Another worrying aspect of chronic antidepressant treatment is the clinical phenomenon called rebound depression. When antidepressant therapy is suddenly discontinued, patients have been reported to relapse into a depressive state, emphasizing the need to slowly phase out SSRI treatment. An analogy may be found with the washout period in preclinical chronic treatment studies, which is commonly used to avoid interference with the pharmacological probes. Arguably, the effects of a sudden discontinuation of treatment are more prominent than the effect of the treatment itself. The latter possibility is also investigated in chapter six by comparing the effects of chronic SSRI treatment on total serotonin content, synthesis and metabolism in presence and absence of a washout period.

The relevance of the data presented in this thesis and the possible consequences thereof for future antidepressant research and drug development will be discussed briefly in chapter seven.
References


General introduction


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Chapter 1


