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Noradrenergic Augmentation Strategies in the
Pharmacological Treatment of Depression and
Schizophrenia
An Experimental Study

by

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Abstract
The pharmacological treatment of depression and schizophrenia, two major psychiatric disorders, is largely based on modulation of central monoaminergic neurotransmission. However, currently available pharmacological treatment alternatives possess a relatively modest clinical efficacy, making them less than optimal. The present series of studies, using in vivo electrophysiological, biochemical and behavioral techniques in rats, aim at the disclosure of mechanisms whereby an augmented clinical efficacy of antidepressant, as well as antipsychotic, drugs may be achieved.

Pindolol, a partial β-adrenoceptor agonist with affinity for the serotonin (5-HT)\textsubscript{1A} receptor, has been claimed to shorten the clinical onset of action of selective serotonin reuptake inhibitors (SSRIs); an effect suggested to result from an antagonistic action of pindolol on somatodendritic 5-HT\textsubscript{1A} autoreceptors, reversing the acute, inhibitory, effect of SSRIs on serotonergic neuronal activity. However, upon systemic administration, pindolol, in contrast to the selective 5-HT\textsubscript{1A} receptor antagonist WAY100635, was unable to reverse the inhibitory effect of an SSRI on 5-HT neuronal activity. This observation, in conjunction with other data from our laboratory, rather indicates that pindolol exerts a weak agonistic activity on somatodendritic 5-HT\textsubscript{1A} autoreceptors and thus may shorten the delayed onset of action of SSRIs by a mechanism unrelated to 5-HT\textsubscript{1A} receptor blockade.

In other experiments, repeated treatment with a noradrenaline reuptake inhibitor (NRI), was found to cause a gradual increase in noradrenaline nerve-terminal output and a partial recovery of the initially suppressed noradrenergic electrophysiological activity during the course of the treatment, consonant with a partial desensitization of the presynaptic inhibitory feedback mechanism. Administration of a low dose of an α\textsubscript{2}-adrenoceptor antagonist enhanced noradrenaline neuronal activity, as well as nerve-terminal release, most markedly in chronically, but also acutely, treated animals; tentatively indicating an advantageous effect of this drug combination in the treatment of at least some forms of depression.

Modulation of central serotonergic function is thought to mediate the clinical action of several classes of antidepressant drugs, and previous data demonstrate a central noradrenergic regulation of midbrain serotonergic neurons. We have now observed that acute administration of the highly selective NRI reboxetine increases the firing rate of serotonin neurons in the dorsal raphe nucleus, which, in turn, results in an enhanced cortical output of serotonin; effects that may have bearing on the clinical action of selective NRIs. Analogous studies of the effects of NRIs on the function of the mesolimbocortical dopamine system revealed several effects. Thus, acute administration of reboxetine increased burst firing, but not basal firing rate, of dopamine neurons in the ventral tegmental area and concomitantly enhanced dopamine output in the prefrontal cortex, but not in the nucleus accumbens. This effect of reboxetine on cortical dopamine release is similar to that caused by α\textsubscript{2}-adrenoceptor antagonists as well as most atypical antipsychotic drugs.

Previous observations have shown that concomitant treatment with an α\textsubscript{2} adrenoceptor antagonist may markedly enhance the effect of a dopamine D\textsubscript{2} receptor antagonist, i.e. a classical antipsychotic drug, on prefrontal dopamine output as well as in the conditioned avoidance response (CAR) test, a preclinical test of antipsychotic efficacy with high predictive validity, supporting the observed advantageous clinical antipsychotic effect of this drug combination. Given the similar effects of α\textsubscript{2}-adrenoceptor blockage and noradrenaline reuptake inhibition on cortical dopamine function, we investigated the effect of reboxetine in the same experimental paradigm. Pretreatment with reboxetine significantly enhanced the effect of the D\textsubscript{2} receptor antagonist on cortical dopamine output as well as in the CAR test, without affecting catalepsy scores. Our data thus indicate that noradrenaline reuptake inhibition may augment the clinical effect of classical antipsychotic drugs in the treatment of schizophrenia, tentatively with particular regard to negative and cognitive symptoms.
Till min familj
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1. Introduction: The catechol- and indolamines

1.1 General characteristics

More than four decades ago the monoamines noradrenaline (NA), dopamine (DA) and serotonin (5-HT) were discovered as neurotransmitters in the brain. Since then the study of these neurotransmitter systems has been intense, largely due to their demonstrated involvement in the control of behavior and their significance for the pharmacological treatment of psychiatric disorders. The central NA, DA and 5-HT systems all originate in a number of small nuclei, primarily located in the brain stem, and following early histological work by Dahlström and Fuxe (1964) the central catecholaminergic and indolaminergic nuclei were designated as A1-A12 and B1-B9, respectively. The monoaminergic nuclei innervate large parts of the brain, as well as the spinal cord, by means of highly arborized neurons, often covered with varicosities along their axons (Molliver 1987, Cooper et al. 1991, Descarries et al. 1996, Pickel et al. 1996). This functional organization allows for a widespread modulatory control of postsynaptic neuronal activity, and may indicate that these systems transmit basic information, with low specific content.

The synthesis of catechol and indolamines is based on the amino acids tyrosine and tryptophan, which initially are hydroxylated by the enzymes tyrosine and tryptophan hydroxylase, respectively. Further enzymatic steps results in the formation of DA, and thereafter NA, from tyrosine, and 5-HT from tryptophan (see figure 1 for details). Interestingly, while tyrosine hydroxylase is the rate limiting enzyme for the synthesis of DA and NA, tryptophan hydroxylase do not seem to be rate limiting for 5-HT synthesis during most physiological conditions. Instead, the availability of tryptophan in the central nervous system appears to limit the amount of synthesized 5-HT (Aghajanian and Asher 1971), and decreasing the intake of tryptophan has been found to reduce the rate of 5-HT synthesis in rat brain (Gessa et al. 1974). In the mammalian brain the monoamines are degraded via the sequential action of the enzymes monoamine oxidase (A and B), catechol-O-methyl transferase and aldehyde dehydrogenase (figure 1). The major metabolites in rat brain of NA, DA and 5-HT are 3-methoxy-4-hydroxy-phenethyenglycol (MHPG), dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindolacetic acid (5-HIAA), respectively. It has been suggested that the amount of accumulated major metabolite of a monoamine in central nerve-terminal areas may provide an accurate reflection of the activity of that transmitter system. Thus, such a measure of turnover may correlate to the relative change in release and subsequent reuptake and degradation of the transmitter (Roth et al. 1976, Zetterström and Ungerstedt 1984).

The electrophysiological activity of monoamine neurons is subject to feedback regulation by inhibitory autoreceptors, located at their cell-bodies, which are activated by transmitter released by axon collaterals originating from neurons in the same nuclei or by extrasynaptic release from
Figure 1.
Major monoamine metabolic pathways in the brain. Numbers in circles indicate the action of the following enzymes: (1) Tyrosine hydroxylase; (2) Aromatic amino-acid decarboxylase; (3) Dopamine-β-hydroxylase; (4) Monoamine oxidase (A/B); (5) Aldehyde reductase; (6) Aldehyde dehydrogenase; (7) Catechol-O-methyl transferase; (8) Tryptophan hydroxylase; (9) Amino-acid decarboxylase. The following abbreviations are used: 5-HIAA, 5-hydroxyindoleacetic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, Homovanillic acid; MHPG, 3-methoxy-4-hydroxy-phenylglycol (Cooper et al. 1991).

dendrites and cell-bodies in close proximity (Wang and Aghajanian 1977, Bunin and Wightman 1998, Callado and Stamford 2000, Cragg et al. 2001). Monoamine release may also be regulated at the nerve-terminal level by a similar autoreceptor-mediated feedback inhibition, caused by the released transmitter (see below). The major mechanism for retrieval, and thus inactivation, of released NA, DA and 5-HT is transport into the intracellular space via carriers, driven by the ionic and electrical gradient over the cell membrane, with high selectivity for each respective transmitter (Trendelenburg 1991, but see Galli et al. 1996).

1.2 Noradrenaline

1.2.1 Central noradrenaline systems and receptors

The gross localization of NA in the mammalian central nervous system was early described by Vogt (1954) who observed NA to be widely distributed in the mammalian brain. By using the fluorescence histochemical technique, in combination with specific lesions, a more detailed description of NA pathways could later be provided (Dahlström and Fuxe 1964, Andén et al. 1966, Ungerstedt 1971). The noradrenergic brainstem cell groups can be divided into two major subgroups, a lateral tegmental group and the locus coeruleus group. The lateral tegmental system has its cells of origin distributed diffusely in the medulla and pons and innervates by its ascending pathways primarily the brainstem, the hypothalamus and parts of the amygdala, but has also descending projections to the spinal cord. The nucleus locus coeruleus (LC), the
source of the majority of the forebrain noradrenergic nerve-terminal projections, is a distinctive cell group located in the periventricular gray, medial to the mesencephalic trigeminal nucleus (figure 2), and contains approximately 1500 neurons on each side of the brainstem in the rat (Swanson 1976). The LC projects with extensively collateralized neurons to most regions of the brain; e.g. the amygdala, the hippocampus as well as several cortical areas (Moore and Card 1984).

Historically, Ahlquist (1948) described two types of adrenoceptors, i.e. α and β receptors, based on the order of potency of a series of molecules. The α adrenoceptors were subsequently subdivided into pre or post synaptic receptors, due to different pharmacological and functional characteristics (Langer 1974, Starke et al. 1974). Binding studies defined the presynaptic α adrenoceptors as being α1 adrenoceptors and most of the postsynaptic ones as being α2 adrenoceptors (see Bylund et al. 1994). Subclassification of the α1 adrenoceptor variants by means of pharmacological, and later also cloning, techniques have resulted in the definition of at least three subtypes, namely the α1A, α1B and α1D subtype (see Docherty 1998). All of the α1 adrenoceptor variants are found in the brain, but with large variations in their expression patterns (Price et al. 1994, see Nicholas et al. 1996). Notably, there is strong expression of α1B adrenoceptor in the serotonergic dorsal raphe nucleus, whereas the expression of α1A adrenoceptor mRNA in the dopaminergic nuclei ventral tegmental area and substantia nigra is weaker, but detectable (Jones et al. 1985). Activation of all known α1 adrenoceptor subtypes results in an increase of intracellular calcium concentrations, mostly mediated by G-protein coupled activation of phospholipase C (Minneman 1988). The α2 adrenoceptor has also been subclassified by similar procedures (Bylund 1988), and there are three subtypes, i.e. the α2A, α2B and α2C adrenoceptor. The previously so-called α2D receptor in the rat is a homolog of the human α2D adrenoceptor (Bylund et al. 1994, see 1.5). A dense population of α2 adrenoceptors, primarily of the α2A subtype and tentatively also of the α2C subtype, are localized to the nucleus locus coerules (Nörenberg et al. 1997, Lee et al. 1998a), where they function as inhibitory somatodendritic autoreceptors (see below). The α2 adrenoceptors found in the LC are thus mainly situated on noradrenergic cell bodies but are also found on presynaptic nerve-terminals, some of which lack tyrosine hydroxylase immunoreactivity (Lee et al. 1998b). Experimental data suggest that α2 adrenoceptors, mainly of the α2A subtype, are also located presynaptically at nerve-terminals, and varicosities, of noradrenergic efferent projections (Langer 1974, Dennis et al. 1987, Scheibner et al. 2001), where they mediate presynaptic feedback inhibition. Moreover, α2 adrenoceptors have also been found on nerve-terminals of other transmitter pathways, i.e. the serotonergic, where they function as inhibitory heteroreceptors (Göther et al. 1981, Frankhuyzen and Mulder 1982). α2 adrenoceptors, mainly of the α2C subtype, are also expressed in noradrenergic postsynaptic target neurons in e.g. the cerebral cortex, hippocampus, striatum, thalamus, hypothalamus and cerebellar cortex (see Nicholas et al. 1996). Activation of α2 adrenoceptors normally results in a hyperpolarization of the target neuron (Aghajanian and Vandermaelen 1982), largely as a result of inhibition of adenyl cyclase activity, mediated by a G-protein (see Limbird 1988).

The β adrenoceptors, which originally was divided into the β1 and β2 subtype based on their different relative affinities for adrenaline and noradrenaline (Lands et al. 1967), now
includes three subtypes, of which the third, the $\beta_3$ adrenoceptor, is not expressed in the brain (see Bylund et al. 1994, Nicholas et al. 1996). $\beta_1$ and $\beta_2$ adrenoceptors are expressed on NA (and adrenaline) target neurons, as well as on glial cells, primarily in the rat cerebral cortex, hippocampus, thalamus and pineal gland (see Nicholas et al. 1996). All $\beta$ adrenoceptors appear to be linked to adenyl cyclase activation via stimulatory G-proteins (Tate et al. 1991).

1.2.2 Regulation of noradrenergic neurotransmission

Major afferent pathways to the LC originate in two medullary nuclei, the nucleus paragigantocellularis and the nucleus prepositus hypoglossi (Aston Jones et al. 1986, 1991), and the glutamatergic innervation from the nucleus paragigantocellularis has been shown to be of importance for the activation of noradrenergic neuronal activity by e.g. footshock and nicotine administration (Ennis and Aston-Jones 1988, Engberg 1989, Valentino et al. 1993). Furthermore, wide-reaching dendrites of cells in the LC appear to be innervated by a plethora of other neuronal pathways, from e.g. the prefrontal cortex, the dorsal raphe nucleus and the ventral tegmental area (Cedarbaum and Aghajanian 1978a, see Aston-Jones et al. 1991), that may modulate the activity, or reactivity, of noradrenergic neurons (Cedarbaum and Aghajanian
1977, Engberg 1992, Jodo et al. 1998). The basal, and evoked, firing rate of noradrenergic neurons in the LC is regulated by $\alpha_2$ adrenergic autoreceptors located in their somatodendritic cell-region. Thus, systemic, or local, administration of an $\alpha_2$ adrenoceptor agonist results in a profound reduction in noradrenergic electrophysiological activity (Svensson et al. 1975), whereas following administration of an $\alpha_2$ adrenoceptor antagonist the basal firing rate, and responsiveness, of noradrenergic neurons increases (Cedarbaum and Aghajanian 1977, Svensson and Usdin 1978, Simson and Weiss 1989). These effects may largely be due to a modulation of the autoinhibitory mechanism within the LC (Cedarbaum and Aghajanian 1978b). However, alterations in the responsiveness of NA neurons may also partly result from a changed inhibitory tone, mediated by adrenergic $\alpha_2$ heteroreceptors, upon non-noradrenergic, tentatively excitatory, terminals innervating the LC (Simson and Weiss 1989, Lee et al. 1999b). Local control of central NA release from nerve-terminals is largely mediated via presynaptic $\alpha_{2A}$ adrenoceptors (Pelayo et al. 1977, Taube et al. 1977, Scheibner et al. 2001). Consequently, when the $\alpha_2$ adrenoceptor antagonist idazoxan is administered locally, via a microdialysis probe implanted in the rat cerebral cortex, a sharp rise in NA output is observed (Dennis et al. 1987).

1.2.3 Functional roles of the central noradrenaline systems

The widespread noradrenergic innervation of the brain has been hypothesized to regulate attention and cognitive function in response to salient environmental stimuli (see Aston-Jones et al. 1999). Hence, electrophysiological activity of noradrenergic neurons in the LC may regulate the level of alertness and has been observed to show large variation in correlation to the sleep-waking cycle as well as to various environmental and behavioral conditions. Generally, the noradrenergic neuronal activity is very low during sleep while a mean firing rate of approximately 2 Hz is observed during quiet waking (Foote et al. 1980, Aston-Jones and Bloom 1981a), and the activity is further increased in response to salient stimuli leading to disruption of ongoing behavior (Foote et al. 1980, Aston-Jones and Bloom 1981b). Activation of the central noradrenergic system is also caused by salient stimuli from the internal environment, e.g. changes in blood volume or pressure, hypercapnia as well as distension of the urinary bladder, the distal colon or the stomach (see Svensson 1987). Furthermore, exposure to environmental stressors may elicit a marked increase in noradrenergic neuronal activity and nerve-terminal release of transmitter (Abercrombie and Jacobs 1987, see Charney et al. 1995). Interestingly, the basal noradrenergic electrophysiological activity during waking is decreased during performance of automatic, repetitive, behavior (Grant et al. 1988), e.g. grooming and feeding, functionally contrasting the behavior of the central serotonergic system, which is activated during similar conditions (see 1.4.3). Manipulations of noradrenergic function in the rat or primate indicate a dual role for NA in the modulation of cognitive function (see Birnbaum et al. 1999). Thus, both depletion of catecholamines or local administration of an $\alpha_1$ adrenoceptor agonist into the cerebral cortex may acutely diminish working memory in experimental animals (Cai et al. 1993, Mao et al. 1999, Birnbaum 1999). On the other hand, $\alpha_2$ adrenoceptor agonists, when administered to catecholamine-depleted
animals, enhance cognitive function, presumably via a postsynaptic mechanism (Cai et al. 1993, Arnsten et al. 1996). The beneficial influence of central noradrenergic activity on cognitive functioning may well reflect a bell shaped curve, with low activity corresponding to drowsiness or unresponsiveness to stimuli while an inappropriate increase in tonic activity, out of environmental context, may give rise to enhanced reactivity to irrelevant stimuli as well as anxiety (see Charney et al. 1995, Aston-Jones et al. 1999).

### 1.3 Dopamine

#### 1.3.1 Central dopamine systems and receptors

During the late 1950s dopamine was shown not only to represent an intermediate step in the synthesis of noradrenaline, but to be an independent neurotransmitter in the brain (Carlsson 1959). The two major dopaminergic cell-clusters in the brain are located in the substantia nigra zona compacta and the ventral tegmental area of Tsai (VTA; figure 2). In addition, there exist other dopaminergic neuronal populations in the brain, e.g. one in the hypothalamus which gives rise to the tuberoinfundibular system (see Cooper et al. 1991). The DA neurons of the substantia nigra innervate primarily the caudate nucleus and putamen, i.e. the striatum, comprising the nigrostriatal DA system, whereas the efferent projections of the VTA have a more diverse distribution, innervating both cortical and subcortical brain regions (figure 3). Thus, the VTA DA neurons innervate brain structures such as the nucleus accumbens (NAC), amygdala, olfactory tubercle, and hippocampus as well as the cingulate and entorhinal cortices (Andén et al. 1966, Ungerstedt 1971, Björklund and Lindwall 1984). The projections from the VTA to these brain regions are referred to as the mesolimbocortical DA system. However, as regards collateralization, single mesolimbocortical DA neurons do not appear to innervate both cortical and subcortical target regions (Fallon 1981, Swanson 1982). The cortical and subcortical projections of the VTA may also be differentiated with regard to autoreceptor regulation as well as reactivity to environmental stimuli (Roth and Elseworth 1995, see below), and may thus be further subdivided into the mesocortical and mesolimbic DA system.

Early studies on the differential regulation of adenylyl cyclase activity by various populations of DA receptors revealed two distinct subsets of receptors which were called dopamine D₁ and D₂ receptors (Spano et al. 1978, Kebabian and Calne 1979). The D₁ receptor was defined by an activating effect on adenylyl cyclase, whereas the D₂ receptor decreases intracellular cAMP levels upon activation. However, both types of receptors, i.e. the D₁- and D₂-like, are associated with other transduction pathways as well (see Jaber et al. 1996). Three more DA receptors have since then been found by means of cloning and subsequent pharmacological analysis. One of the cloned receptors is D₃-like (D₃), whereas two are D₄-like (D₄ and D₅), with respect to the effect on cAMP metabolism and their binding characteristics (see Sokoloff and Schwartz 1995). The D₁ receptor is most strongly expressed in the striatum, NAC and the olfactory tubercle (see Jaber et al. 1996), but clearly detectable levels have also been reported in the cerebral cortex and the hippocampal formation (Boyson et al. 1986, Camps
et al. 1990, Hurd et al. 2001). D₂ receptors are found in the substantia nigra and the VTA, where they are expressed by dopaminergic neurons, and function as presynaptic autoreceptors in the somatodendritic and terminal regions of the nuclei (Weiner et al. 1991, see Jaber et al. 1996). Postsynaptic D₃ receptors are mainly localized to the striatum, olfactory tubercle and NAC, but are also found in other parts of the central nervous system, e.g. in parts of the cerebral cortex and the hippocampus (Weiner et al. 1991, Hurd et al. 2001). While the D₁ and D₂ receptors occur in most major DA projection fields, the D₃, D₄ and D₅ receptors seem to be expressed in appreciable amounts in fewer brain areas. Thus, the D₃ receptor is mainly found postsynaptically in the NAC and in the cerebellum, but is also expressed by dopaminergic neurons in the VTA and substantia nigra, whereas the D₄ receptor is mainly expressed outside the basal ganglia, e.g. in the frontal cortex, medulla, amygdala and hypothalamus (Van Tol et al. 1991, Levesque et al. 1992, see Sokoloff and Schwartz 1995, Jaber et al. 1996). The distribution of the D₅ receptor appears to primarily be restricted to the hippocampus (Tiberi et al. 1991).

1.3.2 Regulation of dopaminergic neurotransmission

Dopaminergic neurotransmission is regulated by a large number of mechanisms, including both intrinsic neuronal factors and various afferent inputs (see Kalivas 1993, Moore et al.
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1999). The feedback regulation of the electrophysiological activity of DA neurons in the VTA and substantia nigra is largely executed by D$_2$-like receptors located in the somatodendritic region of the DA neurons (Bunney et al. 1973a, 1973b). Thus, DA released from dendrites or axon collaterals activates the D$_2$ autoreceptors, thereby increasing a membrane potassium conductance which hyperpolarizes the cell, resulting in a decrease in the basal firing rate (Lacey et al. 1987). D$_2$-like autoreceptors located on dopaminergic nerve-terminals may also regulate both the synthesis and release of DA (Farnebo and Hamberger and 1971, Kehr et al. 1972, Roth 1984, Imperato and Di Chiara 1988). Biochemical and electrophysiological investigations of the autoreceptor mediated control of dopaminergic neurotransmission in different nerve-terminal areas of the mesolimbocortical DA system have revealed regional differences. Thus, an apparent lack of both impulse regulating somatodendritic, and synthesis modulating nerve-terminal autoreceptors in DA neurons innervating the prefrontal and cingulate cortices has been observed (Bannon et al. 1981, Chiado et al. 1984, see Roth 1984). However, recent studies indicate that at least some aspects of mesocortical DA activity are controlled by D$_2$-like receptors (Gessa et al. 2000, see discussion paper IV).

Another mechanism discerning between cortical and subcortical dopaminergic pathways is the inhibitory influence of DA in the prefrontal cortex upon evoked subcortical DA output. Hence, selective disruption of dopaminergic function in the prefrontal cortex has been shown to enhance the magnitude of increase in action potential dependent DA release in the NAC, which occurs in response to an environmental or pharmacological challenge (Deutch et al. 1990, Jaskiw et al. 1990, Rosin et al. 1992). The mechanism whereby cortical DA neurotransmission inhibits subcortical DA release may involve projections from the pyramidal cells in the prefrontal cortex to neurons in the NAC and/or in the VTA (Sesack and Pickel 1992, Harden et al. 1998).

A glutamatergic innervation of the VTA, originating in the prefrontal cortex (Christie et al. 1985, Sesack and Pickel 1992), serves as an excitatory input to the DA neurons (Gariano and Groves 1988, Grenhoff et al. 1988, Svensson and Tung 1989). The glutamatergic input appears to regulate the firing characteristics of the DA neurons, and in response to activation of glutamatergic N-methyl D-aspartate (NMDA) receptors on their cell-bodies, a marked increase in burst-like firing (figure 4) is observed (Charlety et al. 1991, Chergui et al. 1993, Murase et al. 1993). The inhibitory GABA input to DA cells in the VTA is mediated both via afferents from other brain regions and GABAergic interneurons within the nucleus (see Kalivas 1993), which, in turn, also receives a glutamatergic input from the prefrontal cortex (Carr and Sesack 2000).

Several sets of experimental data indicate that central NA activity modulates mesolimbocortical DA activity via different mechanisms. Thus, early biochemical studies indicated that the activity of DA neurons in the VTA decreased following selective destruction of noradrenergic fibers innervating the nucleus, whereas drugs that increase central NA activity may enhance DA turnover (Andén and Grabowska 1976, Hervé et al. 1982). Furthermore, electrophysiological recordings demonstrate that direct stimulation of the LC strongly enhances the activity of DA neurons in the VTA, an effect that is abolished by pretreatment with reserpine or an α$_1$ adrenoceptor antagonist (Grenhoff et al. 1993). The noradrenergic
modulation of the DA neurons seems to primarily involve regulation of burst firing (Grenhoff and Svensson 1989, 1993, figure 4), an effect that has been suggested to be mediated via excitatory α1 adrenoceptors on the dopaminergic cell-bodies (Grenhoff et al. 1993, Grenhoff et al. 1995). Central NA activity has also been observed to affect extracellular levels of DA specifically in the cortical nerve-terminal region (Carboni et al. 1990), primarily via a local mechanism (Gresch et al. 1995). In fact, there is a strong correlation between NA and DA extracellular levels in the prefrontal cortex, a phenomenon that appears to be due to local interactions of the transmitters with the NA transporter mechanism in the nerve-terminal area (Gresch et al. 1995, Tanda et al. 1997, Yamamoto and Novotney 1998). For a more extensive discussion regarding noradrenergic influence on dopaminergic neurotransmission see paper III and IV as well as section 5.4.

1.3.3 Functional roles of central dopaminergic systems

The activity of the various central dopaminergic pathways is associated with the regulation of several important behavioral and cognitive functions. The nigrostriatal dopaminergic pathway was first shown to have significance for behavior with the demonstration of the facilitatory role of DA neurotransmission in the striatum with respect to maintenance and initiation of physical movement (Carlsson et al. 1957, Ungerstedt 1971). This finding gained further importance
following the observation of a preferential degeneration of the nigrostriatal pathway in Parkinson’s disease, and the therapeutic effect of L-DOPA treatment in this devastating disorder (see Hornykiewicz 1973). Central dopaminergic function is also implicated in the mediation of natural and drug induced reward. Thus, in principle all drugs of abuse, as well as natural rewarding behaviors such as feeding and sexual behavior, enhance DA release preferentially in the medial and anterior part of the NAC (di Chiara 1995, Fibiger 1995), whereas accumbal DA output generally seems to be decreased during abstinence from drugs of abuse (Rossetti et al. 1992, Hildebrand et al. 1998 see Koob et al. 1998). Also, electrophysiological recordings of dopaminergic neuronal activity in behaving non-human primates show that a signal associated with an appetitive reward elicits a strong increase in neuronal activity, while aversive stimuli does so less frequently (Schultz 1998). Hence, variation in dopaminergic signaling in response to reward may serve as an incentive signal which may serve to establish the behaviors that elicited the reward (Waelti et al. 2001). Dopaminergic neurotransmission in the prefrontal cortex has been shown to modulate cognitive functions such as working memory and focused attention, and a decreased cortical DA activity has, accordingly, been found to be detrimental for the performance of these functions (Brozoski et al. 1979, Le Moal and Simon 1991). In particular the activation of dopamine D_{1} receptors, tentatively located extrasynaptically on pyramidal cells in the cerebral cortex, seems to be of importance for correct performance in tasks requiring a functional working memory (Sawaguchi and Goldman-Rakic 1994, Castner et al. 2000).

1.4 Serotonin

1.4.1 Central serotonin systems and receptors

Serotonin was initially shown by Brodie and coworkers (1955) to be an independent transmitter in the brain, and by means of the histofluorescence technique Dahlström and Fuxe (1964) subsequently localized 9 discrete serotonergic cell clusters located along the midline of the brainstem. A detailed mapping of the efferent projections of these nuclei showed that the small, caudally located, give rise to intrinsic brainstem projections as well as an innervation of the spinal cord. The serotonergic nuclei located more dorsally in the raphe region, i.e. the median raphe nucleus and the dorsal raphe nucleus (DRN), give rise to several diverse projections, which innervate different parts of the forebrain (Andén et al. 1966, Ungerstedt 1971). Thus, whereas the frontal cortex and the striatum appear primarily innervated by neurons in the DRN, the dorsal part of the hippocampus is rather exclusively targeted by 5-HT cells in the median raphe nucleus, while the ventral part of the hippocampus receives input from both nuclei (Azmitia and Segal 1978, Molliver 1987; figure 2).

Based on the differential antagonism of two ligands on peripheral serotonergic effects, 5-HT receptors were originally divided into the M and D type (Gaddum and Picarelli 1957). Further analyses using radioligand binding, biochemical and cloning techniques have now defined the serotonergic receptors into more than 14 distinct receptors, which are ordered into seven receptor types, i.e. 5-HT_{1} – 5-HT_{7}, (Peroutka and Snyder 1979, Bradley et al. 1986,
Hoyer et al. 1994, Kroese and Roth 1998). The 5-HT₁ receptors are characterized by their negative coupling to adenylyl cyclase and consist of five receptor variants, the A, B, D, E and F subtype (Hoyer et al. 1994). The 5-HT₁₅ receptor is expressed and distributed in 5-HT neurons in the raphe nuclei, where it acts as a somatodendritic autoreceptor, but it is also found on postsynaptic neurons in e.g. the hippocampus and cerebral cortex (Sprouse and Aghajanian 1988, Chalmers and Watson 1991). The 5-HT₁₆ and 5-HT₁₉ receptors act as presynaptic autoreceptors in the central serotonergic system, but in contrast to the 5-HT₁₅ receptors they are mainly found on axons in nerve-terminal areas (Bonaventure et al. 1998, Riad et al. 2000).

All the variants of the 5-HT₂ receptor, i.e. the 5-HT₂₆, 5-HT₂₇ and 5-HT₂₈, mediate their effects through activation of phosphoinositide metabolism (Hoyer et al. 1994), but while the 5-HT₂₆ receptor is only observed in relatively few nuclei in the brain (Duxon et al. 1997), the 5-HT₂₆ and the 5-HT₂₈ receptors have a widespread distribution to several major brain structures, e.g. the cerebral cortex, the basal ganglia and the hippocampus (Abramowski et al. 1995, Cornell-Hébert et al. 1999). In the brain the highest densities of the 5-HT₁ receptor, which is a ligand gated cation channel, are found in the lower brainstem, but low densities also are found in the cerebral cortex as well as in the amygdala and hippocampus (Hoyer et al. 1994, Miquel et al. 2002). The 5-HT₅, 5-HT₆, 5-HT₇ and 5-HT₁₅ receptors, which will not be described in detail here, seem to be positively coupled to adenylyl cyclase, and they are all, to a varying extent, expressed in the brain (Waeber et al. 1998, Sleight et al. 1998, Barnes and Sharp 1999, Vanhonenacker et al. 2000).

1.4.2 Regulation of serotonergic neurotransmission

The DRN, the major serotonergic nucleus with ascending projections (Molliver 1987), receives input from several areas of the brain. A dense glutamateric innervation stems from the lateral habenula nucleus, and extensive projections from the prefrontal cortex, the hypothalamus, preoptic areas as well as structures in the brainstem have been observed (Aghajanian and Wang 1977, Baraban and Aghajanian 1981, Kalén et al. 1985, Peyron et al. 1998). The major inhibitory feedback mechanism of the central serotonergic system is mediated via presynaptic autoreceptors on 5-HT neurons. Thus, extracellular 5-HT, released by a frequency dependent mechanism, may activate somatodendritic 5-HT₁₅ autoreceptors located on serotonergic cells in the DRN inducing a rapid decrease in their firing rate (Aghajanian et al. 1972, Wang and Aghajanian 1977, Sprouse and Aghajanian 1987), maintaining a slow regular spontaneous firing rate of the neurons. 5-HT₁₆ and 5-HT₁₉ autoreceptors, located presynaptically along the nerve-terminals and axons of the 5-HT neurons, may also control serotonergic neurotransmission by reducing nerve-terminal release of 5-HT following their activation by the endogenous transmitter (Middlemiss 1984, Engel et al. 1986, Sharp et al. 1989). Pyramidal neurons of the medial prefrontal cortex (mPFC) have recently been shown to influence the activity of both GABAergic and serotonergic neurons in the DRN, with both inhibitory and excitatory effects on individual neurons (Hajos et al. 1998, Celada et al. 2001). The prefrontal innervation of the DRN is also a part of the inhibitory feedback mechanism via
postsynaptic 5-HT$_{1A}$ receptors located on neurons in the mPFC, which secondarily may reduce an excitatory input to the DRN (Ceci et al. 1994).

Serotonergic cells in the DRN appear to be directly innervated by noradrenergic neurons (Baraban and Aghajanian 1981) and early experiments revealed an excitatory $\alpha$ adrenoceptor mediated noradrenergic tone upon DRN 5-HT neurons, apparently sustaining a basal neuronal activity (Svensson et al. 1975, Baraban and Aghajanian 1980). This effect was later shown to be mediated via $\alpha$ adrenoceptors on the 5-HT neurons, which increases their intrinsic firing rate via alterations in the control of the membrane potential (Vandermaelen and Aghajanian 1983, Aghajanian 1985). NA may also control 5-HT release via a mechanism in the nerve-terminal region; although in contrast to its effect on firing rate, in an inhibitory manner. Thus, extracellular NA may, via activation of inhibitory noradrenergic $\alpha$, heteroreceptors located presynaptically at the serotonergic nerve-terminal, decrease 5-HT output (Göthert et al. 1981, Frankhuysen and Mulder 1982). The inhibitory effect of NA on 5-HT nerve-terminal output may be observed in several regions of the rat brain, e.g., in the mPFC, the hippocampus as well as in the DRN (Maura et al. 1982, Mongeon et al. 1993, Hertel et al. 1999a, Kalsner and Abdali 2001).

1.4.3 Functional roles of central serotonergic systems

In spite of the various afferent inputs to the serotonergic nuclei, electrophysiological studies of the activity of serotonergic neurons in behaving animals indicate that they are rather unresponsive to most specific environmental stimuli. Thus, the firing rate of serotonergic cells in the DRN is not activated by even strong stressors, but mainly seems to reflect the general state of activity of the animal, being slow during sleep, intermediate during quiet waking and slightly increased while the animal is in an aroused state (see Jacobs and Fornal 1999). Still, in microdialysis studies, a large increase in nerve-terminal 5-HT release may be observed in response to various stressful stimuli (see Rueter et al. 1997). However, this effect may not primarily be associated with an effect of stress per se since several other behaviors, such as increased locomotion and waking, also readily enhance central release of 5-HT (Rueter et al. 1997). Interestingly, an exception to the correlation between behavioral arousal and serotonergic neuronal activity is observed while the animal engages in grooming and similar behaviors, during which a subset of the dorsal raphe neurons increase their activity more than two-fold (Fornal et al. 1996). Despite the non-specific, and modest, reactivity of the serotonergic system to specific stimuli, experiments using various selective pharmacological tools indicate several important modulatory roles of 5-HT in the brain. For example, 5-HT$_{1A}$ and 5-HT$_{3}$ receptor ligands appear to affect anxiety-like behaviors (see Barnes and Sharp 1999) whereas 5-HT$_{2A}$ receptors are involved in the mechanism of action of several hallucinogenic drugs (see Aghajanian and Marek 1999). Also, a diminished central serotonergic activity appears to be associated with enhanced sexual behavior and impulsivity (Gessa and Tagliamonte 1974, Linnoila et al. 1982, Harrison et al. 1997), as well as with an increased susceptibility to anxiogenic drugs (Goddard et al. 1995).
1.5 Inter-species differences in brain function and organization

Since most preclinical studies on the mechanisms of action of drugs or drug combinations are performed in rats or mice, but aim to extrapolate the results to human brain functions, the putative differences or similarities in brain function and organization between rodents and primates are important. Evolutionary, rodents and primates probably share a common ancestor approximately 80 million years back in time, and by analyzing the sequence of several translated regions of mRNA, the genomes may be calculated to be approximately 85 percent identical (Makalowski and Boguski 1998). Generally, as regards fundamental biological mechanisms, such as regulation of neurotransmission, no major species differences appear to have evolved during that time, at least not relating to monoaminergic function (see e.g. Raiteri et al. 1990, Hall et al. 1997, Hoffman et al. 1998, Gurevich and Joyce 1999, Feuerstein et al. 2000, Bidmon 2001). Yet, differences in brain anatomy between different species are obvious, and some regions, e.g. the prefrontal cortex, are far more developed in the primate, than in the rodent, brain (Fuster 1997). The prefrontal cortex in primates, in particular the dorsolateral prefrontal cortex, has been described as a brain structure the role of which is to coordinate cognitive functions, allowing for e.g. working memory and inhibitory control (Fuster 1997). Definitions of the prefrontal cortex have primarily been based on the anatomy of its thalamic connections as well as its dense dopaminergic innervation. Based on these definitions, the mPFC of the rat has been considered in principle homologous to the dorsolateral prefrontal cortex of primates (see Fuster 1997). Although this concept is somewhat controversial (Preuss 1995), this notion is supported by the cognitive dysfunction induced in rats by lesions of their mPFC (Bubser and Schmidt 1990, Arnsten 1997).

The basic anatomy of the ascending noradrenergic, dopaminergic and serotonergic transmitter pathways seems to be relatively phylogenetically stable in the mammals (Parent et al. 1984, Moore and Card 1984), with the largest variation to be found in their innervations of target regions. For example, the dopaminergic and noradrenergic innervation of the cerebral cortex seems more organized in specific layers in the primate brain, and also, as regards the dopaminergic innervation, to be much more widespread (Fuster 1997). There also exist interspecies variations in receptor function that are relevant for the interpretation of some of our results as regards their putative clinical significance. Thus, the non-rodent 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> receptors are similar as regards distribution and function, whereas a point mutation in the rodent gene for the 5-HT<sub>1B</sub> receptor alters its binding profile. Therefore, the rodent 5-HT<sub>1B</sub> receptor is functionally different from the rodent 5-HT<sub>1D</sub> receptor as well as from the non-rodent 5-HT<sub>1B</sub> receptor (Oksenberg et al. 1992), and e.g. pindolol has a much higher affinity for the rodent 5-HT<sub>1B</sub> receptors than for the human variant. Also, the rodent α<sub>2</sub> adrenoceptor differ from the corresponding human variant due to its low affinity for the antagonist yohimbine, an effect also caused by a point mutation in its gene (Link et al. 1992, Bylund et al. 1994).
2. Introduction: Clinical Background

2.1 Depression

2.1.1 Characteristics of major depression

Major depressive disorder is a very common, often chronic and intermittent, psychiatric disorder which is characterized by a depressed mood and/or the loss of interest or pleasure in nearly all activities. Symptoms include also impaired sleep and psychomotor disturbances, diminished ability to concentrate, and there is a strong comorbidity with general anxiety disorder (DSM-IV 2000, Rapaport 2001). Apart from its core symptoms major depression might appear heterogeneous, and may thus be further classified. Hence, a depressive episode with a marked decrease in hedonic interest and reduced reactivity to normally pleasurable stimuli, together with terminal insomnia and significant loss of appetite and weight, is described as melancholic depression. Conversely, major depression with atypical features is associated with somewhat sustained mood responsiveness to potentially rewarding events, combined with an increase in food intake as well as hypersomnia (DSM-IV 2000, Sullivan et al. 1998). These variants in the depressive phenotype have been observed to be correlated with differences in biological markers for hormonal and neurotransmitter function in patients, e.g. hypercortisolism in melancholic depressives (Sachar et al. 1970, Price et al. 1986, Gold and Chrousos 1999). However, clinically, many patients do not present a clear picture of either depressive subtype (Levitan et al. 1997).

Theories regarding the alterations in brain function that may cause a depressive episode were originally indirectly derived and based on pharmacological findings. Thus, since a decrease in central catecholamine function, e.g. caused by reserpine, apparently could induce depressive-like symptoms, while pharmacological augmentation of noradrenergic activity, by means of monoamine oxidase inhibitors or tricyclic antidepressants, exerted antidepressant activity, it was hypothesized that major depression was caused by lack of central catecholaminergic tone (Schildkraut 1965). Further studies on the preclinical and clinical effects of contemporary antidepressant drugs subsequently suggested that central 5-HT might also be importantly involved in mood control (Coppen 1967, see Carlsson et al. 1969). In general, clinical investigations of biochemical markers for monoaminergic central activity have not provided direct support for the original monoamine hypothesis of depression (Potter and Manji 1994, Maes and Meltzer 1995, Heninger et al. 1996), and central noradrenergic drive may, in fact, even be enhanced in major depression with melancholic symptoms (Potter and Manji 1994, Wong et al. 2000a). Also, several studies on the effects of rapid depletion of catecholamines or serotonin on the mood of healthy controls do not show any major effects (Salomon et al. 1997, Moreno et al. 1999, see Moore et al. 2000). Clinical and preclinical observations may rather indicate that variations in central monoaminergic activity, in conjunction with several other environmental and phenotypic variables, may affect the vulnerability of an individual to depressive disorder as well as tentatively affect the symptom-profile of the disorder (Heninger et al. 1996, Berman et al. 1999a, Moreno et al. 2000, see Ordway et al. 2002).
2.1.2 Pharmacological treatment of depression I

All major, clinically used, antidepressant drugs share the common effect of enhancing central monoaminergic, particularly 5-HT and/or NA, neurotransmission. Thus, although monoamine reuptake inhibition is the major mechanism of action of the most frequently prescribed antidepressant drugs, other mechanisms, e.g. monoamine oxidase inhibition or α₂ adrenoceptor antagonism, have proven to be efficacious in major depression as well (Frazer 1997), indicating that increased monoamine transmission, mediated by diverse mechanisms, may serve a similar purpose in this case. Interestingly, selective enhancement of central 5-HT or NA neurotransmission may have apparently similar, positive, effects on the core symptoms in depression (Montgomery 1980, Potter et al. 1981, Nathan et al. 1990, see Nelson 1999). On the other hand, particularly beneficial effects of selective serotonin reuptake inhibitors (SSRIs) and noradrenaline reuptake inhibitors (NRIs), on specific symptoms in depression have also been observed, e.g. on anxiety and psychomotor retardation, respectively (Montgomery et al. 1981, Åberg 1981, Åberg-Wistedt 1982, van Praag et al. 1987, see Humble 2000). Furthermore, the clinical efficacy of SSRIs and NRIs are clearly different in other disorders, in the sense that SSRIs, but not NRIs, have a well documented effect in anxiety disorder (Evans and Moore 1981, Den Boer and Westenberg 1988), obsessive compulsive disorder (Thorén et al. 1980, Goodman et al. 1990) as well as premenstrual dysphoria (see Eriksson et al. 2002) whereas NRIs, but not SSRIs, are efficacious in attention deficit hyperactivity disorder (see Popper 1997). Drugs that more or less selectively increase serotonergic or noradrenergic neurotransmission in the brain thus appear to alleviate the core symptoms of major depression, but not necessarily of other psychiatric disorders, by a mechanism serving as a common denominator, that may be located downstream of their initial presynaptic effects. Support for this notion comes from a series of clinical experiments utilizing rapid tryptophan depletion in patients successfully treated for major depression by pharmacological means. Briefly, these studies show that individuals which have responded to an SSRI have a high risk of a brief depressive relapse in response to rapid tryptophan depletion during ongoing treatment (Delgado et al. 1990, 1991, 1999), a vulnerability that is significantly less frequent in individuals successfully treated with an NRI (Delgado et al. 1991, 1999, Heninger et al. 1996). Importantly, rapid tryptophan depletion in a small sample of healthy subjects undergoing chronic treatment with an SSRI did not induce any symptoms of depression (Barr et al. 1997). These investigations, and analogous studies using catecholamine depletion (Delgado et al. 1993, Miller et al. 1996, Heninger et al. 1996), provide evidence that enhancement of serotonergic or noradrenergic central neurotransmission by reuptake inhibition may represent two, largely independent, pharmacological means to induce or sustain a clinical antidepressant action. On the other hand, several mechanisms whereby central monoaminergic systems may influence each other have been delineated (see 1.3.2, 1.4.2), interactions which, in turn, may be of relevance for the clinical effects of antidepressant drugs. For example, SSRIs appear to diminish the basal activity and reactivity of noradrenergic cells in the LC (Nestler et al. 1990, Engberg 1992, Szabo et al. 1999) as well as, at least acutely, decrease the firing
frequency of DA cells in the VTA (Prisco and Esposito 1995). In contrast, augmentation of central noradrenergic tone by administration of idazoxan, which in similarity with the atypical antidepressant drugs mirtazapine and mianserin blocks α₂ adrenergic receptors (Nickolson et al. 1982, Doxey et al. 1983), enhances the electrophysiological activity of monoaminergic neurons in both the DRN and the VTA (Freedman and Aghajanian 1984, Grenhoff and Svensson 1993). Furthermore, several classes of antidepressants, e.g. SSRIs, NRIs and the α₂ and 5-HT₂ receptor antagonist mianserin, appear to specifically enhance DA extracellular levels in the prefrontal cortex (Tanda et al. 1994, 1996a), although the effect of the SSRI fluoxetine, but not that of NRIs, was diminished during chronic treatment (Tanda et al. 1996b). In fact, the acute enhancing effect of various antidepressant drugs on cortical dopaminergic neurotransmission has even been suggested to represent a common denominator for drugs with antidepressant potential (Tanda et al. 1994, 1996a). Another theory regarding the putative common effects of antidepressant drugs, which primarily is based on extracellular electrophysiological recordings from postsynaptic neurons in the hippocampus, suggests that an increase in serotonergic neurotransmission, which seems to be caused by most antidepressants following a chronic treatment regimen, may be a primary mechanism for their clinical antidepressant effect (Haddjeri et al. 1998, Szabo and Blier 2001a); although this notion is not entirely consistent with the above described depletion studies.

2.1.3 Pharmacological treatment of depression II

Following initiation of an antidepressant treatment regimen there is often a delay in onset of action of two to four weeks, which may not be accounted for by pharmacokinetic variables (Overall et al. 1962, Oswald et al. 1972). The slow onset of clinical action is likely to be related to adaptive changes of brain physiology in response to the markedly changed conditions induced by the antidepressant drug (Charney et al. 1981a, Heninger and Charney 1987). Adaptive changes may, in this context, involve many interconnected levels of functional organization, e.g. gene expression, intracellular signaling, receptor density as well as signaling within and between specific neural networks. Following acute administration of a monoamine reuptake inhibitor, a decreased turnover of 5-HT or NA is observed in the brain, with the primary effect depending on the affinity of the used drug (Schubert et al. 1970, Segal et al. 1974, Carlsson and Lindqvist 1978). Thus, a selective inhibitor of the 5-HT transporter preferentially decreases 5-HT turnover, and an NRI reduces NA turnover. The decreased turnover of transmitter is largely due to a decrease in the electrophysiological activity of serotonergic or noradrenergic neurons in the DRN and the LC, respectively (Nyblck et al. 1975, Gallager and Aghajanian 1975); resulting from an increased feedback inhibition of the neurons by their somatodendritic autoreceptors which is activated by the increase in extracellular levels of transmitter, brought about by the reuptake blockade (Svensson and Usdin 1978). The strongly enhanced feedback inhibition of monoaminergic neuronal activity in response to administration of reuptake inhibitors were later shown to clearly diminish the augmenting effect of these types of drugs on extracellular levels of 5-HT or NA, in each transmitters terminal regions (Gallager and Aghajanian 1975, Adell and Artigas 1991, Bel and
L. Linnér

Artigas (1992, Mateo et al. 1998). For example, acute administration of an NRI, in a moderate dose, preferentially enhances extracellular levels of NA in the LC, compared with in the cerebral cortex, due to feedback inhibition, which thus reduces impulse mediated transmitter release and hence nullifies or decreases the effect of the reuptake blockade in the nerve-terminal region (Mateo et al. 1998).

Electrophysiological studies indicated that the feedback inhibition of noradrenergic neurons in the LC was diminished following chronic treatment with a monoamine reuptake inhibitor, resulting in a partially recovered firing rate of the cells (Svensson and Usdin, 1978, 1979). The reduced feedback inhibition was shown to involve desensitization of $\alpha_2$ autoreceptors located in the somatodendritic region of the noradrenergic neurons (Svensson and Usdin 1978), although desensitization of $\alpha_2$ autoreceptors in the nerve-terminal area has also been observed following chronic NA reuptake blockade (Crews and Smith 1978, Lacroix et al. 1991). A clear reduction of the feedback inhibition of serotonergic neuronal activity following chronic inhibition of the reuptake of 5-HT was also found (Svensson 1978, Blier and de Montigny et al. 1983), an effect that accordingly was suggested to be related to desensitization of somatodendritic 5-HT$_{1A}$ autoreceptors on the serotonergic neurons (Svensson 1978, Chaput et al. 1986). The observation of a gradually developing functional desensitization of the autoregulatory feedback mechanism of monoaminergic cells during the course of a chronic treatment regimen with reuptake inhibitors may thus represent an adaptive response, which when implemented allows for an enhanced terminal output of transmitter, tentatively associated with an emerging clinical antidepressant response (see Blier and de Montigny 1994). More recent studies generally support the observation of a downregulated feedback inhibition of serotonergic neuronal activity during chronic treatment with SSRIs (Invernizzi et al. 1994, Le Poul et al. 1995, Arborelius et al. 1995), whereas results concerning the equivalent mechanism in the LC are more divergent (Valentino et al. 1990, Lacroix et al. 1991, Brady 1994).

The acute effect of an SSRI on nerve-terminal 5-HT output is strongly enhanced by the addition of a 5-HT$_{1A}$ receptor antagonist (Hjorth 1993, Arborelius et al. 1996), an effect that theoretically may mimic the result of a downregulation of the serotonergic autoreceptor. Hence, based on the above described theories, such a drug combination might shorten the time of onset of antidepressant action and a clinical study to test this hypothesis was undertaken (Artigas et al. 1994). Since there were no selective 5-HT$_{1A}$ receptor antagonists available for clinical use, the antihypertensive drug pindolol, a $\beta$ adrenoceptor partial agonist with relatively high affinity for the 5-HT$_{1A}$ receptor, was used. The initial study reported, in fact, both a shortened time of onset of action and an enhanced clinical effect of the SSRI by the addition of pindolol. Since then, the addition of pindolol to SSRIs has in most, but not all, subsequent clinical trials been observed to exert an augmenting effect, both as regards time of onset as well as therapeutic efficacy (Péres et al. 1997, Tome et al. 1997 see Artigas et al. 2001 but see Berman et al. 1999b). Analogous clinical trials with regard to NRIs, i.e. addition of an $\alpha_2$ autoreceptor antagonist which acutely enhances their effect on NA nerve-terminal output (Dennis et al. 1987), have so far yielded conflicting results. Thus, in studies with the tricyclic antidepressant imipramine, addition of the $\alpha_2$ adrenoceptor blocker, and antidepressant drug, mianserin enhanced antidepressant activity (Lauritzen et al. 1992, 1994); whereas addition of
the unselective $\alpha_2$ adrenoceptor antagonist yohimbine to a desipramine regimen in treatment resistant patients did not yield any advantageous effects (Charney et al. 1986, Schmauss et al. 1988).

2.2 Schizophrenia

2.2.1 Characteristics of schizophrenia

Schizophrenia afflicts approximately one percent of the global population, and also, secondarily, strongly affects the lives of an even larger amount of close relatives as well as the health care system as a whole (Carpenter and Buchanan 1994, Meltzer 1999). The symptoms of schizophrenia display a wide variation and are traditionally divided into positive and negative symptoms. The positive symptoms may appear as an excess or distortion of normal function, e.g. delusions or hallucinations, whereas the negative symptoms appear to reflect a diminution or loss of normal function, e.g. restrictions in emotional expression or goal-directed behavior (DSM-IV 2000). Cognitive dysfunction is also a prominent symptom in schizophrenia, with impaired learning and short-term memory (Saykin et al. 1991, Heaton et al. 1994). Notably, the degree of cognitive dysfunction in schizophrenia has been observed to represent an important marker of future community functioning of the individual, i.e. outcome of treatment (Green 1996, Meltzer et al. 1999). The etiological background of schizophrenia is not well understood, but neurodevelopmental alterations (see Weinberger and Lipska 1995) in conjunction with a clearly heritable component (NIMH 1999) are likely to be involved.

Based on the antipsychotic action of reserpine and dopamine D$_2$ receptor antagonists, as well as the ability of amphetamine to mimic some of the major symptoms in schizophrenia, it was early hypothesized that schizophrenia may be related to a central hyperdopaminergic state (see Carlsson 1978). However, D$_2$ blockers are not particularly effective in treating negative symptoms in schizophrenia (Crow 1980, Carpenter 1996) and drugs such as phencyclidine, i.e. NMDA receptor antagonists, may mimic most symptoms of schizophrenia (see Javitt and Zukin 1991). Also, administration of amphetamine or DA receptor agonists worsens the positive, but may partly improve negative, symptoms in schizophrenia (Angrist et al. 1982, van Kammen and Boronow 1988, Dolan et al. 1995, Laruelle et al. 1999). Consequently, based on the above mentioned clinical observations, as well as findings in experimental animals (Weinberger and Lipska 1995, Svensson et al. 1995), a notion of a regionally imbalanced central DA system in schizophrenia is emerging. In essence, an increase in subcortical dopaminergic neurotransmission in concert with a diminished cortical DA output, tentatively secondary to dysfunctional interconnections between higher brain structures, may be one of the mechanisms in the pathophysiology of schizophrenia (Weinberger 1987, Davis et al. 1991). Hence, positive symptoms may be related to an increased subcortical dopaminergic neurotransmission whereas a diminished cortical DA activity may tentatively be involved in cognitive and negative symptoms in schizophrenia (Weinberger 1987, Davis et al. 1991, Laruelle et al. 1996, Akil et al. 1999, Svensson 2000, Abi-Dhargam et al. 2002).
2.2.2 Pharmacological treatment of schizophrenia

Classical pharmacotherapy of schizophrenia is based on blockage of central, postsynaptic, dopamine D_2 receptors (Carlsson and Lindqvist 1963, Seeman et al. 1976, Farde et al. 1988). However, while being efficient in ameliorating positive symptoms, the typical antipsychotic drugs exhibit only a limited efficacy on (Leucht et al. 1999), and may even exacerbate, negative symptoms in schizophrenia (Carpenter 1996). In addition, since clinically adequate dosage of typical neuroleptics results in levels of D_2 receptor occupancy as high as 75 percent, the use of these drugs often induce parkinsonism or other extrapyramidal side effects (EPS), which become manifest at approximately 80-85 percent D_2 receptor occupancy (Farde et al. 1992) and which, in turn, may aggravate negative symptoms. In contrast to the clinical profile of typical D_2 receptor antagonists, clozapine, which unfortunately may cause serious side effects such as agranulocytosis, is efficacious at considerably lower levels of D_2 receptor occupancy (Farde et al. 1992), and rarely induces EPS (see Safferman et al. 1991). The diminished induction of EPS and higher efficacy in treatment resistant patients as well as a purportedly, primary or secondary, advantageous effect on negative and certain cognitive symptoms in schizophrenia, has led to the definition of clozapine as the prototypical atypical antipsychotic drug (Kane et al. 1988, Meltzer et al. 1989). Theories regarding the mode of action of typical versus atypical antipsychotics have emphasized the frequent antagonistic effect of the atypicals on 5-HT_{2A} receptors and/or α_1 adrenoceptors (Meltzer et al. 1989, Andersson et al. 1995, Svensson et al. 1995, Ashby and Wang 1996), as well as their differential effect on regional DA output. Thus, whereas classical antipsychotic drugs mainly enhance extracellular DA concentrations in subcortical brain regions, atypical antipsychotics preferentially increase extracellular DA concentrations in the mPFC of experimental animals (Imperato and Angelucci 1989, Moghaddam and Bunney 1990, Nomikos et al. 1994, Youngren et al. 1999). Clozapine displays considerable affinity also for α_1 adrenoceptors (Ashby and Wang 1996), and clinical data have demonstrated that the addition of the selective α_1 adrenoceptor antagonist idazoxan to a D_2 antagonists may cause an augmented antipsychotic effect (Litman et al. 1996). It was subsequently reported that addition of idazoxan to a selective D_2 receptor antagonist resulted in both an enhancement of the D_2-blockage induced effect in a preclinical test predictive for clinical antipsychotic activity, as well as in a markedly enhanced DA output selectively located to the mPFC (Hertel et al. 1999b, see 5.5).
3. Aims of the study

To experimentally analyze the purported mechanism of action of pindolol’s observed augmenting effect on the clinical antidepressant action of an SSRI.

To investigate the functional characteristics of the central noradrenergic system during acute and chronic treatment with a noradrenaline reuptake inhibitor at clinically relevant plasma concentrations, and to test the effect of autoreceptor blockage during these conditions.

To investigate the effect of selective noradrenaline reuptake inhibition by reboxetine on the electrophysiological activity, and nerve-terminal transmitter output, of brain dopaminergic and serotonergic pathways originating in the ventral tegmental area and the dorsal raphe nucleus, respectively.

To test whether selective noradrenaline reuptake inhibition, in similarity with $\alpha_2$ adrenoceptor antagonists, may specifically enhance dopamine output in the mPFC as well as augment the antipsychotic-like effect of a D$_2$ receptor antagonist.
4. Materials and Methods

4.1 Animals

Male albino rats (BK universal, Sollentuna, Sweden) were used in all studies. Rats from the strain BK1:SD (i.e. Sprague-Dawley) were used in all electrophysiological experiments as well as in the microdialysis experiments in paper V, whereas BK1:WR (i.e. Wistar) were used in all other experiments. Animals arrived at least five days prior to experimental use and were housed in the animal facility under standard laboratory conditions with a 12 h light/dark cycle (lights on at 06.00). Animals designated for behavioral experiments, or microdialysis experiments in paper V, were subjected to a reversed light/dark cycle, i.e. lights off at 06.00. All experiments were performed between 08.00 and 18.00. Food and water were available ad lib. All experiments were approved by, and conducted in accordance with, the local Ethical Committee (Stockholms Norra och Södra Försöksdjursetiska Kommittéer).

4.2 Drugs

The following drugs were used:
Citalopram: serotonin reuptake inhibitor (Lundbeck A/S, Denmark), desipramine: noradrenaline reuptake inhibitor (Ciba-Geigy, Switzerland), idazoxan: α1 adrenoceptor antagonist (RBI, USA), imipramine: serotonin and noradrenaline reuptake inhibitor (Ciba-Geigy, Switzerland), (-)-pinindolol: partial β adrenoceptor agonist with affinity for the 5-HT1A receptor (RBI, USA), prazosin: α adrenoceptor antagonist (Pfizer, USA), raclopride: D2 receptor antagonist (Astra Arcus, Sweden), reboxetine: noradrenaline reuptake inhibitor (Pharmacia Corp., USA), tetradotoxin: voltage gated Na+ channel blocker (Sigma, Sweden), WAY-100635: 5-HT1D receptor antagonist (Astra Arcus AB, Sweden), K-OH-DPAT: 5-HT1D receptor agonist (Sigma, Sweden); (see Artigas et al. 2001, Bylund et al. 1994, Carruthers and Twum-Barima 1981, Doxey et al. 1983, Fletcher et al. 1996, Javai et al. 1979, Köhler et al. 1985, Seeman and Van Tol 1993, Wong et al. 2000b). Citalopram, desipramine, idazoxan, imipramine, raclopride, reboxetine, WAY-100635 and 8-OH-DPAT were dissolved in 0.9% saline solution or, for administration via the microdialysis-probe, in perfusion solution. (-)-pinindolol was dissolved in 5.5% glucose with 1 drop of acetic acid. Prazosin was dissolved in 1 drop acetic acid, and then diluted to the correct volume in distilled water. Citalopram and reboxetine were also (paper V) diluted in phosphate buffered saline (Dulbecco’s [i.e. Ca. Mg and NaHCO3]). Before administration to awake animals, drugs solutions were, if needed, adjusted to pH 7.0. Drug administrations were performed by intraperitoneal (i.p.), subcutaneous (s.c.) or intravenous (i.v.) injections as well as, during microdialysis, via the microdialysis-probe. In paper II rats were pretreated according to the following schedules: saline (1.0 ml/kg, i.p.) administered twice daily (09:00/18:00) for 14 days, imipramine (10 mg/kg, i.p.) administered twice daily for 14 days or saline (1.0 ml/kg., i.p.) administered twice daily for 12 days followed by imipramine (10 mg/kg, i.p.) administered twice daily on the 13th and 14th day. Experiments on chronically treated animals were performed 12 - 16 hours after the last drug administration.

4.3 Electrophysiology

4.3.1 Surgery and experimental procedures

Rats weighing 230 – 600 gram were anesthetized with chloral hydrate (400 mg/kg. i.p.; KEBO LAB, Sweden) and maintained under surgical anesthesia throughout the experiments. A vein-catheter for i.v. administration of drugs was inserted in the jugular vein (paper I and II), a tail vein (paper III) or the femoralis vein (paper V) of the rat. The animal was subsequently mounted in a stereotaxic apparatus
(David Kopf, USA) and positioned upon an electrical heating pad (Harvard Apparatus, USA), which kept the rectal temperature at 37 - 38 °C. A hole was drilled in the skull above the LC, VTA or DRN, i.e. approximately -1.0, +3.0 or +1.0 mm anteroposterior (AP) and ±1.0, ±0.9 or ±0.1 mediolateral (ML) relative to lambda, respectively (Paxinos and Watson 1998). Subsequently the dura mater was cut, and, in order to prevent dehydration, saline (1 ml) was administered s.c. in the back of the animal.

4.3.2 Extracellular recordings

Extracellular recording electrodes were pulled in a vertical puller (Narishige, Japan) from glass capillaries (Clark electromedical instruments, UK; outer diameter 1.5 mm, inner diameter 1.17 mm) and filled with 2% pontamine Sky Blue in 2 M sodium acetate. The tip of the electrodes was broken under microscope, yielding an impedance of 2.0 – 3.0 MΩ. Electrodes were lowered into the brain by means of a hydraulic microdrive (David Kopf, USA), and a reference electrode was inserted into the subcutaneous tissue. The signal was amplified and visualized on a digital oscilloscope (Tektronix, USA) connected, via a CED 1401 interface (Cambridge electronic design, UK), to an IBM compatible computer; in which discriminated spikes were stored by the Spike2 software. Drug administrations in cumulative doses, with an interval of 1.5 – 3.0 minutes, depending on the identity of the recorded neuron, were preceded by a control injection of the appropriate vehicle. Recordings were only made from one cell in each animal. Presumed noradrenergic, dopaminergic or serotonergic cells were found approximately 5.5 – 6.0, 7.5 – 8.5 or 5.0 – 6.0 mm from the brain surface and were recognized by their previously described, typical, electrophysiological characteristics (Graham and Aghajanian 1971, Bunney et al. 1973a, Aghajanian and Haigler 1974). Also, when possible, an agonist for the somatodendritic autoreceptor of the recorded neuron, i.e. clonidine, apomorphine or 8-OH-DPAT, was administered at the end of the experiment, in order to further pharmacologically verify the identity of the recorded cell. At the end of each experiment, a negative current of 5 μA was passed through the electrode for 10 minutes, leaving a dye spot (Pontamine Sky Blue), which later was used to identify the location of the recording site by visual examination of brain slices stained with neutral red. Only data from animals with correct electrode placement within the respective nucleus were included in the subsequent analysis.

4.3.3 Data analysis

Quantitative analysis of neuronal firing characteristics was performed by means of the CED spike2 software. Average firing rate was calculated as the ratio between the number of spikes and the time elapsed, expressed as spikes per second (Hz). In dopaminergic neurons in the VTA, burst firing, with burst onset defined as an interval of less than 80 ms and burst termination as the next interspike time interval exceeding 160 ms (Grace and Bunney 1984), was also determined. Burst firing was quantified as the percent ratio of spikes in bursts and the total number of spikes, calculated over a period of 500 consecutive interspike time intervals. Average firing rate was presented as spikes per second (paper I and II) or as percent of control or baseline (paper I, III and V) whereas burst firing was presented as a percentage variable. All data is shown as mean ± S.E.M. and was statistically evaluated using the student t-test for dependent or independent samples as well as one (treatment) or two-way (treatment × dose or time) analysis of variance (ANOVA) for repeated measures, followed by the Newman-Keuls test for multiple comparisons with a criterion of p < 0.05.
4.4 Microdialysis

4.4.1 Surgery and microdialysis

Rats weighing 240 – 400 gram were anaesthetized with sodium pentobarbital (60 mg/kg, i.p.: Nordvac, Sweden) and mounted in a stereotaxic frame (David Kopf, USA) equipped with a heating pad. Additional administrations prior to surgery consisted of atropine (0.05 mg, s.c.; NM Pharma Sweden), saline (1 ml, s.c.) and Chloromycetin (1%, eye-salve; Warner Lambert Nordic AB, Sweden). Also, after surgery, before suturing the wound, a local anesthetic (Bupivacain: Norcox Pharma AB, Sweden) was administered. Concentric dialysis probes were stereotaxically implanted in either the mPFC or the NAC. Stereotaxic coordinates were (in mm): AP +3.0 or +1.6; ML +0.6 or +1.4; dorsoventral (DV) -5.2 or -8.2, respectively, relative to bregma and dorsal surface (Paxinos and Watson 1998). In some implanted rats (paper III), a second dialysis probe was implanted in the ipsilateral VTA at the coordinates: AP +3.8 mm, ML 0.7 mm, DV -8.7 mm, relative to lambda and dorsal surface. In paper II a transversal probe, implanted at the coordinates: AP +1.7 mm, DV -1.7 mm relative to bregma and skull surface, was used for microdialysis of the frontal cortex (FC). During probe implantation in paper V a sterilized silica tube (50 × 0.94 mm; Sikema AB, Sweden), attached to a bent (90°) steel tube (20 × 0.64 mm; Kinnvall AB, Sweden), was applied via the cut above the skull, subcutaneously into the back of the neck of the animal. The steel tube was subsequently fixed to the skull together with the microdialysis probe. During microdialysis experiments a polyethylene tube (PE-10, Merck Eurolab, Sweden) was attached to the steel tube, allowing for s.c. administration without handling the animal. Dialysis occurred through a semipermeable membrane (copolymer of acrylonitrile and sodium methallyl sulfonate) with an inner diameter of 0.24 mm, molecular cutoff at 4000 Da (AN69 Hospal; Hospal Med, Sweden) and an active surface length of 4.0, 2.25, 1.0 or 8.0 mm in case of mPFC, NAC, VTA or FC dialysis, respectively. All microdialysis probes were handmade in our laboratory. The recovery of DA and several monoamine metabolites across the membrane of similar types of microdialysis probes has previously been estimated to be slightly less than 10 % (Nomikos et al. 1992). After surgery the animals were housed individually and given free access to food and water. Dialysis experiments were conducted approximately 48 h after surgery in freely moving animals in a bright room, except in paper V where experiments were performed in a dark room. The dialysis probe was perfused with a modified ringer’s solution (147 mM sodium chloride, 3.0 mM potassium chloride, 1.3 mM calcium chloride, 1.0 mM magnesium chloride and 1.0 mM sodium phosphate; pH 7.4) at a rate of 2.5 μl/min set by a microinfusion pump (Harvard Apparatus, USA). In paper V, citalopram (0.5 μM) or citalopram (0.5 μM) and idazoxan (10 μM) was included in the perfusion solution during the entire experiment. The dialysate was loaded directly into the sample loop of the injector (Valco Instruments, USA) which was controlled, via a PE Nelson 900 interface (Perkin-Elmer, USA), by a personal computer running the turbochrom 4.1 software (Perkin-Elmer, USA) to automatically inject samples to the analytical system based on a predetermined program. Upon completion of experiments, the animals were killed by an overdose of anesthetic and the brains were taken out and preserved in 10% formalin in 25% sucrose. Each brain was subsequently sliced, stained with neutral red and examined for probe placement. Only data from experiments where the probe had been correctly placed according to the atlas of Paxinos and Watson (1998) was included in the analysis.

4.4.2 Biochemical assay

Concentrations of NA, DA, 5-HT, DOPAC and 5-HIAA were determined by high performance liquid chromatography (HPLC) coupled to electrochemical detection. Separation of NA, DA, 5-HT and their metabolites was accomplished by reversed phase liquid chromatography through a C-18 column (Supelcosil 3 μm, 150 × 4.6 mm), using a water/methanol based acetate buffer as mobile phase (see details in respective paper), delivered at a rate of 0.8 ml/min. Samples were quantified by sequential
oxidation (+370 mV / +410 mV; 5-HT and metabolites) or sequential oxidation and reduction (+300 or +400 mV / -100 or -200 mV; NA or DA and metabolites) using a coulometric detector (Coulochem II, model 5200; ESA) feeding output both to a two-pen chart recorder (Kipp and Zonen, Netherlands) as well as to a personal computer running the turbochrom 4.1 software. The detection limit for NA, DA, 5-HT and metabolites was approximately 0.8, 0.2, 0.2 and 20 fmol/min, respectively, based on a signal to noise ratio of 2:1.

4.4.3 Data analysis

Raw data was analyzed using the turbochrom software and was subsequently presented as dialysate concentration over time (fmol/min; paper II) or as mean percent change of basal concentration (paper II, III, IV and V) ± S.E.M, where 100 percent is defined as the average of the last two samples preceding drug or vehicle administration. Drug effects were evaluated both as single observations at each time-point as well as mean output, i.e. based on the average of four consecutive samples following administration (paper II and III). Data were statistically analyzed using one (treatment or time) or two-way (treatment × time) ANOVA for repeated measures, followed by Newman-Keuls test for multiple comparisons with a criterion of p < 0.05 to be considered significant.

4.5 Behavioral experiments

4.5.1 Conditioned avoidance response

Rats weighing 300 – 380 gram were placed in a conventional shuttle-box (530 × 250 × 225 mm), divided into two compartments by a partition. Upon presentation of the conditioned stimulus (CS), 80 dB white noise, the rat had 10 s to move into the opposite compartment in order to avoid the unconditioned stimulus (UCS), an intermittent shock of approximately 0.6 mA in the grid floor (inter-shock interval 2.5 s, shock duration 0.5 s). The following variables were recorded: [1] avoidance (response to CS within 10s); [2] escape (response to CS + UCS within 50s); [3] failure (failure to escape within 50s). Following an adaptation time of five minutes each day, the animals were trained for five consecutive days in a session of 20 trials randomly distributed over 15 minutes. All experimental sessions consisted of 10 trials randomly distributed over 7.5 minutes, and were performed 20, 90 and 240 minutes after the last drug administration. Experimental manipulations were always preceded by a 10 trial pretest session. Experiments in individual rats were performed every third day.

4.5.2 Catalepsy

Animals were placed on an inclined (60°) grid and, excluding the first 30s, the time the rat remained in the same position was measured for a maximum of 2.5 minutes. Experiments were performed 30, 60, 120 and 240 minutes after last drug injection. The catalepsy was scored from 0-5 according to the time (square root transformation) the animal remained immobile: score 0 = 0 – 0.08, 1 = 0.09 – 0.35, 2 = 0.36 – 0.80, 3 = 0.81 – 1.42, 4 = 1.43 – 2.24, 5 ≥ 2.25 min (Ahlénius and Hillegaart, 1986).
4.5.3 Data analysis

Since the data obtained from both behavioral experiments in this study is not normally distributed, non-parametric statistics were used for statistical evaluation. Analysis was hence performed by means of the Friedman two-way ANOVA, followed by the Wilcoxon matched-pairs signed-ranks test (conditioned avoidance response) or by means of the Kruskal-Wallis one-way ANOVA, followed by the Mann-Whitney U-test (Catalepsy).

4.6 Measurements of drug plasma concentrations

4.6.1 Pretreatment and plasma collection

Wistar rats weighing 270 – 310 gram at the time of plasma collection were pretreated with imipramine as described in section 4.2 or with reboxetine (0.15 – 13.5 mg/kg i.p.). Seventeen hours after the last administration of imipramine or 30 minutes after the administration of reboxetine, rats were killed, and blood was collected in test tubes containing 150 µl heparin (500 IU/ml; Apoteket AB, Sweden) which immediately was put on ice. After centrifugation (2500 or 3000 rpm; 4 °C; 25 or 10 min for reboxetine and imipramine, respectively) the supernatant was collected and frozen.

4.6.2 Chromatographic analysis

Imipramine and desipramine in plasma was analyzed by HPLC with ultraviolet detection. Five hundred µl of plasma was extracted with 5 ml of 0.3 M sodium phosphate and 400 µl of diisopropylether; then 100 µl of organic layer was injected into a silica column with an eluent consisting of 25% methanol and 0.4% ammonia in acetonitrile. The ultraviolet detector was set at 254 nm. The lower limit of quantification for both imipramine and desipramine was 10 nM. Racemic reboxetine in plasma was determined by a similar methodology as imipramine and desipramine. One milliliter of plasma was extracted using solid phase extraction columns C2. The final extract was evaporated to dryness and resolved in 100 µl of the HPLC mobile phase (27% acetonitrile in 10 mM phosphate buffer, pH 4.9). A fraction (25 µl) of the resolved extract was then injected on to a Zorbax Eclipse XDB-phenyl analytical column. Reboxetine content was measured with UV detection at 210 nm. The inter- and intraday coefficient of variance of the method was below 5% and the limit of quantification for reboxetine was 5 nM. Data was presented as nmol ± S.E.M.
5. Results and discussion

5.1 Effects of (–)-pindolol in combination with citalopram on central serotonergic neuronal activity: comparisons with a selective 5-HT\textsubscript{1A} receptor antagonist

It has been hypothesized that the observed effect of pindolol to accelerate the onset of the antidepressant effect of SSRIs is due to blockage of somatodendritic 5-HT\textsubscript{1A} autoreceptors located on serotonergic cell bodies in the raphe nuclei. Hence, by diminishing the acute inhibitory effect of 5-HT reuptake inhibition on serotonergic neuronal activity, secondarily allowing for a larger initial increase in nerve-terminal extracellular levels of 5-HT, a faster antidepressant action was suggested to be obtained (Artigas et al. 1994, 1996; Romero et al. 1996, see 2.1.2). However, some experimental evidence also indicates that (–)-pindolol, which is the enantiomer with 5-HT\textsubscript{1A} receptor affinity (Schoeffler and Hoyer 1988), may have agonist activity on 5-HT\textsubscript{1A} autoreceptors \textit{in vivo} (Hjorth and Carlsson 1986, Clifford et al. 1998). Therefore, in order to further analyze the mechanism involved in the purported augmentation by pindolol of the antidepressant effect of SSRIs, we investigated the effect of (–)-pindolol on the inhibitory response of serotonergic neurons in the DRN to acute administration of citalopram, a highly selective 5-HT reuptake inhibitor. In electrophysiological single cell recordings \textit{in vivo}, (–)-pindolol exerted an inhibitory effect on serotonergic neurons in the DRN of rats, an effect that could be reversed by the 5-HT\textsubscript{1A} receptor antagonist WAY-100635 (figure 5a), in congruence with other observations (Fornal et al. 1999, Haddjeri et al. 1999, Sprouse et al. 2000). Also, in difference to WAY-100635, (–)-pindolol was unable to reverse the inhibition of serotonergic cell firing caused by citalopram (figure 5b). On the other hand, pretreatment with a high dose of (–)-pindolol, although partly inhibiting serotonergic cell firing when given alone, still significantly diminished the citalopram induced inhibition, thus keeping up basal firing rate (figure 6). This observation

\textbf{Figure 5.}
Representative rate-meter recordings from two serotonergic neurons in the dorsal raphe nucleus showing the effect of (–)-pindolol and WAY 100635 on (A): basal firing rate and (B): citalopram induced inhibition of firing rate activity. All drug administrations were intravenous.
is partly in agreement with a previous study where pretreatment with pindolol for two days completely blocked the suppressant effect of paroxetine on the spontaneous activity of 5-HT cells (Romero et al. 1996), an effect that appear to be dependent on the dose of pindolol used (Haddjeri et al. 1999). Notably, the effect size of pindolol pretreatment on the SSRI induced inhibition of serotonergic neuronal activity was not large in our experiments (figure 6), and may not necessarily explain the enhancing effect on the SSRI induced increase in nerve-terminal 5-HT output (see below). At any rate, pindolol appears to act as a partial agonist upon somatodendritic 5-HT_{1A} autoreceptors, a contention also supported by observations in other model systems (Artigas et al. 2001), and its pharmacological effect may consequently vary depending on the experimental conditions.

Most microdialysis experiments have observed an augmenting effect of pindolol on the SSRI induced increase in extracellular levels of 5-HT (Dreschfield et al. 1996, Hjorth 1996, Gobert and Millan 1999, Dawson and Nguyen 2000, but see Gartside 1999), when given in a dose equivalent to the high dose in the above described electrophysiological experiments. This may, in part, reflect a stabilizing effect of pindolol on the response of serotonergic neuronal activity to administration of an SSRI (see above). However, the effect can also be related to the fact that pindolol may block serotonergic nerve-terminal 5-HT_{1A} autoreceptors in the rat (Assie and Koek 1996). Hence, pindolol may enhance the acute effect of an SSRI on 5-HT output by diminishing an increased inhibitory tone upon nerve-terminal release secondarily elicited by the SSRI (Sharp et al. 1997, Gobert et al. 1997, Gobert and Millan 1999, but see Miguez et al. 2002), an effect which, however, is likely to be exclusive to the rodent brain (see 1.5).
5.2 Central noradrenergic function during acute and chronic treatment with tricyclic antidepressants: effects of $\alpha_2$ adrenoceptor blockage

The occurrence of a gradual desensitization of somatodendritic autoreceptors during chronic reuptake inhibition, and the tentative benefit of acute blockage of these receptors for a clinical antidepressant effect has not been as thoroughly investigated in the noradrenergic central system as in the serotonergic (see 2.1.2). Also, some controversy exists to whether noradrenergic somatodendritic autoreceptors, in similarity with serotonergic, are desensitized during the course of a chronic treatment regimen with a selective reuptake inhibitor (Svensson and Usdin 1978, Valentino et al. 1990, Lacroix et al. 1991, Brady 1994). Furthermore, in recent experiments in rats undergoing chronic treatment with an SSRI the administration of a low dose of a 5-HT$_{1A}$ receptor antagonist resulted in a firing rate clearly above baseline, an effect that was not observed in control animals (Arborelius et al. 1996), and which may serve as a secondary marker of a downregulated feedback mechanism. For these reasons we examined the effects of subacute and chronic NA reuptake inhibition, by means of the tricyclic antidepressant drug imipramine and its major metabolite desipramine, on the basal function of the central noradrenergic system during ongoing treatment. In order to compare with the above mentioned effects of autoreceptor blockage upon serotonergic electrophysiological activity during chronic SSRI treatment, as well as to document the putative clinical utility of the combination, we also investigated the effect of a low dose of an $\alpha_2$ adrenoceptor antagonist upon noradrenergic function during the above described treatment conditions.

In single cell electrophysiological experiments it was observed that the basal firing rate of LC NA neurons in rats undergoing chronic treatment with imipramine was significantly higher than after subacute treatment, but still clearly suppressed in comparison with the firing rate in rats chronically treated with saline (figure 7). Thus, a partial desensitization of the somatodendritic feedback inhibition of noradrenergic neuronal activity appears to occur during chronic treatment with imipramine, a notion that is particularly strengthened by the fact that the plasma concentrations of imipramine and its major metabolite

![Figure 7.](image)

Mean (spikes/s ± SEM) basal firing frequency of noradrenergic neurons in the locus coeruleus (LC) of rats treated chronically (see 4.2) with either saline ($n=18$) or imipramine ($n=16$) or subacutely with imipramine ($n=13$), measured 12-14 hours after the last dose of drug or vehicle. Data were analyzed by t-test for independent samples. ***p < 0.001 compared with chronic and subacute imipramine. *p < 0.01 compared with subacute imipramine.
Table 1. Mean (± SEM) plasma concentration of imipramine and desipramine in rats treated chronically or subacutely (see 4.2) with imipramine. Plasma was collected 17 hours after last dose of drug.

<table>
<thead>
<tr>
<th>treatment</th>
<th>[imipramine]_{baso} (nmol/L)</th>
<th>[desipramine]_{baso} (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>chronic imipramine</td>
<td>61 ± 15</td>
<td>580 ± 66</td>
</tr>
<tr>
<td>subacute imipramine</td>
<td>&lt; 10</td>
<td>43 ± 5</td>
</tr>
</tbody>
</table>

desipramine was increased by one order of magnitude in the chronic treatment group (table 1). Indeed, the plasma concentrations of desipramine in chronically treated animals were essentially equivalent to those observed in patients treated with imipramine (Brosen et al. 1986). Still, despite the very low plasma concentrations of desipramine and imipramine, the average firing rate of the noradrenergic neurons was lower in the subacutely treated animals when compared with the firing rate in chronically treated rats, thus indicating that desensitization has taken place. On the other hand, in similarity with the original investigation of the effect of chronic NA reuptake inhibition on noradrenergic neuronal activity during ongoing treatment (Svensson and Usdin 1978), the noradrenergic electrophysiological activity was still clearly reduced in comparison with control animals (figure 7). Indeed, in patients undergoing treatment with desipramine, plasma MHPG levels, a measure of central NA turnover, are decreased in acutely, but also, although to a lesser degree, in chronically treated individuals (Charney et al. 1981b). The remaining partial inhibition of LC cell activity during chronic NA reuptake blockade represents a quantitative difference in comparison with analogous results from the serotonergic system, where a more thorough desensitization of the somatodendritic feedback inhibition may occur during a chronic regimen with an SSRI (Blier et al. 1983, Chaput et al. 1986, Arborelius et al. 1995). Two recent electrophysiological studies investigating the noradrenergic neuronal activity during ongoing subacute or chronic treatment with the NRIs desipramine or reboxetine did not observe any decrease in feedback inhibition of firing rate following chronic treatment (Szabo et al. 2000, Szabo and Blier 2001b). However, following chronic desipramine treatment the number of active noradrenergic neurons was increased (Szabo et al. 2000).

The output of NA in the rat frontal cortex during subacute and chronic treatment with imipramine was clearly enhanced in comparison to rats chronically treated with saline (figure 8). The apparently,

Figure 8. Mean (± SEM) basal output of noradrenaline in the frontal cortex (FC) of rats treated chronically (see 4.2) with either saline (n=6) or imipramine (n=7) or subacutely with imipramine (n=7), measured 14-16 hours after the last dose of drug or vehicle. Baseline values were calculated as the mean of two consecutive samples immediately before injection of saline. Data were analyzed by means of one-way (treatment) ANOVA followed by Newman-Keuls test for multiple comparisons. *p < 0.05, **p < 0.01 compared with chronic saline.
Figure 9.
The acute effect of intravenous (i.v.) administration of idazoxan (0.125 mg/kg) on (A): the firing rate of a single noradrenergic neuron in the locus coeruleus, in a rat treated chronically (see 4.2) with imipramine, shown in a representative rate meter recording; and (B): the mean (± SEM) firing rate (Hz) of noradrenergic neurons in the locus coeruleus in rats treated chronically (see 4.2) with either saline (n=12) or imipramine (n=9) or subacutely with imipramine (n=6), or in rats acutely treated with desipramine (0.25 mg/kg, intravenously; n=11). Experiments on chronically treated animals were performed 12-16 hours after the last dose of drugs or vehicle. Data were analyzed by means of two-way (treatment × time) ANOVA followed by Newman-Keuls test for multiple comparisons. *p < 0.05, **p < 0.01 compared with in-group pre-idazoxan value. "p < 0.05, "p < 0.01 for between-group comparisons of the effect of idazoxan.

Although not significantly, higher basal NA output in the chronic compared with the subacute imipramine treatment group (figure 8) may be related to a downregulation of nerve-terminal α₂ autoreceptors, which has been suggested to occur during chronic NA reuptake inhibition (Crews and Smith 1978, Lacroix et al. 1991, Mateo et al. 2001). However, this observation may also be related to the large differences in plasma concentrations of imipramine and desipramine between the two treatment groups (table 1). In fact, the relatively large increase in extracellular concentrations of NA in rats treated subacutely, i.e. for two days, with imipramine, in spite of the very low levels of active drug found in plasma, indicate i) that relatively large variations in plasma concentrations of reuptake inhibitor do not strongly affect nerve-terminal output of transmitter during ongoing treatment, and ii) that there is a clear possibility that noradrenergic nerve-terminal output is strongly enhanced, not only during chronic administration, but also in the acute phase of antidepressant treatment with NRIIs in clinically effective doses. Two recent microdialysis studies measuring the reactivity of the presynaptic, α₂ adrenoceptor mediated, feedback inhibition of central noradrenergic output in rats which have undergone chronic treatment with desipramine have both observed desensitization. Hence, by investigating the effects of clonidine on NA nerve-terminal output in rats following wash-out of the drug, Sacchetti et al. (2001) noted a decreased response of somatodendritic α₂ autoreceptors, whereas Mateo et al. (2001) observed a desensitization of nerve-terminal α₂ autoreceptors in the cingulate cortex following chronic treatment. In general, investigations on homeostatic reactions of neurotransmitter systems in response to reuptake inhibition are complicated by the fact that either one observes the system during exposure, while receptor agonist/antagonist challenges may be confused by the concomitant exposure; or alternatively, one observes the
Figure 10.
Effects of saline (open arrow) and idazoxan (0.3 mg/kg, subcutaneously; filled arrow) on noradrenaline output in the frontal cortex (FC) of rats treated chronically (see 4.2) with either saline (n=6) or imipramine (n=7) or subacutely with imipramine (n=7). Experiments were performed 16-18 hours after the last dose of drug or vehicle. Data were analyzed by two-way (treatment × time) ANOVA followed by Newman-Keuls test for multiple comparisons. *p < 0.05, ***p < 0.001 compared with last baseline value.

system after exposure, and eventual wash-out, while rebound phenomena (see Svensson and Usdin 1979) may bias results.

Administration of a low dose of the α₂ adrenoceptor antagonist idazoxan to rats pretreated with imipramine or alternatively with desipramine, given acutely in a dose designated to result in a similar inhibition of noradrenergic cell firing as during chronic imipramine treatment (Ceci and Borsini 1996), resulted in a marked recovery of the firing rate of noradrenergic neurons. This effect was more pronounced in chronically compared with acutely drug treated animals (figure 9), whereas the effect on saline treated rats was small. Equivalently, idazoxan potently enhanced NA output in the frontal cortex in rats chronically treated with imipramine, but had a much smaller effect in subacutely treated animals and no effect in the control group (figure 10). The enhanced activating effect of idazoxan on noradrenergic function in rats undergoing long-term treatment with imipramine may thus reflect a desensitization of the α₂ adrenoceptor mediated feedback inhibition, both at the somatodendritic and nerve-terminal level. However, this effect may, in part, also be related to differences in drug plasma concentrations.
5.3 Effects of selective noradrenaline reuptake inhibition on the electrophysiological activity of dopaminergic and serotonergic neurons in the brain

The central noradrenergic system has been shown to functionally interact with both dopaminergic and serotonergic central pathways, both at the somatodendritic and nerve-terminal level (see 1.3.2 and 1.4.2), and there is ample evidence suggesting that both of these transmitter systems may be involved in the clinical effects of antidepressant drugs (see 2.1.2, Jimerson 1987, D’Aquila et al. 2000). Consequently, antidepressant drugs which primarily enhance noradrenergic neurotransmission may secondarily affect dopaminergic and serotonergic function and may, tentatively, mediate a part of their clinical effect by this interaction. Investigations of the effect of selective NA reuptake inhibition on the activity of dopaminergic neurons in the VTA and serotonergic neurons in the DRN, may therefore provide additional information regarding the mechanism of action of these type of drugs. To this end we used the highly selective NRI reboxetine, which is successfully used as an antidepressant in Europe (Berzewski et al. 1997, Burrows et al. 1998). In contrast to desipramine, which previously has been used as a prototypical selective NRI, reboxetine has low affinity for the α adrenoceptors (Richelson and Nelson 1984, Wong et al. 2000b). Intravenous administration of reboxetine, over a large dose range, did not significantly affect the firing rate of dopaminergic neurons in the VTA (figure 11a). However, even at low doses of reboxetine the burst firing activity of the cells was increased, an effect that was significant at the higher doses used.
(figure 11b). An earlier study using desipramine in analogous experiments did not observe any stimulatory effect (Einhorn et al. 1988), although burst firing was not included in the analysis. However, in a recent study investigating the action of amphetamine upon dopaminergic activity, a high dose of the NRI nisoxetine enhanced burst firing of DA neurons in the VTA (Shi et al. 2000). The specific, enhancing, action of NA reuptake inhibition on burst firing of mesolimbocortical DA neurons may be of considerable interest in view of the important role of this type of signaling for transmitter release and expression of immediate early genes in DA target areas (Chergui et al. 1996, 1997, see figure 4).

**Figure 12.** Effects of intravenous (i.v.) administration of reboxetine (0.15–4.80 mg/kg) on (A): mean (± SEM) firing rate, expressed as percent of baseline, of serotonin neurons in the dorsal raphe nucleus; and (B/C): electrophysiological activity of single serotonergic neurons, shown in representative rate meter recordings. Note that WAY100635 reverses the inhibitory effect of a high dose of reboxetine on serotonergic neuronal activity. Data were analyzed by t-test for dependent samples. Numbers of recorded cells are shown in each bar. *p < 0.05, **p < 0.01 compared with effect of saline injection.

At low doses, reboxetine significantly enhanced the firing rate of serotonergic neurons in the DRN, with a maximal effect of approximately 40 percent above the baseline firing rate (figure 12a). However, at higher doses reboxetine had an inhibitory influence on serotonergic neuronal activity, an effect previously reported by Wong et al. (2000b). Since this inhibitory effect was reversible by means of the 5-HT1A receptor antagonist WAY-100635 (figure 12c) it is likely to be caused by an increase in extracellular levels of 5-HT in the DRN, mediated by inhibition of the 5-HT transporter by reboxetine at high, unselective, doses. Our results from the recordings in the DRN are in agreement with a recent study by Millan et al. (2001) who reported an enhancing effect of reboxetine on serotonergic neuronal activity in the DRN, although they did not observe any significant stimulatory effect of reboxetine on dopaminergic activity in the VTA. However, in similarity with Einhorn et al. (1988), they did not include burst firing in their analysis of dopaminergic electrophysiological activity. One study has failed to observe any significant effect of ongoing treatment with reboxetine on serotonergic basal firing rate in the DRN during acute or chronic treatment (Szabo and Blier 2001b), although in this study no statistical analysis was presented (see discussion paper V). The mechanism whereby NA reuptake inhibition increases the activity of central dopaminergic and serotonergic neurons might involve direct activation of excitatory a1 adrenoceptors located on their cell bodies.
Results and Discussion

(Vandermaelen and Aghajanian 1983, Grenhoff et al. 1995). Indeed, pretreatment with a low dose of prazosin inhibited the effect of reboxetine on the burst firing of dopaminergic neurons (figure 11b). However, indirect excitatory effects mediated via e.g. cortical projections to both nuclei, as well as other alternative mechanisms, may also be involved in the noradrenergic regulation of dopaminergic and serotonergic neuronal activity (see discussion paper III and V).

5.4 Effects, and sites of action, of selective noradrenaline reuptake inhibition on nerve-terminal extracellular levels of dopamine and serotonin; comparisons with selective serotonin reuptake inhibition

An increase in burst firing of dopaminergic neurons in the VTA has been shown to result in a markedly increased nerve-terminal output of transmitter (Gonon 1988, see figure 4), and stimulation of the DRN enhances 5-HT release in the brain (Sharp et al. 1990). The excitatory effects of reboxetine on the firing characteristics of dopaminergic and serotonergic neurons may consequently have as functional outcome an increase in nerve-terminal release of each respective transmitter, which, in turn, may be of importance for the clinical action of NRIs. In microdialysis experiments, systemic administration of low doses of reboxetine significantly enhanced extracellular DA concentrations in the rat mPFC, whereas no effect, even at very high doses, was observed in the NAC (figure 13a). The selective increase in cortical DA output elicited by reboxetine is similar to the effect of both other NRIs (Carboni et al. 1990, Tanda et al. 1994) and α₁ adrenoceptor antagonists (Gresh et al. 1995, Hertel et al. 1999c), which, in turn, also increase nerve-terminal NA output (Dennis et al. 1987)

**Figure 13.**
Effects of intraperitoneal (i.p.) administration of reboxetine (0.15-13.5 mg/kg) on (A): average (±SEM) dopamine output during four consecutive samples after injection, expressed as percent of baseline, in the rat nucleus accumbens (NAC; n=7) and the medial prefrontal cortex (mPFC; n=7); and (B): mean plasma concentrations of reboxetine in rats 30 minutes after administration. Each dot indicates a single observation. Solid line and gray area represents mean (± ½ S.D) serum concentration of reboxetine in humans undergoing chronic treatment (Ohman et al. 2001). Data were analyzed by one-way (concentration) ANOVA followed by Newman-Keuls test for multiple comparisons. ***p < 0.001 compared with effect of saline.
as well as burst firing of dopaminergic neurons in the VTA (Grenhoff and Svensson 1993). Local administration of reboxetine, via the microdialysis probe, into the VTA, did not affect cortical DA output, whereas local administration into the mPFC resulted in a dose dependent increase in extracellular DA levels in the brain region (table 2), an observation identical to the effects of the α adrenoceptor blocker idazoxan (Hertel et al. 1999c). Hence, the increase in burst firing of DA neurons caused by reboxetine may not represent a critical determinant of the augmented cortical DA output induced by systemic administration of the drug. Indeed, enhanced cortical NA release may increase cortical DA output via an effect at the nerve-terminal level (Gresch et al. 1995). This is so since released DA appears to be removed from the extracellular space largely by the NA reuptake mechanism (Carboni et al. 1990), which shows high affinity for DA (Raiteri et al. 1977), and extracellular DA levels may therefore be modulated by drugs affecting the noradrenaline transporter, or which by other mechanisms enhance mPFC NA output (Gresch et al. 1995, Yamamoto and Novotney 1998). Recent studies using mice deficient of the NA transporter gene clearly demonstrate the important role of the transporter for regulation of cortical, but not accumbal, DA levels (Morón et al. 2002), and it has even been suggested that DA may be released by noradrenergic nerve-terminals in the cerebral cortex (Devoto et al. 2001). Interestingly, intra-accumbal administration of high concentrations of reboxetine also increased extracellular levels of DA in the NAC (table 2). Thus, the lack of effect of systemic reboxetine on accumbal DA release may reflect the previously observed inhibitory action of cortical dopaminergic neurotransmission on subcortical DA release (see 1.3.2 and Discussion paper III).

Figure 14.
Effects of systemic intraperitoneal (i.p.) administration of reboxetine (3 mg/kg; n=8) or citalopram (6 mg/kg; n=5) on dopamine output, expressed as the mean (± SEM) percent of baseline, in the medial prefrontal cortex (mPFC) of rats. Arrow indicates time of injection of drug. Data were analyzed by two-way (administration × time) ANOVA. ***p < 0.001 compared with baseline.
Plasma concentrations of reboxetine in rats were similar to, or below, steady state plasma concentrations in patients undergoing chronic treatment with reboxetine (figure 13b, Öhman et al. 2001), indicating that cortical nerve-terminal output of DA may be enhanced during treatment with reboxetine in humans. However, the administration of citalopram, in a dose resulting in a clinically relevant drug plasma concentration (6 mg/kg, i.p.; Cremers et al. 2000), did not significantly affect DA extracellular levels in the mPFC (figure 14). Consequently, these data, as well as other results (Pozzi et al. 1999, Bymaster et al. 2002), are not in accordance with the proposition that an increase in cortical DA output is a common denominator of almost all antidepressant drugs (see 2.1.2, Tanda et al. 1994).

During experiments in which cortical 5-HT output was quantified, several measures were taken to increase the sensitivity and specificity of the method. Hence, experiments were performed in rats during their active phase, in a dark room, and all drug administrations were performed via an earlier implanted subcutaneous inlet, thereby diminishing potential bias from awakening or stress. Also, to be able to obtain detectable levels of 5-HT in the dialysate, as well as to be able to differentiate noradrenergic effects from 5-HT reuptake inhibition, a low amount of citalopram was added to the perfusion solution. During these conditions, administration of reboxetine significantly enhanced nerve-terminal output of 5-HT in the mPFC (figure 15a).

**Figure 15.**
Effects of systemic subcutaneous (s.c.) administration of reboxetine (3 mg/kg; n=6) or citalopram (3 mg/kg; n=5) on (A) serotonin and (B) 5-hydroxyindol acetic acid (5-HIAA) output, expressed as the mean (± SEM) percent of baseline, in the medial prefrontal cortex (mPFC) of rats. Citalopram (0.5 μM) where present in the perfusion solution during the whole experiment. Arrows indicate administration of vehicle or drug. Data were analyzed by two-way (administration × time) ANOVA. *p < 0.05, **p < 0.01, ***p < 0.001 compared with baseline, “p < 0.01, “”p < 0.001 compared between treatment groups.
Figure 16.
Effect of local infusion of increasing concentrations of reboxetine (10-1000 µM) on serotonin output, expressed as mean (± SEM; n=4) percent change of baseline, in the medial prefrontal cortex (mPFC). Data were analyzed by one-way (time) ANOVA followed by Newman-Keuls test for multiple comparisons. *p < 0.01, ***p < 0.001 compared to baseline.

This effect may be related to the increase in neuronal firing rate of serotonergic cells in the DRN induced by reboxetine, since local administration of increasing doses of the drug into the mPFC did not result in a significant increase until the concentration in the perfusion solution was high enough to inhibit 5-HT reuptake (figure 16, see Wong et al. 2000b). In other studies (Tanda et al. 1994, Sacchetti et al 1999, Millan et al. 2001, Page and Lucki 2002), where an SSRI was not included in the perfusion solution, no effect of desipramine or reboxetine on nerve-terminal release of 5-HT was observed, even at very high doses. However, it has been noted that, in microdialysis experiments, an SSRI in the perfusion solution may be required to detect the effect of stimulation of serotonergic neuronal activity on nerve-terminal release of transmitter (Sharp et al. 1990, Brodin et al. 1990). Acute administration of citalopram significantly reduced extracellular 5-HT and 5-HIAA levels in this experimental setup (figure 15b), an effect probably mediated by increased extracellular 5-HT levels in the DRN which causes inhibition of serotonergic neuronal activity, whereas 5-HT reuptake already is blocked at the terminal level, due to the presence of citalopram in the perfusion solution (see Hjorth and Auerbach 1994). Consequently, since the SSRI decreased nerve-terminal 5-HT release in these experiments, reboxetine must enhance cortical serotonin output by a mechanism other than 5-HT reuptake inhibition, e.g. by an increase in basal serotonergic neuronal activity. The observed enhancing effect of selective NA reuptake inhibition on cortical serotonergic neurotransmission might be involved in the antidepressant effect of these types of drugs.
5.5 The influence of selective noradrenaline reuptake inhibition on dopamine D<sub>2</sub> receptor blockade induced changes in behavior and central DA output related to a preclinical model of antipsychotic efficacy

As observed in earlier experiments described in this study, reboxetine, in similarity with α<sub>2</sub> adrenergic/alpha-adrenergic antagonists as well as the atypical antipsychotic drug clozapine, enhances the electrophysiological activity of dopaminergic neurons in the VTA (Grenhoff and Svensson 1993, Melis et al. 1999, figure 11); and specifically increases cortical DA output (Moghaddam and Bunney 1990, Nomikos et al. 1994, Hertel et al. 1999c, figure 13a), primarily by means of a local mechanism (Gresch et al. 1995, Gessa et al. 2000, table 2). We (Hertel et al. 1999b) recently showed that when adding the α<sub>2</sub> adrenergic antagonist idoxazol to the D<sub>2</sub> receptor antagonist raclopride the antipsychotic-like effect of low doses of raclopride was potentiated in the conditioned avoidance response (CAR) test, a preclinical test with high predictive validity for a clinical antipsychotic effect (see Wadenberg and Hicks 1999). Hence, idoxazol augmented the antipsychotic-like effect of raclopride, an effect that was accompanied by a strong increase in cortical DA output, caused by the drug combination (Hertel et al. 1999b).

Interestingly, previously published clinical data demonstrate an augmenting effect of idoxazol as well as other, less selective, α<sub>2</sub> adrenergic antagonists on the therapeutic efficacy of classical antipsychotics, especially as regards negative symptoms (Mizuki et al. 1990, Litman et al. 1996, Berk et al. 2001). Due to the similarities between the effects of α<sub>2</sub> adrenergic antagonism and NA reuptake inhibition on central dopaminergic function,

*Figure 17.*

Effects of raclopride alone, or in combination with reboxetine (6 mg/kg, intraperitoneally [i.p.]) on (A): Conditioned avoidance response; each bar represents the median avoidance (+ semi-interquartile range; n = 11 in all groups). Statistical evaluation was performed by means of Friedman two-way ANOVA, followed by the Wilcoxon matched-pairs signed-ranks test. (B): Catalepsy; each bar represents the median catalepsy score (+ semi-interquartile range; n = 8 in all groups). Statistical evaluation was performed by the Kruskal-Wallis one-way ANOVA, followed by the Mann-Whitney U-test. *p < 0.05, **p < 0.01 compared to respective control group (saline/saline or reboxetine/saline). ’p < 0.05, ’’p < 0.001 for comparisons between saline/raclopride and reboxetine/raclopride treatment group.
we evaluated the potential augmentation by reboxetine of the antipsychotic-like effect of the selective D$_2$ receptor antagonist raclopride, utilizing the same experimental paradigm as in our previous study with idazoxan.

The suppressant effect of raclopride on the CAR, when given in a dose resulting in approximately 65% D$_2$ receptor occupancy in the rat (Wadenberg et al. 2000), was significantly augmented by the addition of reboxetine, without any concomitant effect on catalepsy scores (figure 17). Thus, NA reuptake inhibition and α$_2$ adrenoceptor blockage, two drug regimens that primarily act on, and enhance, central NA nerve-terminal output (Dennis et al. 1987), may both serve to augment the antipsychotic-like effect of D$_2$ receptor antagonists, tentatively via their common effect on cortical DA activity. Indeed, as observed also with idazoxan, reboxetine, when added to raclopride, strongly and selectively enhanced the extracellular concentrations of DA in the mPFC (figure 18a), an observation that is consistent with previous experiments using other NRIs under similar conditions (Carboni et al. 1990, Yamamoto and Novotney 1998, Westerink et al. 2001). The large increase in prefrontal DA output caused by the drug combination as well as the negligible effect of raclopride alone, may indicate that the increase in DA output in the mPFC induced by NRIs is diminished by concomitant activation of nerve-terminal D$_2$ autoreceptors, which during normal conditions may be under low dopaminergic tone (see Westerink et al. 2001).

**Figure 18.**
Effects of saline or raclopride (0.1 mg/kg, subcutaneously [s.c.]) administration on (A) dopamine and (B) dihydroxyphenylacetic acid (DOPAC) output in the medial prefrontal cortex (mPFC) or (C) dopamine output in the nucleus accumbens (NAC) in animals pretreated with saline or reboxetine (6 mg/kg, intraperitoneally [i.p.]). Arrows indicate time of administrations. Each point represents the mean percent (± SEM) change from baseline (n ≥ 4 in all groups). Data were analyzed using two-way (treatment × time) ANOVA followed by Newman-Keuls test for multiple comparisons. *p < 0.05, **p < 0.01, ***p < 0.001 for difference from saline/saline treatment group. *p < 0.05, **p < 0.01 for comparisons between saline/raclopride and reboxetine/raclopride treatment group.
This interpretation is also supported by the reduction of mPFC extracellular DOPAC levels caused by reboxetine, when administered alone (figure 18b). Pretreatment with reboxetine did not affect the raclopride induced increase in DA output in the NAC (figure 18c). The results indicate that adjunctive treatment with NRIs may reduce the level of D2 receptor occupancy required for a full antipsychotic effect, resulting in a reduced risk of EPS and, tentatively, less risk of cognitive impairment (see Castner et al. 2000). In addition, given the antidepressant effect of NRIs alone, an enhanced therapeutic effect on cognitive or negative symptoms may also be suggested on this ground. Two subsequent, small, clinical trials investigating the putative beneficial effect of adding reboxetine to antipsychotic pharmacotherapy in schizophrenia have yielded contrasting results. Thus, Schutz and Berk (2001) did not observe any difference in treatment response following addition of reboxetine to ongoing haloperidol treatment, whereas most recently Raedler et al. (2002) found that addition of reboxetine did result in augmentation.
6. General discussion

Clinical studies utilizing rapid monoamine depletion provide evidence that a critical mechanism whereby antidepressant drugs sustain a clinical effect is their common ability to enhance central monoaminergic neurotransmission (see 2.1.2). Still, since the clinical onset of an antidepressant drug regimen may require weeks of treatment, long term alterations in brain function to the drug is likely to be involved. The slow onset of antidepressant action has been argued to partly be due to an increased feedback inhibition of the monoaminergic transmitter systems, resulting in a less than optimal initial facilitation of monoaminergic neurotransmission during the early stages of treatment (see 2.1.2). Several studies show that the acute effects of threshold doses of an SSRI or NRI on nerve-terminal transmitter output are hampered by an initially larger effect in the nuclei of the transmitter systems, leading to an inhibition of their neuronal activity, which, in turn, decreases the effect of the drug on nerve-terminal release of transmitter (Adell and Artigas 1991, Mateo et al. 1998). However, larger doses of a reuptake inhibitor may overcome this threshold effect and thus also acutely induce robust increases in nerve-terminal transmitter output (Arborelius et al. 1996, Mateo et al. 1998). In the present study we show that during ongoing treatment with an NRI, nerve-terminal output of NA in the frontal cortex is clearly enhanced, not only after chronic treatment, but also during the initial days of treatment (figure 8). Importantly, the plasma concentrations that were present in the experimental animals during these observations were either in the clinically relevant range or even below this range (table 1). Corresponding studies on the serotonergic system also indicate that SSRIs enhance serotonergic output in the brain acutely, also when given in a clinically relevant dose (Arborelius et al. 1996, Cremers et al. 2000). Interestingly, in some conditions in which SSRIs and NRIs are used, i.e. premenstrual dysphoria (see Eriksson et al. 2002) and attention deficit attention disorder (Popper 1997), respectively, a similar delay in onset of clinical action is not observed; which may indicate that these disorders are more readily affected by an increase in postsynaptic tone of the transmitter. Also, the highly selective NRI reboxetine has been shown to possess a rapid onset of action on several symptoms in seasonal affective disorder (Hilger et al. 2001). Such observations might seem to argue against the importance of gradual downregulation of presynaptic autoreceptor function for the clinical efficacy of monoamine reuptake inhibitors. Still, a desensitization of somatodendritic autoreceptors, which will not only modify basal, but also evoked, neuronal activity, may be of principal importance for the impact of various sensory stimuli on the activity of the serotonergic or noradrenergic systems (Simson and Weiss 1989, Murase et al. 1992).

The observation that pindolol may shorten the time of onset of an SSRI in the treatment of depression has been claimed as a proof of principle for the theory of gradual desensitization of autoreceptor functioning as a homeostatic response allowing for the emergence of an
antidepressant effect during monoamine reuptake inhibition (see Artigas et al. 1996). It was thus hypothesized that pindolol may shorten the clinical onset of action of an SSRI by blocking the serotonergic somatodendritic autoreceptors, thereby reducing the need for desensitization of the receptor. However, the present study, as well as several other recent studies, clearly indicates that pindolol does not potently reverse the inhibitory effect of SSRIs on serotonergic neuronal activity, but rather may act as a partial agonist at the 5-HT\textsubscript{1A} autoreceptor. Also, the plasma concentrations of pindolol needed to obtain augmentation of serotonergic output in microdialysis studies are approximately two orders of magnitude higher than those observed in patients undergoing clinical trials with pindolol as add-on to an antidepressant (Cremers et al. 2001). Pindolol has recently been observed to enhance cortical catecholaminergic nerve-terminal output by a β adrenoceptor mediated mechanism (Gobert and Millan 1999). Hence, the augmentation by pindolol of the clinical antidepressant effect of SSRIs might not be construed as proof of the utility of autoreceptor blockade in conjunction with simultaneous reuptake inhibition, but may rather support the augmenting effect of increasing catecholaminergic activity in patients undergoing treatment with an SSRI (see below). Since a clinical study investigating the effect of the clinically utilizable selective 5-HT\textsubscript{1A} receptor antagonist NAD-299 (Robalzotan; Arborelius et al. 1999) upon the delay in onset of action of an SSRI has yet to be performed, it is still not known whether serotonergic autoreceptor antagonism may be advantageous during treatment with an SSRI.

In similarity with previous studies on the serotonergic system the present study show that addition of an α\textsubscript{2} adrenoceptor antagonist to ongoing treatment with an NRI strongly increases nerve-terminal release of NA, not only, as earlier observed, during the initiation of treatment (Dennis et al. 1987), but even more potently following chronic treatment (figure 10). The augmentation of noradrenergic neurotransmission by addition of an α\textsubscript{2} adrenoceptor blocker may, based on the demonstrated importance of sustained central monoamine output for the antidepressant effect, tentatively enhance the antidepressant action of an NRI. However, clinical studies on the putative augmentation of the antidepressant action of NRIs by addition of yohimbine do not support this notion (see 2.1.2). In fact, some studies report an increase in anxiety using this drug combination (Holmberg and Gershon 1961, Schmauss et al. 1988). Further clinical studies in this area, using more selective antagonists with preferential affinity for the presynaptic α\textsubscript{2A} adrenoceptor, may be of interest. Thus, considering the notion of a bell shaped curve for the correlation between noradrenergic tone and cognitive function (see 1.2.3), a very large increase in noradrenergic neurotransmission, which may be achieved by combining an NRI with an α\textsubscript{2} adrenoceptor antagonist (figure 10), might well be beneficial in a subgroup of depressed patients characterized by anergia, but with low anxiety.

It has been hypothesized that a unifying feature of all antidepressant drugs is an increase in central serotonergic neurotransmission following chronic treatment (see 2.1.2). Indeed, in the present study it was observed that selective NA reuptake inhibition enhances serotonergic neuronal activity, and that this increase results in an augmented nerve-terminal release of 5-HT in the forebrain (figures 12 and 15), an effect that tentatively may be further enhanced during chronic treatment (Mongeau et al. 1994, Yoshioka et al. 1995). These results may lend further strength to the notion of an increase in central serotonergic function as one major important
feature of most antidepressants. However, considering that SSRIs have a much larger, and in several aspects different, effect on central serotonergic output compared to NRIs, and that several clinical depletion studies clearly indicate that the antidepressant effect of NRIs, but not that of SSRIs, may be sustained even during severely reduced central serotonergic function (see 2.1.2), this notion may seem less plausible. Still, approximately 10 – 15 percent of the individuals which have recovered from major depression in response to an NRI relapse following rapid tryptophan depletion, while the relapse frequency is about 50 – 60 percent in SSRI responders (Delgado et al. 1991, 1999). It may thus be tenable that at least some specific part of the antidepressant effect of NRIs may be mediated by their effect on serotonergic systems.

A fairly exclusive action of antidepressant drugs that selectively enhance central noradrenergic function is their acute stimulatory effect on central dopaminergic function. Thus, in contrast to an SSRI, given in a clinically relevant dose, reboxetine strongly enhances extracellular levels of DA in the mPFC (figure 14), and also increases burst firing of mesolimbocortical DA neurons (figure 11). Notably, these effects are shared by other NRIs than reboxetine as well as by α₂ adrenoceptor antagonists (see 5.4). The enhancing effects of these types of antidepressant drugs on central dopaminergic neuronal activity may have bearing on the pharmacological treatment of anhedonia in depression. Thus, it may be suggested that by increasing basal activity, or reactivity, of DA neurons, which normally are activated by rewarding stimuli (see 1.3.3), NRIs may enhance reward related behavior. DA in the prefrontal cortex has been associated with modulation of cognitive activity, and a decrease in cortical dopaminergic tone has been shown to diminish several cognitive functions (see 1.3.3). Importantly, cognitive deficits are often observed in depressed patients (Brown et al. 1994, Landro et al. 2001), and reboxetine, which thus increases cortical DA output, appear to enhance cognitive function in depressed patients significantly more efficiently than an SSRI (Ferguson et al. 2001). The addition of an NRI or an antidepressant drug with α₂ adrenoceptor blocking properties has been shown to result in remission in patients resistant to treatment with an SSRI alone ( Hawley et al. 2001, Rapaport et al. 2001, Carpenter et al. 2002 see Nelson 2000). Tentatively the selective increase in cortical DA output caused by NRIs and their effect on cognitive functioning may represent one important factor in the clinical augmentation by NRIs of SSRI refractory depression.

Investigations in depressed patients frequently reveal a relative metabolic inactivity of the prefrontal cortex (Baxter et al. 1989), i.e. hypofrontality, a marker that is associated with disease severity and psychomotor retardation (Videbech 2000, Kimbrell 2002), and which may normalize following successful antidepressant treatment. Notably, the markers of brain metabolic activity, i.e. glucose utilization or blood flow, may primarily reflect the activity of brain astrocytes, which, in turn, are activated by e.g. regional glutamate release from active neurons (see Magistretto and Pellerin 1999). Astrocytes, which comprise a large fraction of the cells in the brain provide nearby neurons with a stable flow of energy, which the astrocytes may store for future use in the form of glycogen, and they also are active in the removal of several transmitters from the extracellular space (Tsacopoulos and Magistretti 1996, see Laming et al. 2000). The activity and vigor of astrocytes in the forebrain may consequently have bearing on several psychiatric functions, and a reduction in the density of astrocytes in the cortex has been

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observed in depressed subjects (Öngür et al. 1998, Rajkowska et al. 1999), a phenomenon that also may be related to the reported decrease in cortical volume in depression (Lai et al. 2000, Brenner et al. 2002). Interestingly, astrocytes express both serotonergic and noradrenergic receptors and particularly NA has been observed to exert a stimulatory effect on their short- and long-term metabolic activity (Takita et al. 1992, Allaman et al. 2000). Hence, by enhancing cortical noradrenergic tone as well as the extracellular range of actions of NA by noradrenaline reuptake inhibition (Callado and Stamford 2000) an enhanced or stabilized, metabolic activity of brain astrocytes may be achieved during chronic drug treatment. Indeed, drugs enhancing central noradrenergic function have been claimed to be superior in comparison to SSRIs in the treatment of psychomotor retardation in depression (see 2.1.2).

Hypofrontality has also been observed in schizophrenic patients, where it primarily has been associated with negative and cognitive symptoms (Ingvar 1987, Weinberger and Berman 1988, Andreasen 1992, Wolkin et al. 1992, Hazlett et al. 2000, Potkin et al. 2002). Interestingly, addition of α2 adrenoceptors, which enhance cortical dopaminergic and noradrenergic activity, to an ongoing treatment regimen with a classical antipsychotic seems to diminish not only positive but also negative symptoms in schizophrenia (see 5.5). In the present study we show preclinical evidence for that addition of an NRI to a classical antipsychotic may allow for a reduction in the dose needed to achieve an antipsychotic effect (figure 17, see 5.5), which secondarily may reduce the risk for an increase in negative symptoms or EPS. This effect may well be related to the marked increase in cortical DA output caused by the drug combination. Thus, since cortical DA release has been shown to reduce the reactivity of subcortical dopaminergic systems (1.3.2), which in turn may be abnormally activated in schizophrenia (Laruelle et al. 1996, 1999, Breier et al. 1997), as well as in ketamine induced psychosis (Kegeles et al. 2000), a sustained increase in cortical dopaminergic activity may tentatively diminish the level of subcortical postsynaptic D2 receptor blockade required to achieve the antipsychotic effect of classical antipsychotics. In addition, an increase in cortical catecholaminergic function may, during long-term treatment, also positively affect negative and cognitive symptoms directly (see above, Dolan et al. 1995, Friedman 1999, Castner et al. 2000, Abi-Dargham 2002). Indeed, the antipsychotic drug zotepine, which appears to increase prefrontal DA extracellular concentrations by concomitant NA reuptake inhibition and D2 receptor blockade (Rowley et al. 2000), seems to possess an advantageous clinical profile, both with regard to its effect on negative symptoms as well as its low EPS liability (Petit et al. 1996, Kasper et al. 2001). Early clinical studies of the putative beneficial effect of reboxetine as add-on to antipsychotic drugs in the treatment of schizophrenia has been disparate in their outcome (see 5.5, Schutz and Berk 2001, Raedler et al. 2002, see also Whitehead et al. 2002). However, ongoing studies may more clearly indicate whether NRIs actually may enhance the antipsychotic effect of classical neuroleptics, and, moreover, provide evidence to support, or refute, the notion that an increased cortical dopaminergic output per se represents a critical component in the mechanism of action of atypical antipsychotic drugs.
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