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Perspective

Prospects for Improved Antidepressants

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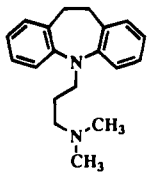
Introduction

Effective drug treatments for depression have been available for more than 3 decades.[1] However, the first generation of tricyclic antidepressants (TCAs) such as the monoamine reuptake inhibitors imipramine (**1**) and amitriptyline (**2**) were associated with anticholinergic and cardiovascular side effects, while the early monoamine oxidase inhibitors (MAOIs) like iproniazid (**3**) and phenelzine (**4**) were plagued by serious and sometimes life-threatening hypertensive crises precipitated by interaction with tyramine-containing foodstuffs.[1] Second-



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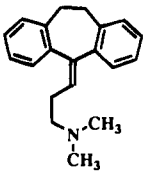

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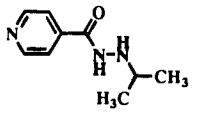

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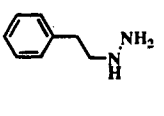

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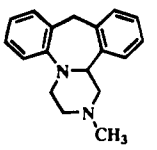
generation antidepressants have tended to be more selective in their pharmacology and atypical in structure and have generally lacked the characteristic side effects of first-generation agents (Table 1). They have increased the likelihood of a clinical response with a reduction in unwanted toxicity.[1] Nevertheless, both the early examples of the genre, such as mianserin (**5**) and trazodone (**6**), and the later contenders, the selective serotonin reuptake inhibitors (SSRIs) and the reversible inhibitors of MAO-A (RIMAs), have brought their own particular pattern of adverse reactions. However, all of



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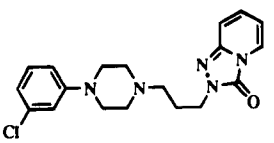

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these drugs have a common mechanism of action in acutely raising synaptic levels of those neurotransmitters chiefly believed to be involved in depressive pathophysiology, *viz.*, norepinephrine (NE) and 5-hydroxytryptamine (5-HT).[1,2] This concept was the basis for the biogenic amine hypothesis for depression, which postulated that deficiencies of monoamines particularly NE and 5-HT were responsible for symptomatology (Figure 1). It remains the inspiration for new drug design, despite the realization that longer-term adaptive changes in receptor sensitivity may better explain the delayed onset of action of all antidepressants.[2] Moreover, no antidepressant is effective in all depressed patients, the usual rule being that *ca.* 70% of patients will respond to some degree, only one-half of whom will experience a full response.

The major advantage of second-generation antidepressants is their relative safety in overdose.[3] Epidemiological surveys of poisonings associated with antidepressant overdose on both sides of the Atlantic Ocean have suggested that first-generation TCAs have a higher incidence of fatalities than second-generation non-TCAs when prescription volumes are compared, whatever the age of the patient, while MAOIs fall in between.[3-6] The TCAs amitriptyline and dothiepin (**7**)

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Table 1. Characteristics of Three Generations of Antidepressants

generation	time of first introduction	examples	efficacy in >70% of patients	onset of action (<2–3 weeks)	side effects: anti-cholinergic	side effects: cardio-vascular	toxic in overdose
first[a]	1958	imipramine, iproniazid	−	−	+	+	+
second[b]	1975	mianserin, trazodone, SSRIs, RIMAs	−	−	−	−	−
third	>2000	none	+	+	−	−	−

[a] Tricyclic antidepressants (inhibitors of monoamine reuptake) and monoamine oxidase inhibitors. [b] Drugs of atypical structure and atypical pharmacology. −, absent; +, present.

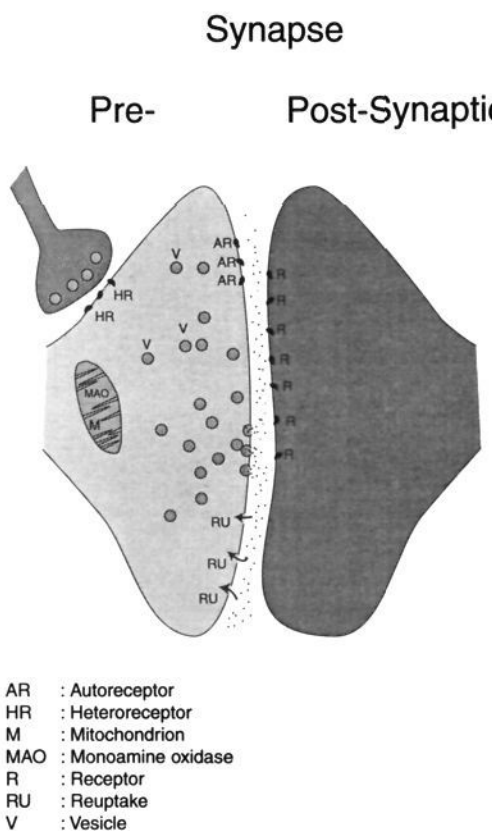
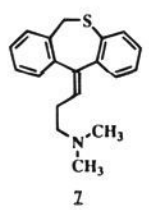


Figure 1. Schematic drawing of a synaptic contact illustrating the role of autoreceptors, heteroreceptors, and postsynaptic receptors in neurotransmission. Antidepressants can acutely raise synaptic levels of neurotransmitters by various mechanisms including inhibition of reuptake or monoamine oxidase and auto- or heteroreceptor antagonism.

appear to be implicated in the majority of deaths and have, together with desipramine (**8**), the highest incidences of fatality. Furthermore, a recent prospective Australian study[7] confirmed that dothiepin was significantly worse than other TCAs in precipitating general seizures and cardiac arrhythmias, the latter being the principal cause of death in antidepressant overdosage.[8] Nevertheless, older TCAs like amitriptyline remain the mainstays of daily practice, probably because of their familiarity and low cost.[4]



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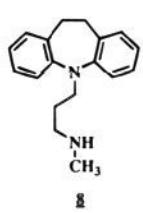

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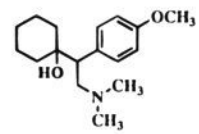
Second-generation antidepressants continue to be developed, and recent introductions include the monoamine reuptake inhibitor venlafaxine (**9**), the RIMA moclobemide (**10**), the trazodone-like 5-HT_2 antagonist and 5-HT reuptake inhibitor nefazodone (**11**), and the SSRIs paroxetine (**12**), sertraline (**13**), and citalopram (**14**). The SSRIs are now being extended with agents such as duloxetine (**15**) which additionally inhibit reuptake of NE and have a TCA-like profile but lack the traditional side effects and toxicity. They are all based upon the same mechanistic theme of enhancing monoaminergic neurotransmission, and it is improbable that any of them will offer advantages over previous examples of the genre in terms of faster onset of action or broader efficacy. There is a need for a third generation of



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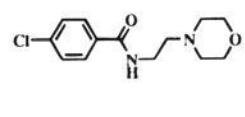

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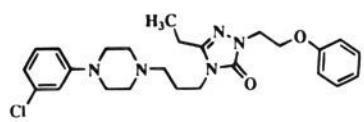

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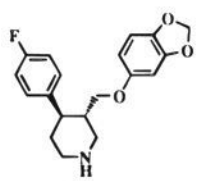

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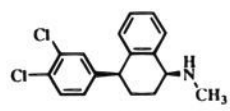

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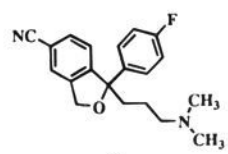

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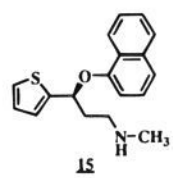

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antidepressants which will retain the gains already made in terms of fewer side effects and lesser toxicity in overdose but will additionally act in the first few days of treatment and/or in a greater proportion of patients (Table 1).[1] Such drugs will help to reduce the enormous economic burden placed upon society by depressive illness. Estimates for the U.K.[9] and the U.S.A.[10] put the total annual costs of depression at £3.5 and $44 billion, respectively. Direct pharmaceutical costs represent a minor proportion of these totals, and the major component of treatment cost is the cost of treatment failure.[11] The largest contributors to the direct costs of depression are in- and out-patient care, while indirect costs generated through lost productivity are even more substantial.[9,10] The overall costs of depression to society

Table 2. Combination Treatment for Depression: Proposals for and Examples of Neurotransmitter Potentiation[a]

desired result	mechanism or target	examples	controlled clinical trials
enhanced neurotransmitter availability	reuptake inhibition/MAO inhibition	TCAs[b]/MAOIs	amitriptyline/tranylcypromine,[19] moclobemide[20]
	NE reuptake inhibition/α_2-antagonism	TCAs/mianserin[c]	imipramine,[22,23] desipramine[23]/mianserin
	α_2-antagonism/MAO inhibition	mianserin[c]/MAOIs	mianserin/phenelzine[21]
	α_2-antagonism/β_2-agonism	mianserin[c]/clenbuterol[d]	
	5-HT reuptake inhibition/ 5-HT supersensitivity	TCAs (SSRIs)/lithium	TCAs/lithium[18]
	5-HT reuptake inhibition/ 5-HT auto (α_2-hetero-) receptor antagonism	SSRIs/methiothepin[d] (mirtazapine)	
	5-HT reuptake inhibition/ 5-HT somatodendritic receptor antagonism	SSRIs/5-HT_{1A} antagonists	
	5-HT auto- (α_2-hetero-) receptor antagonism/MAO inhibition	methiothepin[d] (mirtazapine)/ MAOIs	
	5-HT autoreceptor antagonism/ α_2-heteroceptor antagonism	methiothepin[d]/ mirtazapine	
accelerated downregulation of postsynaptic receptors	β-adrenoceptors	TCAs/clenbuterol[d]	
	β-adrenoceptors	TCAs/α_2-antagonists	imipramine,[22,23] desipramine[23]/mianserin
	5-HT_2 receptors	TCAs/5-HT_2 agonists	

[a] 5-HT = 5-hydroxytryptamine; MAO = monoamine oxidase; NE = norepinephrine; SSRIs = selective serotonin reuptake inhibitors; TCAs = tricyclic antidepressants. [b] SSRIs such as fluoxetine[24] should be avoided because of the risk of a severe serotonin syndrome. [c] Idazoxan, an unproven antidepressant, is more selective. [d] Unproven as antidepressants.

are similar to those estimated for major diseases like coronary heart disease and acquired immune deficiency syndrome (AIDS).[12]

There are currently no third-generation antidepressants (Table 1).[1,13] Until such drugs become available, drug-refractory patients may be treated by judicious use of combinations of antidepressant modalities based rationally upon sound pharmacological principles.[1] The fact that combined therapy can often lead to immediate response in a previously refractory patient suggests that the goal of a single agent is more than ephemeral since there appears to be no absolute mechanistic barrier such as delayed sensitivity changes in relevant receptors.[2] Potential targets for the design of third-generation antidepressants have been identified.[1,13] These include receptor multiplicity, intracellular events beyond the receptor at the level of second messengers and G-proteins, and the hypothalamic–pituitary–adrenal (and thyroid) axis.

There are two major classes of affective disorders in which antidepressants are used, the unipolar types, in which depression is the sole recurring feature, and the bipolar disorders, where patients suffer cycles of depression and mania. Antidepressants and lithium are used to some extent in both classes of disorders but are respectively the drugs of choice for unipolar and bipolar patients. Lithium does have antidepressant as well as antimanic properties, whereas antidepressants do not affect mania except possibly to precipitate it. Some antidepressants are used, however, in various forms of anxiety disorders, including generalized anxiety, phobia, panic, and obsessive-compulsive disorders (OCDs), as well as in atypical depression. Targeted antidepressant therapy for such disorders may become the norm in the future.

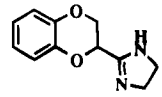
Combination Therapies

The efficacy of first- and second-generation antidepressants is limited even when they are given in full therapeutic doses. In primary care, however, low and inadequate doses are frequently the norm, while many depressed patients receive inappropriate treatment with drugs lacking specific antidepressant properties such as benzodiazepines.[14] Although these are common reasons to suggest treatment failure, there remains a substantial minority of patients who fail to respond to an adequate trial of an antidepressant that is, doses of TCAs like imipramine up to 300 mg daily or the MAOI phenelzine at 90 mg daily for at least 4 weeks, either because the drugs are truly ineffective or because side effects interfere with the therapeutic effect.[15,16] Strategies to deal with refractory or resistant depression are now well established, including augmentation of full doses of other antidepressants with lithium or triiodothyronine.[17] Other useful combinations in such patients include TCAs with MAOIs or neuroleptics and the addition of tryptophan or its 5-hydroxy derivative to a regimen of lithium with MAOIs or clomipramine.

Much of the clinical evidence is anecdotal, double-blind trials are rare, and large placebo-controlled studies are unavailable. While the efficacy of lithium augmentation is relatively well established in a series of small placebo-controlled trials,[18] that of triiodothyronine is not.[15,16] Controlled trials of combinations of TCAs and MAOIs, including recent studies of amitriptyline with the nonselective inhibitor tranylcypromine[19] or the more modern RIMA moclobemide,[20] have shown no added advantage over either antidepressant given alone. Phenelzine also added no extra benefit to mianserin therapy.[21]

The efficacy of combination therapy is entirely explicable in traditional terms of deficiencies of brain monoamines in depression, whereby, for example, lithium or tryptophan further enhance the raised central 5-HT neurotransmission resulting from administration of classical antidepressants.[1,2] However, a far wider range of possibilities exists for neurotransmitter potentiation (see Figure 1), based upon the not mutually exclusive mechanisms of enhanced neurotransmitter availability or accelerated downregulation of postsynaptic receptors, many of which have been demonstrated in experimental animal studies and some of which are only now being tested in clinical situations (Table 2).[1] The recent availability of compounds of a highly selective pharmacological nature with restricted receptor interactions may provide better tests of the concept. Nevertheless, the α_2-antagonist mianserin restored the efficacy of the mixed reuptake inhibitor imipramine in the treatment

of imipramine-resistant melancholia[22] and enhanced the effect of both imipramine and the selective NE reuptake blocker desipramine in poststroke depression.[23] Although the combination with desipramine was less effective than that with imipramine,[23] suggesting an involvement of the 5-HT antagonistic properties of mianserin with the 5-HT reuptake inhibition of imipramine, a proper test of the concept would have been to combine desipramine with the selective α_2-antagonist idazoxan (**16**), a compound of unproven value in depression. Other possibilities for enhancing noradrenergic transmission include combinations of reuptake inhibitors with centrally active β_2-agonists such as clenbuterol (Table 2).



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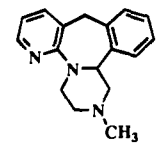
Largely untested potential exists for enhancing serotonergic transmission with 5-HT autoreceptor blockade combined with selective inhibition of 5-HT reuptake or with α_2-antagonists which release 5-HT via their effects upon α_2-heteroceptors located on 5-HT terminals (Table 2).[1,25] Selective 5-$HT_{1D\beta}$ autoreceptor antagonists are not yet available for clinical use, and the best example, methiothepin, has recently been identified as an inverse agonist and additionally blocks dopamine receptors.[25,26] However, the α_2-adrenoceptor antagonist mirtazapine (**17**) is the first example of a clinically effective antidepressant which can release 5-HT by an action upon α_2-heteroceptors.[27] It may also influence both 5-HT and NE neuronal firing activity via antagonism of α_2-somatodendritic receptors.[28] Somatodendritic 5-HT_{1A} receptors, which function to slow down the activity of 5-HT neurons, may also represent a good target. 5-HT_{1A} antagonists given with drugs which release or inhibit the reuptake of 5-HT may be effective combinations, and a pilot study using the β-blocker pindolol, which is not selective but has high affinity for 5-HT_{1A} receptors, has demonstrated rapid improvement of depressed patients refractory to SSRIs.[29]

Certain compounds combine additive or synergistic mechanisms in one molecule and represent interesting drugs if the mechanisms are operational at about the same dose.[1] They include mirtazapine (**17**), rolipram (**18**), ABT-200 (**19**), sercloremine (**20**), and bazinaprine (**21**). Mirtazapine is from the same series of tetracyclic antidepressants as mianserin and setiptiline (**22**) but differs from them in its ability to not only enhance NE release by α_2-adrenoceptor blockade but also enhance 5-HT release by means of antagonism of α_2-heteroceptors,[27] as well as enhancing neuronal cell firing in the locus coeruleus and raphe nuclei via antagonism of α_2-somatodendritic receptors.[28] Although mirtazapine is an effective antidepressant, studies in drug-refractory depression have yet to be performed. Rolipram enhances both presynaptic and postsynaptic effects of NE via phosphodiesterase inhibition, raising central cAMP levels and stimulating tyrosine hydroxylase.[1] Although several double-blind clinical trials have suggested equivalent efficacy and tolerance to standard TCAs, rolipram has not demonstrated superior efficacy to placebo at the doses tested, and development has been stopped. The racemic ABT-200 resembles mianserin in combining NE



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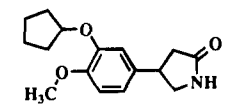

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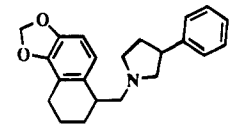

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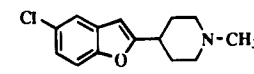

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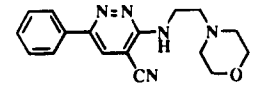

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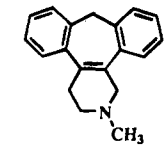

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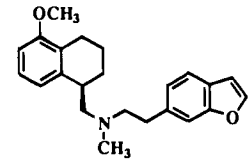
reuptake inhibition and α_2-adrenoceptor antagonism,[30] effects which are selectively possessed by each of the individual enantiomers. Only preliminary phase II data have been published, where the drug appeared to have potential antidepressant effects.[31] A sister compound, A-80426 (**23**), is a potent and selective α_2-antagonist *in vitro* which has additional modest inhibitory effects upon 5-HT reuptake.[32] However, it was ineffective as an α_2-antagonist *in vivo*, lacked efficacy in animal models of depression, and was discontinued. Clinical data have not been reported on either sercloremine, a selective RIMA which inhibits 5-HT reuptake with similar potency, or bazinaprine, which combines RIMA activity with inhibition of dopamine (DA) reuptake.[1] However, brofaromine (**24**), a more potent RIMA than sercloremine but also possessing 5-HT reuptake-inhibiting properties *in vivo*,[1] is an effective antidepressant whose development was recently stopped in phase III.



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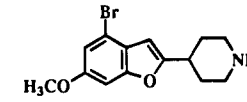

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Receptor Multiplicity

Molecular cloning techniques have allowed characterization of a wide variety of receptors.[33] The largest multigenic family of membrane receptors are those coupled to G-proteins, which are built on the model of the ancient bacteriorhodopsins. Despite a stereotyped structure of seven transmembrane helices and a common mode of action through coupling to an effector system or ion channel via a trimeric GTP-binding protein (Figure 2), such receptors display an extraordinary diversity in being involved in the recognition of signals from *inter alia* biogenic amines, neuropeptides, and protein hormones. The multiplicity of these receptors may provide opportunities to design more selective and perhaps superior drugs for the treatment of depression.[1,13]

α_2-Adrenoceptors. A considerable body of evidence has accumulated to suggest an underlying noradrenergic dysfunction in depression, based not only upon the common effects of many antidepressant treatments but

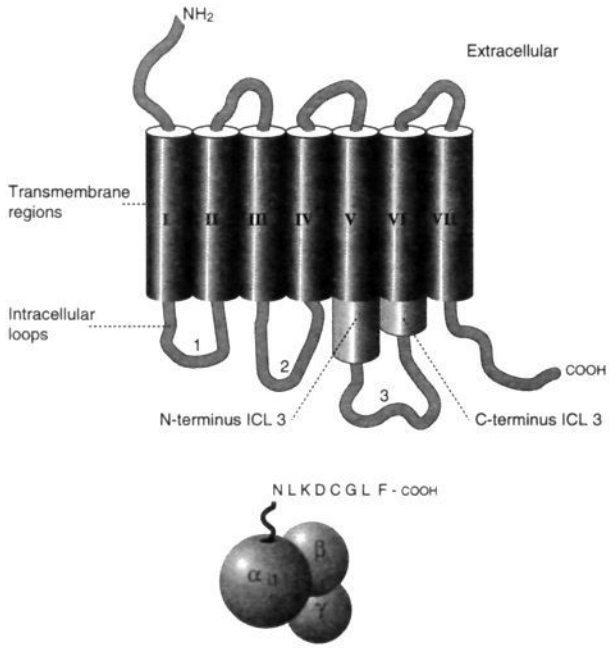
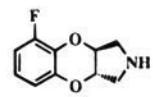


Figure 2. Schematic representation of a G-protein-coupled receptor illustrating the location of intracellular loops and the N- and C-termini of intracellular loop 3 (ICL 3). The α-helical transmembrane regions of the protein are represented as cylinders. The carboxy terminus of the α-subunit is important for the interaction of the G-protein with the receptor. It is not known with which intracellular part of the receptor this interaction occurs. N, asparagine; L, leucine; K, lysine; D, aspartic acid; C, cysteine; G, glycine; F, phenylalanine.

also upon direct studies of adrenoceptor function in blood platelets and during challenge tests with drugs affecting noradrenergic responses.[34,35] Depression seems to be associated with a downregulation of α_2-adrenoceptors. Release of NE in the central nervous system (CNS) involves both exocytotic and carried-mediated mechanisms. Release by exocytosis is autoregulated by presynaptically located α_2-adrenoceptors, where NE acts as an agonist to inhibit its own release (Figure 1).[36] Carrier-mediated NE release occurs via the same membrane transporters responsible for reuptake and can therefore be inhibited by certain antidepressants particularly TCAs.[37] α_2-Adrenergic autoreceptors have been identified in human cortex, and their blockade leads to a situation of raised synaptic NE levels akin to that following reuptake inhibition.[38] Release-inhibiting α_2-adrenergic heteroceptors have also been demonstrated to exist on serotonergic terminals in human cortex, where their blockade facilitates 5-HT release (Figure 1).[39]

The pharmacological prospects for α_2-adrenoceptor antagonists are manifold,[40] not least of which is their potential application as antidepressants in their own right or as adjuncts to traditional inhibitors of monoamine reuptake.[1] However, convincing evidence of antidepressant efficacy exists only for the three tetracyclic α_2-antagonists mianserin (**5**), mirtazapine (**17**), and setiptiline (**22**), all of which block central histamine and 5-HT receptors to a similar or greater degree.[1] Nevertheless, mianserin and mirtazapine have 5- and 30-fold selectivity, respectively, for α_2- over α_1-adrenoceptors, while mirtazapine has a 10-fold selectivity for pre- over postsynaptic α_2-adrenoceptors.[41] Many potent and selective α_2-adrenoceptor antagonists have been synthesized, and two, idazoxan (**16**) and fluparoxan (**25**), have been evaluated in limited clinical trials as antidepressants. Although idazoxan may be equivalent under some circumstances to amitriptyline[42] and bupropion,[43] the published information on its efficacy is unconvincing, and large placebo-controlled trials are required to establish the value of such drugs. They are useful, however, in challenge tests to explore the physiological role of α_2-adrenoceptors in sickness and health.[35]



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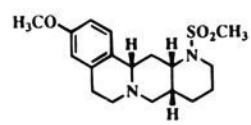
Many of the new selective antagonists, including idazoxan, are imidazoline derivatives which may both facilitate and inhibit NE release by interaction at presynaptic α_2-adrenergic and imidazoline-preferring receptors, respectively.[44] The endogenous agonist for imidazoline receptors appears to be agmatine (decarboxylated arginine), a non-catecholamine ligand at α_2-adrenoceptors which may act as a neurotransmitter in the brain.[45] The net effect upon NE release of imidazolines is uncertain, unlike that of non-imidazolines such as the two recent lipophilic examples RS-15385-197 (**26**) and the quinoline **27**, both of which had nanomolar affinities for central α_2-adrenoceptors.[46,47] Such considerations may also affect the value of putative antidepressants which combine α_2-adrenoceptor antagonism with NE reuptake inhibition; the non-imidazoline ABT-200 (**19**) promotes NE release and is potentially antidepressant in man,[30,31] whereas assorted fenmetazole (**28**) analogues do not and have not been evaluated in depression.[48]



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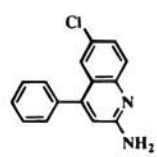

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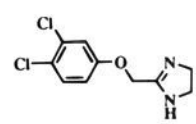

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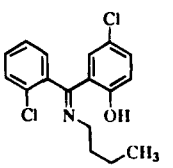
The potential of α_2-adrenoceptor antagonists as antidepressants probably lies in their ability to promote release of NE and/or 5-HT by virtue of their affinity for presynaptic α_2-autoceptors and/or α_2-adrenergic heteroceptors. Both actions may depend upon selectivity for α_2-adrenoceptor subtypes, of which four have been identified pharmacologically including three characterized by molecular cloning.[49-51] At least three genes, on chromosomes 2, 4, and 10, code for α_2-adrenoceptors in man, and three rat genes have been expressed. The human blood platelet, often used as a proxy for central α_2-adrenoceptors, contains prazosin-insensitive α_{2A}-adrenoceptors. Although species and organ heterogeneity exist, there is general agreement that α_2-autoreceptors in the brain are different from the prazosin-sensitive α_{2B}- and α_{2C}-types and probably belong to the prazosin-insensitive α_{2A}- or α_{2D}-families. Pharmacologi-

cal identity, α_{2A} in the rabbit and α_{2D} in the rat, has been claimed for both α_2-autoreceptors and α_2-heteroceptors,[52] while guinea pig cortex contains α_{2D}-autoreceptors.[53] Postsynaptic α_2-adrenoceptors, which represent *ca.* 80% of the total population of α_2-adrenoceptors in rat cortex, are α_{2D} in nature.[54] Cortical α_2-autoreceptors in man may also belong to the α_{2D}-subtype since they are stereoselectively blocked by (+)-mianserin,[38] while the (−)-enantiomer is highly selective toward α_{2A}- rather than α_{2D}-sites in bovine brain.[55] This confounds previous notions that tetracyclic antidepressants were selective for α_{2B}-adrenoceptors[1] and suggests that antagonists selective for α_{2D}-adrenoceptors may have the best chance to be antidepressant via enhancement of NE release. α_2-Adrenoceptor antagonists may also affect neuronal cell firing mediated via the raphe nucleus.[25] Blockade of somatodendritic α_2-autoreceptors and release-modifying terminal autoreceptors enhances release of NE and facilitates 5-HT transmission.

Although pharmacological identity has been claimed for α_2-auto- and -heteroceptors in rat cortex,[52] other studies have suggested that they are different.[38,56-57] In particular the relative affinities of the mianserin enantiomers for the four subtypes[55] suggest that their equipotency in blocking α_2-heteroceptors on 5-HT terminals[38,56] is due to interaction at probable α_{2B}- or, less likely, α_{2A}-sites. Whatever the true nature of the α_2-heteroceptor, the tetracyclic antidepressant mirtazapine, in addition to its ability to release NE via antagonism of α_2-autoreceptors, enhances 5-HT neurotransmission in rats *in vivo*.[27,58] This effect was not seen with mianserin under the same conditions, although mianserin was able to enhance NE neurotransmission.[59] (+)-Mirtazapine exhibited similar potency at the α_2-autoreceptors on NE terminals and at the α_2-heteroceptors on 5-HT terminals, whereas the (−)-enantiomer was selective for α_2-heteroceptors since it blocked the effects of the agonist NE on 5-HT release but not on NE release.[58] This stereoselectivity was also demonstrated electrophysiologically in rat dorsal raphe, where only the racemate and not the (−)-enantiomer was capable of enhancing the firing activities of 5-HT neurons and antagonizing the suppressant effect of clonidine on 5-HT neuronal firing, effects not involving α_2-heteroceptors.[28] This suggests that selective α_2-adrenoceptor antagonists can be developed which will modify NE and/or 5-HT neurotransmission. The α_2-heteroceptor represents an attractive target for modulation of 5-HT neurotransmission,[13,60] and antagonists at this receptor could be useful antidepressants in their own right or as an important element in combination treatment (see Table 2).

Alternative mechanisms to α_2-adrenoceptors for stimulating NE release in cerebral cortex have been identified but have not yet been studied for their potential role in antidepressant action. Thus, the excitatory amino acid *N*-methyl-D-aspartate (NMDA) induces NE release through NMDA receptors located presynaptically on noradrenergic axons.[61] NMDA-stimulated release of NE is modulated by inhibitory presynaptic receptors of the α_2-adrenoceptor[62] and histamine H_3 receptor types.[63] Thyrotropin-releasing hormone (TRH) and a metabolically stable analogue are also able to facilitate cortical NE release.[64] Neither NMDA agonists nor H_3 antagonists are yet known for their antidepressant properties, although TRH analogues possess psychotropic activity but have not been evaluated in depression.[1] However, the NMDA receptor complex has been suggested to play a more fundamental role in the pathophysiology of depression and the action of antidepressants.[65,66] Thus, functional antagonists of the NMDA receptor complex appear to have antidepressant-like properties in animals and downregulate β-adrenoceptors after chronic treatment, while chronic antidepressant treatment reduces glycine and glutamate binding in rat cortex. Further support for a potential role is provided by observations that the downregulating effect of chronic imipramine treatment upon β-adrenoceptors is in part dependent upon unimpaired glutamatergic transmission.[67]

GABA Receptors. Another important mechanism of NE, and other neurotransmitter, release is mediated through γ-aminobutyric acid (GABA) receptors. GABA is the majority inhibitory neurotransmitter in the mammalian CNS. Multiplicity of GABA receptors is restricted to two main types: $GABA_A$, associated with the chloride channel and involved in fast inhibitory signal transmission, and $GABA_B$, which operates in a modulatory fashion.[33,68,69] The $GABA_A$ receptor complex, which consists of a pentameric structure assembled from four possible subunit families, has additional binding sites for, among others, benzodiazepines, barbiturates, and picrotoxin and is the locus of action for anxiolytics, sedative hypnotics, anticonvulsants, and anesthetics. A hypothesis of deficient GABA-ergic transmission in depression enjoyed popularity in the 1970s and 1980s, leading to clinical evaluation of two largely $GABA_A$ agonists, fengabine (**29**) and progabide (**30**). Neither agent proved to be superior to placebo.[70] Although many antidepressants block $GABA_A$ receptors and despite advancement of a $GABA_A$-ergic predominance hypothesis for depression,[71] recent attention has focused upon the $GABA_B$ receptors.



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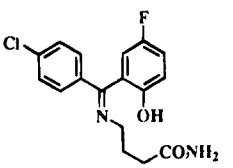

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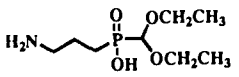
Challenge testing of GABA function in depression has been limited to the $GABA_B$ receptor.[35] The agonist baclofen produced an increase in growth hormone secretion in normal subjects, a response which was unaltered in depression before or during TCA treatment and which is probably mediated via a postsynaptic $GABA_B$ receptor.[72] Depressed patients consistently display a blunted growth hormone response to α_2-adrenoceptor agonists, and it is significant that GABA release is enhanced by presynaptic α_2-heteroceptors[73] and inhibited by similarly located $GABA_B$ autoreceptors.[74] (−)-Baclofen also appeared to worsen the condition of some depressed patients.[75] $GABA_B$ receptors have been classified pharmacologically into two major groups depending upon their relative sensitivity to baclofen, while the baclofen-sensitive group has been further subdivided into three types.[68] Since no $GABA_B$ receptor has yet been cloned, the structural characteristics of the proposed subtypes are unknown. Compel-

ling evidence indicates that a major function of $GABA_B$ receptors is to mediate inhibition of neurotransmitter release, not only of GABA itself but also of the monoamines more familiar in depression such as NE, 5-HT, and DA.[68] $GABA_B$ receptor antagonists should therefore enhance the release of neurotransmitters.[69]

The first $GABA_B$ antagonists were phosphinic acid analogues of baclofen, including phaclofen and 2-hydroxysaclofen, but they were inactive *in vivo*.[69] Penetration of the blood–brain barrier was first demonstrated with a series of (3-aminopropyl)phosphinic acids; GCP35348 (**31**), after systemic administration, antagonized the response to baclofen applied iontophoretically to rat cerebral cortex. The first orally active compound, GCP36742 (**32**), resembled desipramine in its ability to upregulate rat cortical $GABA_B$ receptors upon multiple dosage.[76] However, both of these compounds are relatively weak antagonists whose affinity for the receptor has been vastly amplified by amino substitution, as exemplified by the selective and potent antagonists GCP52432 (**33**) and GCP54626(**34**). Both compounds have nanomolar potency in antagonizing baclofen-induced inhibition of GABA release in rat cerebral cortex and are orally effective.[69] **33** seems to be highly selective toward the $GABA_B$ autoreceptor subtype, while **31** is more inclined to the $GABA_B$ heteroceptor mediating inhibition of glutamate release, but both are equally effective against the third-type mediating inhibition of somatostatin release.[68] Antagonists selective for those $GABA_B$ receptors mediating inhibition of NE and/or 5-HT release will have considerable interest as a novel type of putative antidepressant.[13,69]



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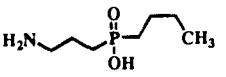

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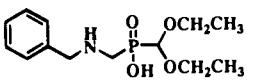

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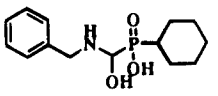

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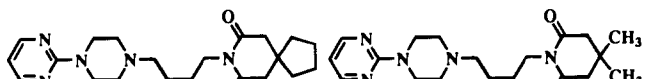
Serotonin Receptors. At least 14 serotonin receptor subtypes have now been identified.[77] The nomenclature is sometimes confusing since the original $5\text{-}HT_{1C}$ receptor is now regarded as being in the $5\text{-}HT_2$ family ($5\text{-}HT_{2C}$), while the old $5\text{-}HT_2$ or S_2 receptor is now designated $5\text{-}HT_{2A}$. Several serotonin receptors are under scrutiny as targets for drugs for affective disorders. It is paradoxical that in many instances both agonists and antagonists are investigated as antidepressants. With the existing uncertainty about the mechanism of antidepressant effects and the role of serotonin receptor subtypes, research approaches will have to be eclectic and proceed with considerable tolerance for uncertainty.

Foremost in this respect is the $5\text{-}HT_{1A}$ receptor. It is implicated in the therapeutic effects of buspirone (**35**),[78] which is a partial agonist for the $5\text{-}HT_{1A}$ receptor.[79] Although widely used as an antianxiety agent, it does not relieve anxiety immediately but acts only after 1 week or more of treatment. This is similar to the action of antidepressants in anxiety disorders. It may therefore be more appropriate to classify buspirone as an antidepressant, and antidepressant effects have been demonstrated in double-blind clinical trials.[80] Newer azapirone agonists for $5\text{-}HT_{1A}$ receptors, such as gepirone (**36**)[81] and ipsapirone (**37**),[82] are in development as antidepressants. A major obstacle in using the therapeutic efficacy of the azapirones as evidence that activation of $5\text{-}HT_{1A}$ receptors is implicated in antidepressant effects is their common metabolite 1-pyrimidylpiperazine (1-PP), which is a blocker of α_2-adrenoceptors.[83] Following oral administration, buspirone, for example, is biotransformed by 90% to 1-PP after first-pass metabolism by the liver.[84] Essential information on the significance of $5\text{-}HT_{1A}$ receptor stimulation for the treatment of affective disorders should come from highly selective new compounds which are not biotransformed to pharmacologically active metabolites. Such compounds include flesinoxan (**38**), which is investigated in clinical trials in depression,[85] and SR 57746A (**39**), which is active in animal models.[86]



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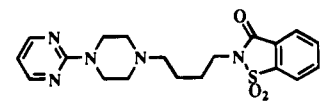

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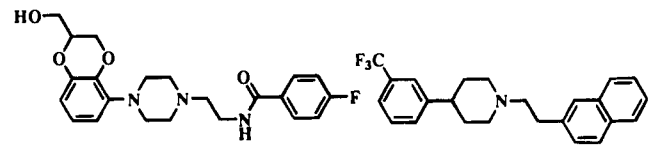

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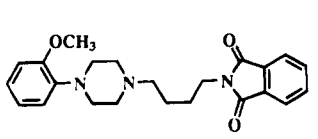
Identification of "silent" (complete) and selective antagonists for the $5\text{-}HT_{1A}$ receptor has been difficult.[87] The location of the receptor in the brain and on the neuron determines its sensitivity for partial agonists. Compounds which are antagonists for the postsynaptic receptors in the hippocampus are often agonists on the autoreceptors in the raphe nuclei.[88,89] A compound such as NAN 190 (**40**) had long been considered as an antagonist but was later shown to have agonistic effects.[90] Interpretation of data obtained in cell lines with expression of receptor clones is even more difficult since the cell responds extremely sensitively to ligands when very high expression is obtained. Even potent antagonists like spiroxatrine can behave as agonists in a cell line with high-expression cloned $5\text{-}HT_{1A}$ receptors.[91] The most recent discovery[92] of a selective full agonist is WAY 100635 (**41**) which is related to the earlier WAY 100135 (**42**). Only **41** has antidepressant-like effects in an animal model, while **42** seems to resemble anxiolytics, but **41** has recently been discontinued owing to lack of oral activity. Two other potential $5\text{-}HT_{1A}$ antagonists are available, SDZ 216-525 (**43**) which has been shown to have partial agonist activities at the somatodendritic $5\text{-}HT_{1A}$ receptor[88] and MM-77 (**44**) which may be a full antagonist at postsynaptic receptors.[93] The demonstration that pindolol enhances the antidepressant effect of SSRIs will give a strong impetus to the search for selective and full $5\text{-}HT_{1A}$ antagonists.[29]



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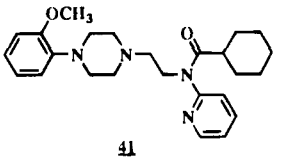

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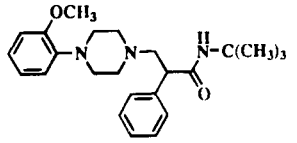

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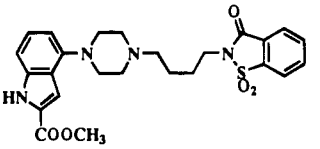

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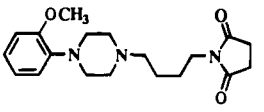

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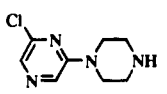
The 5-HT_{2C} receptor is receiving increasing attention as a target for novel antidepressant compounds. Many behavioral effects of SSRIs can be mimicked by direct agonists for the 5-HT_{2C} receptors.[94,95] The oldest 5-HT_{2C} agonist is MK 212 (**45**).[96,97] This compound has been tested in man in preliminary open trials, but its development was not continued because the results were insufficiently convincing. In France, oxaflozane (**46**) is available as a treatment for dysthymia but has not been well studied in major depressive disorders.[98] Oxaflozane



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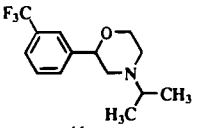

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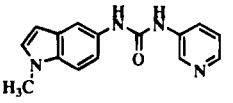
acts as a prodrug for the 5-HT_{2C} agonist [*m*-(trifluoromethyl)phenyl]morpholine which is structurally related to the widely used clinical research tool (*m*-chlorophenyl)piperazine (mCPP). mCPP is a major metabolite of the antidepressant trazodone and has predominantly 5-HT_{2C} agonistic effects. It has been evaluated in an open trial with severely depressed patients where it induced remission of symptoms in four of the six patients.[99] There is a strong need for new and selective agents in order to further evaluate and possibly exploit the therapeutic potential of 5-HT_{2C} agonists. Such compounds may also affect appetite because the appetite-suppressing effect of fenfluramine seems to be mediated by 5-HT_{2C} receptors.[100]

Similarly to the situation with the 5-HT_{1A} receptor, both 5-HT_{2C} receptor agonists and antagonists are under investigation as potential antidepressants.[101,102] The efficacy of SSRIs in depression is in favor of agonists, but a role for antagonists is supported by the 5-HT_{2C} antagonistic effects of mianserin and other nonselective antidepressants. This information is not a sufficient basis to expect therapeutic effects for selective 5-HT_{2C} antagonists. However, early reports that mCPP induces panic attacks in panic disorder patients and that obsessive compulsions are enhanced in OCD patients have suggested that 5-HT_{2C} receptors mediate fear and therefore that antagonists for the 5-HT_{2C} receptor should be beneficial in anxiety disorders.[103] Such expectations have hardly waned despite a wealth of more recent studies showing that mCPP is well tolerated by both healthy individuals and persons with various kinds of psychopathology.[104-106] The first reports on a selective 5-HT_{2C} antagonist, SB 200646A (**47**), have appeared, and information on its therapeutic effects is eagerly awaited.[101]



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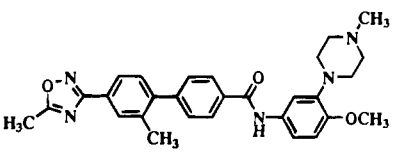
Antagonists for 5-HT_3 receptors have enriched the armamentarium of clinicians who need to give chemotherapy. These compounds are highly effective as antiemetic agents. In addition, animal experiments gave reason to suggest efficacy of 5-HT_3 antagonists in psychiatric disorders such as schizophrenia and anxiety, but no information is yet available on results with such compounds in affective disorders.[107]

Purely on the grounds that 5-$HT_{1D\beta}$ receptors are located presynaptically in the human brain,[108] it should be possible to potentiate serotonergic transmission with blockers for this autoreceptor in analogy with α_2-autoreceptor antagonists like mianserin and mirtazapine. An action at 5-$HT_{1D\beta}$ autoreceptors, particularly when combined with an intrinsic inhibition of 5-HT reuptake in the same molecule, might achieve a more selective and effective enhancement of serotonergic transmission and even to efficacy in drug-refractory patients (see also Combination Therapies and Table 2). Few compounds are available as yet to exploit this receptor for therapeutic purposes, and the best example, methiothepin, is a nonselective antagonist[108] and may even act as an inverse agonist.[26] GRL 127935 (**48**) is a potent and selective 5-$HT_{1D\beta}$ antagonist from a series of benzanilides,[109] but its development as an antidepressant was recently discontinued. An extra compilation is the marked species difference in pharmacology between rat (5-HT_{1B}) and human (5-$HT_{1D\beta}$) receptors, arising from only one amino acid substitution in the otherwise common receptor structure.[110]



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Other receptors for serotonin are at this moment of importance for therapeutic applications unrelated to affective disorders.

Alternatives to Lithium

Lithium is the most commonly used drug for the management of bipolar disorders, both for acute mania and maintenance therapy.[111] It is also effective in the acute treatment of depression, both given alone and particularly when used to augment mainline antidepressant treatment in refractory patients.[18] Although opportunistic clinical strategies have identified anticonvulsants and calcium antagonists as the leading alternatives to lithium,[1] the efficacy of carbamazepine[111] and valproate[112] on the one hand and verapamil[113] on the other has been established in mania rather than depression. Furthermore, substantial efforts to capitalize on the structural and mechanistic variety offered by

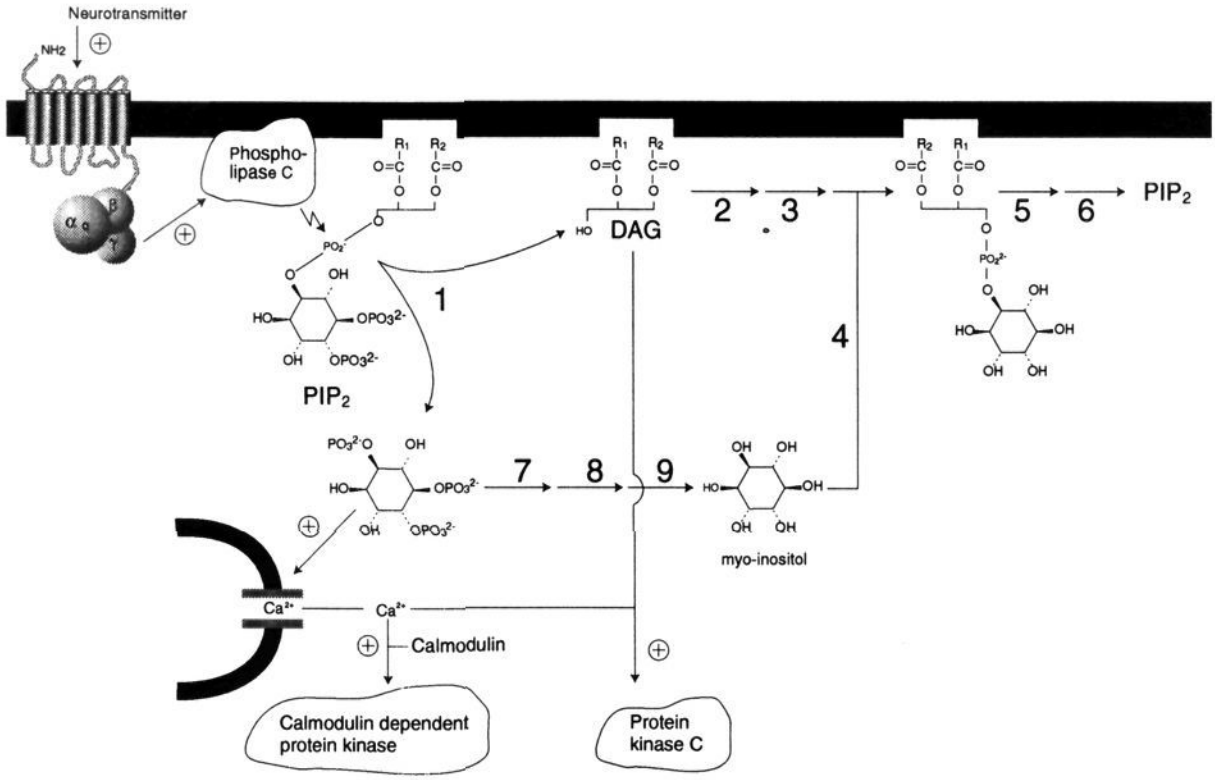


Figure 3. Phosphoinositol cycle. Activation of the receptor enables the catalytic hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP_2) to inositol 1,4,5-triphosphate. The latter molecule induces a release of calcium from intracellular stores, which in turn leads to activation of calmodulin-dependent protein kinase as well as protein kinase C. The latter enzyme is coactivated by diacylglycerol (DAG). In eight enzymatic steps PIP_2 is regenerated. The lithium ion interferes with several of the steps symbolized by 7–9. R_1COOH, arachidonic acid; R_2COOH, stearic acid.

anticonvulsants and calcium antagonists have not been made despite the potential rewards in terms of novel psychotropic, including antidepressant, actions.[1,13] Rather, the wide spectrum of biochemical actions of lithium itself has attracted the attention on the basis that lithium mimetics are a more feasible option.[13] Thus, in addition to its enhancement of 5-HT transmission, supposed to be involved in its augmenting effect upon traditional inhibitors of monoamine reuptake, lithium affects G-protein-regulated phenomena such as receptor-activated phosphatidylinositol and cAMP turnover leading to altered function of interregulated cAMP-dependent protein kinases (Figures 3 and 4).[114–116] Lithium may thus affect not only second-messenger systems but also protein phosphorylation, properties it may share to some extent with classical antidepressants.[117,118] The first putative antidepressant known to affect second-messenger systems was rolipram (**18**), which raised cAMP levels by selective inhibition of calcium-independent phosphodiesterase IV. Although rolipram was never marketed because of insufficient efficacy, its mechanism of action remains a viable target for new antidepressant design,[1] especially in view of the emerging multiplicity of cyclic nucleotide phosphodiesterase isozymes in mammalian brain.[119] Finally, inositol itself, given in high doses, may have antidepressant properties.[120]

Signal Transduction Pathways as Molecular Targets for Putative Antidepressants. Antidepressants may be clinically effective not because they directly influence monoamine availability but because they modulate the converging postsynaptic signals induced by the endogenous ligands.[121] Signal transduction pathways are able to affect the functional balance between neurotransmitter systems and are attractive targets to explain the molecular mechanisms of action of antidepressants. Since prolonged administration of antidepressants is required for obtaining therapeutic responses, neuroadaptive changes are important for mediating the clinical effects of these drugs.[2] Several lines of evidence indicate that neuroadaptation after treatment especially occurs at a level beyond the receptor. Unravelling the functional alterations of components of the intracellular signal transduction pathway may ultimately lead to new biochemical target mechanisms for innovative antidepressants including substitutes for lithium or lithium mimetics. Furthermore, understanding of the biochemical basis for the delayed onset of action of antidepressants may lead to new treatment strategies for faster acting antidepressants.[2]

The largest known family of cell surface signal-transducing proteins is that of the G-protein-coupled receptors (GPRs).[33,122] Extracellular messages received through these membrane-bound receptors are transferred by heterotrimeric (α-, β-, γ-subunits) G-proteins to a variety of intracellular effectors (Figures 3 and 4). Activation by agonists induces a conformational change in the GPRs. As a result, the GDP/GTP exchange at the α-subunit is facilitated, leading to dissociation of α-GTP and $\beta\gamma$-subunits. The intermediate agonist–receptor–$G_{\alpha\beta\gamma}$ (non-guanine nucleotide bound) ternary complex represents the well-known high-affinity state of these GPRs. The dissociated complexes (α-GTP and $\beta\gamma$-subunits) can both regulate the activity of specific effector proteins.[123] Due to the intrinsic GTPase activity

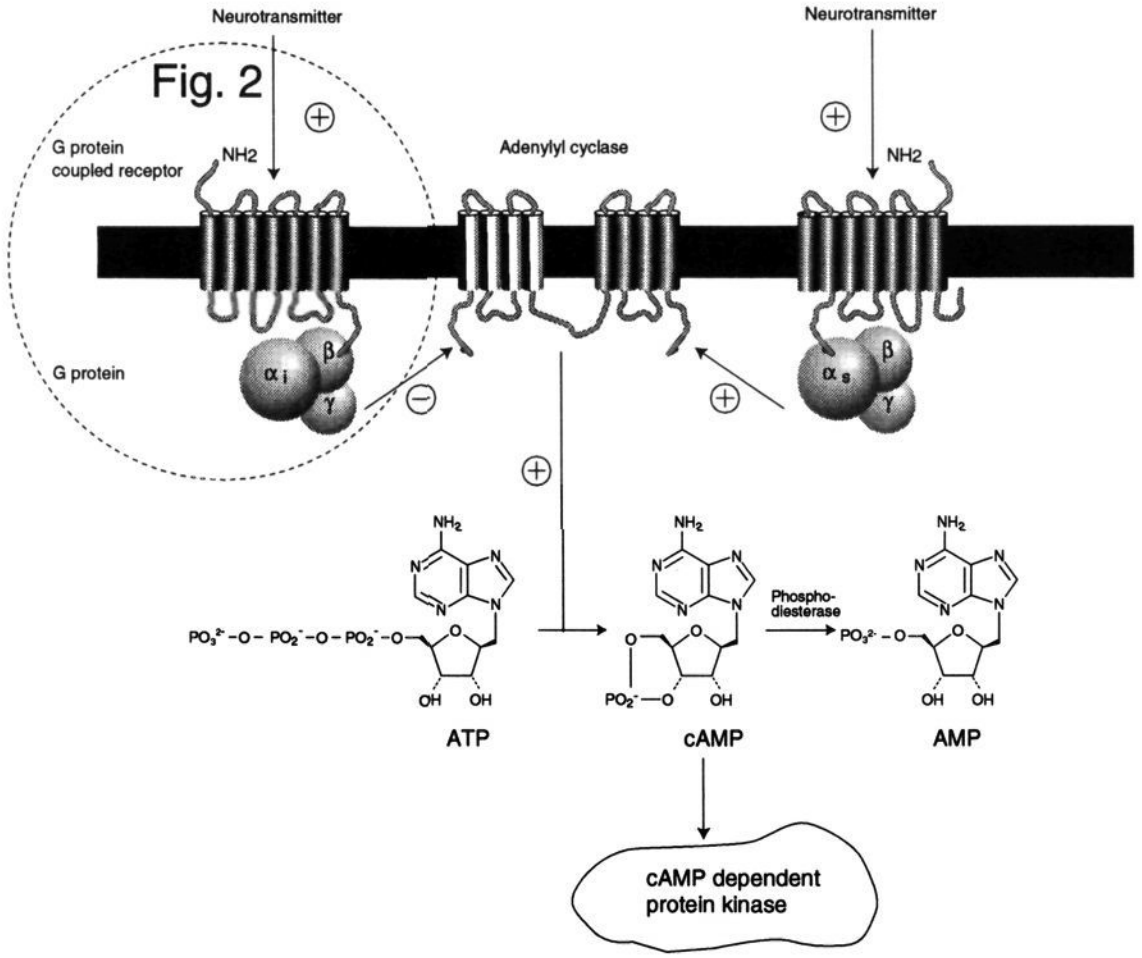


Figure 4. Receptor activation leading to production of cyclic adenosine 3,5-monophosphate (cAMP). Note α_i- (inhibitory) and α_s- (stimulatory) subunits determine inhibition or activation of adenylyl cyclase. The encircled area is enlarged in Figure 2.

of the α-subunit, GTP is hydrolyzed to GDP. As a result α-GDP reassociates with $\beta\gamma$, completing the cycle with the formation of the inactive G-protein. G-proteins are grouped into subfamilies based upon the overall identity of the primary structure of their α-subunits, and the subfamilies have functional correlates in terms of effector interaction: *e.g.*, G_s stimulates adenylyl cyclase (AC), G_i inhibits AC, and G_q stimulates phospholipase C-b.[124] At least 16 isotypes of cDNAs encoding α-subunits have been identified.[125]

Neuroadaptive changes can occur at different levels in the cascade of GPR-mediated signal transduction and at different time intervals. The fastest adaptive change is a reduction of responsiveness of many G-protein-coupled systems after prolonged exposure to agonists, a process known as desensitization. As the currently available antidepressants all enhance monoaminergic neurotransmission (monoamine reuptake or oxidase inhibition), they will indirectly give rise to desensitization. Interestingly, desensitization processes do not occur with nuclear receptors, and steroid treatment does not lead to tolerance.[126] Rapid desensitization involves phosphorylation of receptors which can reduce the coupling of receptors to G-proteins and also promotes binding of proteins such as arrestins to the phosphorylated receptors.[127] Two distinct types of kinases are important: the second-messenger-activated cAMP-dependent protein kinase and protein kinase C (PKC) and the second-messenger-independent GPR kinases, which are specific for agonist-occupied or -activated receptors.[128] cAMP-dependent protein kinases can be activated by specific membrane permeable cAMP analogues, whereas PKC can be activated by phorbol esters and bryostatin-1. Several protein kinase inhibitors are known. They mainly originated from anticancer research, since many oncogenes give abnormal activation of certain protein kinases.[129] The structure of the catalytic subunit of cAMP-dependent protein kinase complexed with MgATP and a 20-residue inhibitor peptide has been resolved.[130]

Inhibitors of the various events of the desensitization process will be valuable tools to study the possible role of desensitization in the mode of action of antidepressants. Furthermore, modulation of the desensitization process by drugs might represent a subtle mechanism to increase neurotransmission by endogenous ligands. Two principal mechanisms can be envisaged.

(a) Inhibition of receptor/effector phosphorylation by kinases and/or subsequent interactions with arrestins. This could be accomplished by disrupting specific protein−protein interactions necessary for the initial interaction/recognition or by inhibiting the phosphorylation at the catalytic domain of these kinases. The homologous desensitization of β_2-adrenergic receptors by β-adrenergic receptor kinase (βARK) can be inhibited by polyanions (heparin and dextran sulfate) and poly-

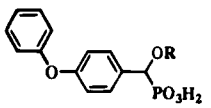
cations (poly(lysine)), as well as by synthetic peptides derived from the intracellular loops of the β_2-adrenoceptor.[131]

(b) Inhibition of the translocation of protein kinases from the cytosolic to membrane cell fractions by interference with the isoprenylation of specific kinases. Inhibition of posttranslational modification has successfully been applied in another therapeutic area, where inhibition of the farnesylation of *ras* proteins has produced potential anticancer agents.[132] Alternatively, translocation may be inhibited by interference with the interaction of specific kinases (βARK1 and -2) with $G_{\beta\gamma}$-subunits. These kinases are not isoprenylated. Their activity is stimulated by $G_{\beta\gamma}$ addition, which promotes βARK association with membranes.[133] A 3D model for the interaction of a putative α-helix at the C-terminus of βARK1 with a two-stranded coil in $G_{\beta\gamma}$ has been postulated.[134]

Apart from desensitization, a slower but more pronounced form of neuroadaptation after antidepressant treatment takes place at the DNA level. There is increasing evidence that the currently available antidepressants affect gene expression. Chronic administration of virtually all antidepressants, regardless of their acute biochemical effects, results in downregulation of 5-HT_2 and/or β_1-adrenergic receptor/effector systems in rat brain, the time course of which parallels the onset of antidepressant action in patients with major depression.[1,13] Antidepressants may exert complex transcriptional and posttranscriptional effects, leading to modulation of receptors and G-proteins and modification of signal transduction. The changes in gene expression are orchestrated, at least in part, by 5-HT, NE, and glucocorticoids.[135] These individual components seem to be linked, resulting in integration of the multiple signals by the cells to regulate gene expression.

Most antidepressants affect G-protein-regulated phenomena such as receptor-activated cAMP and phosphatidylinositol/1,2-diacylglycerol turnover. These second messengers modify the functions of the interregulated cAMP-dependent protein kinase and PKC, leading to altered concentrations of transcription factors. Within the nucleus, these transcription factors interact with promotors on the genes to regulate the initiation of RNA transcription, the first step in the expression of a gene.[121] In nerve terminals, the control of the mRNA of presynaptic receptors has been questioned because there is no retrograde transport for cystolic molecules.[126] Corticosteroids directly interact with nuclear receptors, which belong to the ligand -activated enhancer binding factors. After initial binding to DNA sequences (hormone-response DNA elements) with its C-region, the agonist-bound nuclear receptor activates transcription.[126] In addition, other regulation steps can be modified, such as posttranscriptional processing (RNA splicing and polyadenylation), translation, and posttranslational protein modification (glycosylation, isoprenylation, and phosphorylation).

The impact of chronic antidepressant treatment on the signal transduction pathways, as well as the relevance of disturbances in these intracellular processes concerning the pathophysiology of affective disorders, has gained increasing attention in recent years. In this respect, the receptor/G_s/adenylyl cyclase (AC) system and its intracellular "cross talk" with PKC seem to be especially relevant. Several experimental data point toward this particular system. (1) $G_s\alpha$ levels are elevated in the cerebral cortex from postmortem brains of manic-depressive patients.[136] (2) In lymphocytes of unipolar depressed patients, G_s function as measured with $G_{pp(NH)p}$ binding is sharply reduced, while non-G_s functions are almost unchanged. G_s function is normalized in antidepressant-treated euthymic affective patients. The lymphocytes of manic patients show distinct hyperfunctional G-proteins. Lithium treatment normalizes these functions.[137] (3) The activity of the adenylate cyclase system is significantly decreased in the leukocytes of depressed patients as compared to normal control subjects. This is suggested to be the result of abnormal signal transduction mechanisms and an abnormal G-protein function.[138] (4) *Ex vivo* and *in vitro* studies indicate that components of the signal transduction cascade such as G-proteins, AC, myoinositol-1-phosphatase, and protein phosphorylation appear to be involved in the biochemical effects of lithium.[139-141] Several of these effects are probably due to competition of Li^+ with Mg^{2+} at the level of Mg^{2+}-dependent reactions, as recently demonstrated for human myoinositol-1-phosphatase.[142] Alternative inhibitors, for example, **49** and **50**, for this enzyme already exist, and the availability of its crystal structure will encourage the synthesis of new selective compounds.[13] (5) Desipramine



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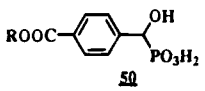

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treatment attenuates AC responses to isoproterenol and GTP in rat cortex. This effect cannot be attributed to desensitization of βARs, as the subsensitivity develops before the decrease in the number of βARs. Intraventricular pretreatment with pertussis toxin reverses these desipramine-induced effects. Therefore, the functional decoupling of the βAR/G_s system in rat cerebral cortex after desipramine administration requires an active G_i-mediated signal transduction system.[143] (6) Acute administration of high concentrations of various antidepressants directly affects *in vitro* the function of G-proteins, especially G_s. These effects were not observed with MAOIs, antipsychotics, or anxiolytics. Functional photoaffinity labeling of G-proteins indicates an increase in B_{max} and K_d of G_s. These effects can be the result of an increased GPTase activity and accelerated dissociation of $G_{\alpha\beta\gamma}$ into G_α and $G_{\beta\gamma}$.[117] (7) In rat brain and cell cultures, chronic, but not acute, exposure to antidepressants produces downregulation of βARs, accompanied by an increase in the ratio of the dissociation constants for the low- and high-affinity states of the βAR. Chronic incubation of cultured cells with desipramine reduces the amount of PKC_α, whereas coincubation with PKC inhibitors attenuates the βAR downregulation. Therefore, the effects of antidepressants on GPR-mediated systems is thought to be mediated via intracellular "cross talk" with PKC.[116,144] (8) Chronic, but not acute, treatment with TCAs, atypical antidepressants, or electroshock significantly increased the AC activity in synaptic membrane preparations from rat cerebral cortex.[118,145] These effects were not due to a change in the amount of the cyclase or G-protein

subunits. Further experimental data suggest that chronic treatment with antidepressants increases the G_s–AC coupling, probably by altering components of the membrane or cytoskeleton.[145] As a result, an enhanced binding of cAMP to the regulatory (R) subunit of the cAMP-dependent protein kinase has been found. As chronic desipramine treatment did not affect the expression of the mRNA of the R subunits (*in situ* hybridization experiments), these effects are thought to be due to translational changes of the enzyme.[118] (9) Studies *ex vivo* reveal regionally specific effects of antidepressants on G-protein α-subunits, as well as on their mRNA levels.[121,146] In the hippocampus most antidepressants decrease the levels of $G_{\alpha s}$ and $G_{\alpha i}$. A coordinated deamplification of again the βAR/G_s/AC system by antidepressants in the hippocampus has been proposed.[144] This might be the mechanistic basis for the increase in the 5-HT_{1A}-mediated responses as measured by electrophysiological techniques in the hippocampus.[25]

Modulation of the activity of G-proteins might be a very attractive concept for innovative antidepressants. Interfering with the receptor/G-protein or G-protein/effector activation by mimicking or blocking these protein–protein interactions could be an alternative for receptor agonists and antagonists. In contrast to the traditional search for selectivity for G-protein-coupled receptors, selectivity for G-proteins or their effector molecules becomes the objective. A complicating factor is the divergency (one receptor can couple to multiple G-proteins) and convergency (multiple receptors can couple to one G-protein) of signaling pathways. In view of potential side effects, this divergency might be a big concern with drugs acting at the G-protein level. We should however realize that the cellular environment highly influences the selectivity for G-proteins, since greater selectivity of receptor–G-protein coupling is found in intact cells than in reconstituted systems. Furthermore brain specific α-, β-, and γ-subunits have been identified. As an example, α_0 is only expressed in neuronal tissue and represents the major fraction of α-subunits in the brain. On the other hand, the divergency offers new prospects to interfere with only one of the pathways downstream from the neurotransmitter/receptor activation.

No compounds based upon these mechanisms are as yet under development as potential antidepressants. However, enormous progress has been made in our understanding at the molecular level of the biochemical events underlying the function of G-proteins. Especially their α-subunits are very well characterized with respect to their molecular structure versus function and their association with other proteins (receptor, βγ-subunit, and effector) and the lipid bilayer.[147] Recently the 3D structure of retinal transducin ($G_{t\alpha}$) in its GDP-bound form as well as bound to GTPγS has been described.[148,149]

It is well known that the receptor/G-protein interaction can be modulated by peptides derived from the C-terminus of the G_α-proteins, specific parts of the intracellular loops of the GPRs, and several amphiphilic peptides. The C-terminus of G_α is one site through which G-proteins interact with their receptors. Inhibition of the GPR/G-protein interaction can be obtained by *inter alia* pertussis-catalyzed ADP ribosylation of the cysteine residue present near the C-terminus,[150] an Arg to Pro mutation in the C-terminus in the *unc* mutant,[151] and antibodies[152] to and synthetic peptides derived from the C-termini.[153] Altered receptor interactions have been observed with α_q/α_{i2}-chimeras in which the C-terminal amino acids have been exchanged.[154] The 3D structure of an 11-amino acid peptide derived from the C-terminus of $G_{t\alpha}$ bound to rhodopsin has been studied with nuclear magnetic resonance.[155] Conformational differences (β-turns) in this peptide bound to the unexcited versus the excited rhodopsin were proposed based upon the results obtained with analogues and NMR studies.[155]

With respect to the GPRs, several extensive molecular studies have focused on their G-protein-binding domains. Four regions appear to be important for interaction with G-proteins and determining specificity at the receptor site: the intracellular loop i2, the N- and C-termini of intracellular loop i3, and a portion of the C-terminal part of the receptor. This is supported by experimental data from deletion, proteolytic cleavage, and mutation studies and experiments with chimeric receptors. The signal transduction of the βAR/G_s system can be blocked by synthetic peptides derived from i2 of the βAR,[156] whereas the dopamine D_2R/G_i system can be uncoupled by synthetic peptides derived from parts of i3 of the D_2R.[157] Coexpression of the i3 fragment of the α_{1B}AR with the complete α_{1B}AR and α_{1C}AR inhibited the inhibition of phospholipase C mediated by these receptors but not by the M1 muscarinic receptor nor of the dopamine D_{1A}-mediated adenylate cyclase activity.[158] The C-terminus of the intracellular loop i3 of the βAR directly activates the G_s-protein as it dose dependently enhances the AC activity and increases the initial rate of $GTP_\gamma S$ binding.[156] Structural determinants for G_{i2} activation have been proposed derived from the sequence of the insulin-like growth factor receptor (GF-IIR), which despite its single transmembrane configuration couples directly to G_{i2}.[159]

Several amphiphilic peptides such as mastoparan, a wasp venom tetradecapeptide, substance P, and their analogues directly activate certain G-proteins by a mechanism similar to that of agonist-activated GPRs.[160,161] Direct activation of G-proteins is not limited to peptides, as amiodarone, used in the therapy of cardiac arrhythmias, directly activates PTX-sensitive G-proteins at therapeutic relevant concentrations.[162]

In addition to the receptor/G-protein interaction, the G-protein/effector interaction can also be modulated.[147] Several effector-activating regions have been identified for the $G_{s\alpha}$/AC and $G_{t\alpha}$/PDE (cGMP-dependent phosphodiesterase) systems.[163,164] The $G_{t\alpha}$/PDE interaction can be blocked by a peptide derived from the γ-subunit of PDE (residues 24–26).[164] A segment of $G_{t\alpha}$ (residues 293–314) activates PDE through binding to the inhibitory γ-subunit of the latter. The question of selectivity has been addressed by studying analogues of this peptide. NMR conformational analysis suggests an α-helical structure to be important for activity.[165]

The peptides described above are used as tools to map the receptor/G-protein or G-protein/effector interface. Besides being potential leads for peptidomimetics, these peptides can also be valuable to set up assays to screen for nonpeptides. Clearly drugs acting at the signal transduction pathway have to cross the plasma membrane. This should not be an insurmountable hurdle,

since several drugs on the market, *e.g.*, lithium, steroids, and xanthines, act intracellularly.

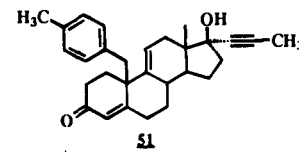
The structure–function relationships of the individual components of G-protein-coupled pathways, together with transcription factors and nuclear receptors, will receive greater attention in the future. As a result more information upon neuroadaptive responses after antidepressant treatment at the molecular level will become available. This type of research will shed new light upon regulation of receptor/effector systems, the potential molecular mechanisms of action of antidepressants, the biochemical basis for their delayed onset of action, and possibly the pathophysiology of depression. It is likely that these insights can be translated into new innovative antidepressants.

Hypothalamic–Pituitary Axis

HPA Axis. Disturbances of the hypothalamic–pituitary–adrenal (HPA) axis are common in patients with endogenous depression, characterized by hypersecretion of cortisol even in the face of dexamethasone suppression but with maintenance of the diurnal rhythm.[166,167] Levels of adrenocorticotropic hormone (ACTH) appear to be unchanged, but patients display a blunted secretion of ACTH in response to corticotropin-releasing hormone or factor (CRH or CRF).[168] Elevated levels of CRH have been demonstrated in the cerebrospinal fluid of patients with endogenous depression,[169] and the number of CRH-binding sites is reduced in the frontal cortex of patients who committed suicide.[170] The current hypothesis to explain the disturbances in the HPA axis in major depressive disorders is that there is a disinhibition of the axis combined with a hypersensitivity of the adrenals, although the precise biochemical mechanisms involved are still a matter for dispute.[166,167] Whatever the precise cause, it is likely that both corticosteroids and CRH are involved in the etiology of depression and therefore could represent novel targets for the design of new antidepressant treatments.

Corticosteroids. Hypercortisolemia may be an important factor in the occurrence of depression. Treatment of depressed patients with antidepressants or electroconvulsive therapy resulted in decreased cortisol levels accompanied by a normalization of dexamethasone suppression.[166,167] The decline in dexamethasone-suppressed cortisol levels usually preceded or coincided with good clinical outcome. Although depressed patients frequently have hypercortisolemia, they do not develop the symptoms of Cushing's syndrome, which is characterized by a persistent elevation of plasma corticosteroids.[166,167] On the other hand, depression is a frequent feature of Cushing's syndrome and is significantly more common in Cushing's syndrome than in other pituitary diseases. Cushing's patients have increased cortisol, normal CRH, and increased or normal ACTH levels. Correction of Cushing's syndrome is usually followed by recovery from depression. Lowering of plasma cortisol levels in Cushing's patients by treatment with inhibitors of corticosteroid synthesis, such as metapyrone, aminoglutethimide, and ketoconazole, reduces the severity of the associated depression.[166,167]

Steroid synthesis inhibition has also been investigated as a therapeutic strategy in the treatment of endogenous depression.[166,167] All of the published studies are based upon open trials or case reports,[171–174] but the consensus view is that such drugs are effective and rapid in action.[166] One of the drawbacks of this strategy is that the enzymes involved in the synthesis of cortisol are identical with those which participate in the biosynthesis of other steroid hormones such as testosterone, progesterone, estradiol, and aldosterone. Those inhibitors used so far—metapyrone, aminoglutethimide, and ketoconazole—are insufficiently selective for the purpose of inhibiting only cortisol synthesis. An alternative approach is to use selective glucocorticoid antagonists. Indeed, initial open trials with the mixed glucocorticoid/progesterone antagonist mifepristone (RU 486) have suggested antidepressant efficacy.[175] Those selective antagonists that have been described, *e.g.*, RU 43044 (**51**), are inactive *in vivo* due to very rapid metabolism.[176] The antidepressant potential of selective glucocorticoid antagonists remains hypothetical.[176,177]



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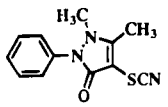
Corticosteroids influence neuronal function by binding to intracellular receptors and subsequent alteration of the genomic action of the target cell.[176] All five classes of steroid receptors—glucocorticoid (GR), mineralocorticoid (MR), estrogen, androgen, and progesterone receptors—are ligand-dependent transcription factors. The GR and MR systems are widely distributed in the brain, and the former may be involved in the feedback action of corticosteroids on stress-activated mechanisms and the HPA axis. There are numerous links between glucocorticoids and monoamine neurotransmitters.[135] Thus, innervations of the hippocampus by midbrain serotonergic and locus ceruleus noradrenergic neurons are under GR control. These projections are inhibited by the glucocorticoid feedback at GR leading to desensitization of 5-HT and NE neurons and receptors. Chronic stress or corticosterone treatment leads to a reduction in hippocampal GR and thereby antagonizes the negative feedback mechanism. Antidepressants seem to regulate GR corticosteroid receptor gene expression which may explain their normalization of HPA axis hyperactivity.[178] On the other hand, they seem to have little effect upon MR gene expression, which has different transcriptional specificity.[179]

CRH. Numerous studies have demonstrated a hypothalamic–limbic system 'overdrive' with excessive secretion of CRH causing heightened HPA axis activity in depression.[166,167] Depressed patients display blunted ACTH responses to CRH, which can be explained by a downregulation of CRH receptors. Both the increased CRH levels and the blunted ACTH responses could be normalized by means of a number of antidepressant treatments.

CRH is an important regulator of the release of ACTH from the pituitary. In addition to its endocrine role, CRH also acts as a neurotransmitter that activates and coordinates endocrine, behavioral, and autonomic responses to stress and fear.[180–182] The secretion of CRH as well as the ACTH responses to CRH levels in man is inhibited by glucocorticoids, while CRH secretion is activated by several biogenic amines especially 5-HT

through 5-HT_{1A} and 5-HT_2 receptors and NE via α_1-adrenoceptors. Animal experiments have shown that the depression-related effects of CRH stem from a central action. Central injections of CRH produced behavioral syndromes which resemble responses that can be observed after confronting an animal with a stressor, and these effects could not be blocked by hypophysectomy or adrenalectomy. There are clear inhibitory effects of the CRH antagonist α-helical CRH_{9-41} and CRH antisera on several responses in stress.[181] The development of selective CRH antagonists would allow direct testing of the hypothesis that CRH hypersecretion is responsible for certain of the cardinal features of depression.[182]

Selective antagonists at central CRH receptors must be sufficiently lipophilic to pass the blood–brain barrier. However, most reported CRH antagonists are CRH-related peptides which cannot penetrate into the brain.[184-186] Recently a number of patents have appeared on small heterocyclic molecules which can block CRH effects, including a series of oxopyrazoline thiocyanates and disulfides (*e.g.*, **52**).[13] These compounds appear to penetrate the blood–brain barrier and have been claimed as antidepressants. No data on their therapeutic efficacy are currently available. The cloned human CRH receptor provides a new tool for the further refinement of selectivity and potency.[187]



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HPT Axis. TRH (thyrotropin-releasing hormone) is released from nerve terminals in the median eminence of the hypothalamus and is transported to the anterior pituitary. In the pituitary it releases TSH (thyrotropin) which activates the thyroid to release L-tetraiodothyronine (T4) and L-triiodothyronine (T3). T4 and T3 feed back at both pituitary and hypothalamic sites to control the secretion of TSH and TRH, respectively.[188] There are three major findings that support a role of the hypothalamic–pituitary–thyroid (HPT) axis in affective disorders. Firstly, patients with thyroid disease, particularly primary hypothyroidism, frequently exhibit prominent depressive symptoms.[188,189] Secondly, many patients with affective disorders have abnormalities in HPT function, most demonstrably shown by a blunted TSH response after TRH administration, which might be explained by a TRH receptor downregulation.[190] Increased concentrations of TRH in the cerebrospinal fluid of depressed patients have been reported by some authors[191,192] but not by others,[193] and there is still considerable controversy about whether both the blunted TSH response and the increased TRH concentrations represent a "state" or "trait" marker. A third argument in favor of a role for the HPT axis in depression is the use of hormones of this axis both to treat depression and to accelerate and/or potentiate the effects of TCAs.[16,188,194]

Prange *et al.*[195] first reported that, in a series of women with unipolar depression, the intravenous injection of TRH caused an antidepressant response that was prompt, partial, and brief. Following this report, many studies on TRH in depression were performed, but the results can at best be described as equivocal. This might be caused by the poor blood–brain barrier penetration of TRH. Analogues of TRH are available which are more metabolically stable and retain psychotropic activity.[1] None of them has been tested in depression, but it is interesting to note that one is able to facilitate cortical NE release in a manner akin to α_2-adrenoceptor antagonists.[64]

TSH has never been used for the treatment of depression. One study, in depressed women, investigated whether a TSH injection before a standard imipramine regimen improved clinical outcome. The patients who received TSH improved much faster than their controls.[194] An explanation for this effect might be the thyroid stimulation caused by TSH. A number of investigations have evaluated the direct effects of T3 on the therapeutic effects of tricyclic antidepressants. Since the first report by Prange *et al.*[196] that T3 accelerated the onset of the antidepressant effects of imipramine in depressed patients, the augmentation by T3 of antidepressant treatment has become standard clinical practice especially in drug-refractory patients.[15,16] The evidence is largely based upon open trials or small numbers in controlled studies, and there are several that have demonstrated addition of T3 to be ineffective.[16]

Future Directions

There is concern about the economic burden placed upon society by depressive illness. The changing health care environment, in which the costs as well as the benefits of drugs will play an important role, necessitates the development of new and more effective antidepressants. The older and cheaper TCAs are still widely used because of their familiarity and low cost, despite their clear overdosage risks and an enhanced awareness of the dangers of suicide by depressed patients. Safer alternatives like second-generation antidepressants have made little impact on improving the effectiveness of treatment although they have reduced the risks. However, the worldwide availability of generic fluoxetine within a few years may tip the balance by providing a safer and equally cheap alternative to TCAs. A further consequence of generic SSRIs may be to limit development of new antidepressants which offer only marginal improvements over those already available and to encourage pharmaceutical companies to go for real innovation. Alternatively, antidepressants may be targeted toward anxiety disorders.

Combination therapy utilizing currently available treatments can be effective in some patients who fail to respond to a single drug, but new agents are needed which will act more rapidly and more effectively. Several targets have been identified for the design of new antidepressants, including variations on the long-running receptor agonist/antagonist theme utilizing the growing multiplicity of subtypes particularly of α_2-, 5-HT, and GABA receptors. Additional targets exist beyond the receptor, particularly at the level of second-messenger and G-protein-coupled phenomena. Effects on gene transcription may be the final common denominator of both future and current antidepressants. The HPA axis remains crucial in depression, and drugs are becoming available to manipulate its function. Whatever the target chosen, it is unlikely that third-genera-

tion antidepressants will be introduced until the next century, when the health care environment may dictate that only such truly innovative products will achieve premium pricing.

Biographies

Chris L. E. Broekkamp received his Ph.D. degree from the medical faculty of the University of Nijmegen and, following postdoctoral work at Nijmegen and Vancouver, British Columbia, joined Synthélabo in Paris as Project Leader in behavioral pharmacology. Since 1983, he has been in the Department of Neuropharmacology at N.V. Organon, Oss.

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Roger M. Pinder received his B.Sc. degree in chemistry from the University of Hull followed by a Ph.D. on the synthesis and pharmacology of adrenoceptor antagonists. After a 2-year postdoctoral fellowship with Professor Alfred Burger at the University of Virginia, Charlottesville, VA, he joined the Chemical Defense Establishment at Porton Down, Wiltshire, to work on hallucinogens and dopamine agonists. In 1974, he moved to New Zealand and became editor of the journal *Drugs*. In 1977, he joined Organon as head, and subsequently manager, of the CNS-active drugs program. From 1988 to 1992, he was Program Manager, Cardiovascular Drugs, and became Director of Research, Coordination Pharma, at the corporate headquarters of Akzo Nobel in Arnhem. In July of 1995, he rejoined Organon as Medical Director CNS.

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