Selectivity of Antidepressants:
From the Monoamine Hypothesis of Depression
to the SSRI Revolution and Beyond

Michael J. Owens, Ph.D.

In 2003, available pharmacotherapy for mood disorders was based almost entirely on observations from the 1950s and 1960s that agents that enhance monoamine transmitter function are effective antidepressants. Preclinical studies show that chronic administration of all effective antidepressants increases the efficiency of post-synaptic 5-HT transmission. Many antidepressants also modify noradrenergic function in the central nervous system. For the majority of antidepressants, these long-term changes in serotonergic and/or noradrenergic function result from direct antagonism of serotonin and/or norepinephrine transporters (also termed "uptake sites"). Pharmacotherapy that is highly selective for one transporter over another has been demonstrated to be effective and tolerable, whereas agents that act on multiple transporters may not necessarily achieve better efficacy and may be associated with additional adverse events. Nevertheless, the rationale is in place to suggest that antidepressants that block both the serotonin and norepinephrine transporters might provide better efficacy, which can only be determined by empirical testing.   *(J Clin Psychiatry 2004;65[suppl 4]:5–10)*

Available pharmacotherapy for mood disorders is based almost entirely on observations from the 1950s and 1960s that agents that enhanced monoamine transmitter function are effective antidepressants. This article briefly reviews the historical role of monoamines in the treatment of affective disorders, with a particular focus on the roles of the neurotransmitters norepinephrine and serotonin. The relevance of affinity and selectivity of antidepressants for the serotonin and norepinephrine transporters is also examined.

EVOLUTION OF NEUROCHEMISTRY

Knowledge of the physiologic roles of specific hormones dates to 1894, when the British physicians George Oliver and Edward Albert Schafer first demonstrated that extracts of the adrenal gland raise blood pressure.[1] Their work led to the first isolation of a hormone, epinephrine, and marked the beginnings of a new field of study that would continue throughout much of the next century. A little more than 25 years later, the German pharmacologist Otto Loewi demonstrated that neurons release these chemical hormones, acetylcholine in this case, and the field of neurochemistry was born.

Norepinephrine

While the eventual discovery of norepinephrine and the elucidation of its role in the central nervous system (CNS) involved the work of many individuals, the efforts of Ulf Svante von Euler (whose contributions to the field of pharmacology led to a Nobel Prize in Medicine) and Peter Holtz were particularly notable. Working independently, von Euler and Holtz demonstrated in the 1940s that peripheral nerves contain norepinephrine, which acts as a neurotransmitter.[2] Several years later, Raymond Perry Ahlquist postulated that there were 2 different types of adrenergic receptors, termed α- and β-adrenoceptors, which predominantly mediated excitatory and inhibitory responses, respectively, in the various experimental tissue preparations,[3] thereby explaining why different sympathetic amines would have different effects. Ahlquist observed that norepinephrine was a potent excitatory catecholamine and generally had a low inhibitory activity on smooth muscle cells, while epinephrine was equally potent as both an excitor and inhibitor of smooth muscle. As a consequence, it was postulated that norepinephrine would be more potent at sites where sympathetic

neurotransmission would be excitory and mediated by α-adrenoceptors, rather than at sites where mediation by β-adrenoceptors would occur.

Much of the scientific establishment remained unconvinced that norepinephrine-containing neurons were present in the brain until the mid 1950s, when Martha Vogt[4] used spectrophotofluorometry to locate norepinephrine in the brain, suggesting that it functioned as a neurotransmitter in the CNS as well as in the peripheral nervous system, as had been demonstrated previously. Arguably, with this discovery modern neurochemistry was launched. The evidence from Vogt has since led to the further classification of adrenoceptors as $\alpha_{1A, B, D}$; $\alpha_{2A, B, C, D}$; and β_1, β_2, and β_3, which in turn led to the development of selective adrenoceptor agonists and antagonists.

Serotonin

Meanwhile, extraction of 5-hydroxytryptamine (5-HT) as serotonin from beef blood[5] and as enteramine from gastrointestinal tract mucosa[6] was accomplished in the 1940s by independent teams in the United States and Italy. In 1952, Erspamer and Asero[6] reported that the chemical identity of serotonin and enteramine was identical. Two years later, Twarog and Page[7] demonstrated that relatively high concentrations of 5-HT were found in the mammalian brain, and their findings were followed by suggestions that serotonin played a role in human behavior (see below).[8]

The hypothesis that serotonin was associated with mood and behavior was enhanced by the observation[9] that the molecular structure of serotonin was similar to that of the psychedelic lysergic acid diethylamide (LSD), which was known to cause effects that could be construed as resembling psychosis. Also intriguing were the observations that reserpine, an antihypertensive agent that depletes monoamine stores (including 5-HT), not only caused depression in some individuals taking the medication for hypertension, but also modified the action of LSD in the brain of laboratory animals by lowering levels of serotonin.[9]

CATECHOLAMINE/MONOAMINE HYPOTHESIS OF DEPRESSION

By the 1960s, initial enthusiasm over the connection of serotonin to mood seemed to wane, while the scientific community, especially in the United States, focused on the relationship between catecholamines and affective disorders. Monoamine oxidase inhibitors, beginning with the antituberculosis agent iproniazid, and tricyclic antidepressants (TCAs), including imipramine, became available in the 1950s and 1960s and were used effectively to treat depression. Interest in the effects of these agents on catecholamine metabolism led Julius Axelrod to conduct a series of experiments to understand the distribution of norepinephrine in the body.

Axelrod, who shared the 1970 Nobel Prize in Medicine with von Euler and Sir Bernard Katz, discovered the mechanism by which most neurotransmitters were released, activated, stored, and recaptured in the brain.[10] When Axelrod[11] injected tritium-labeled norepinephrine into rodents, the radioactive substance accumulated in organs known to have large numbers of sympathetic nerve endings. Yet, when the sympathetic nerve branches were cut and degenerated, norepinephrine no longer collected in those tissues. Axelrod correctly concluded that nerve endings transported norepinephrine back into the nerve terminal via some unidentified transporter or uptake mechanism. Axelrod's research helped explain how the actions of the neurotransmitter were terminated as well as how monoamines were distributed through the body and then recycled.

Additional research in the 1960s by Axelrod and others demonstrated that the TCAs, such as imipramine and amitriptyline, blocked the uptake of norepinephrine in brain tissue.[12] Within several years, it was demonstrated that TCAs blocked serotonin uptake as well.[13]

Despite what was known of serotonin by the mid 1960s, the action of reserpine in causing depression by depleting catecholamines and the reversal of these effects by TCAs formed the basis of the catecholamine hypothesis of depression.[14] That hypothesis, which suggested that depression was due to a deficiency of norepinephrine, diverted the attention of most scientists and drug companies away from serotonin through much of the next decade.

Still, the Swedish researcher Arvid Carlsson noted that clomipramine was more potent in blocking 5-HT than catecholamine uptake[15,16] and promulgated that serotonin transporter antagonists may be antidepressant in their own right. As an aside, Carlsson is perhaps best known for his work on dopamine and its role as a neurotransmitter in normal and pathophysiologic states. He was among the first to suggest that synaptic transmission involved not only electrical signals (via action potentials) but also chemical messengers. As a consequence of his efforts, serotonin and dopamine were recognized as neurotransmitters,[17] and he shared the Nobel prize with Kandel and Greengard in 2000.

Although fluoxetine was first reported in the literature in 1974, zimelidine was the first selective serotonin reuptake inhibitor (SSRI) to make it to the market in the early 1980s. Its efficacy led to the earnest development of many other SSRIs by competing pharmaceutical companies. The efficacy of SSRIs led to a refinement of the monoamine hypothesis of depression to postulate that the biochemical basis of depression was a consequence of a deficiency in one or more of the monoamines norepinephrine, serotonin, and dopamine. This hypothesis is incorrect. Current research suggests that there may be a partial but unclear role for these neurotransmitters in mediating the pathophysiology of depression, but they are clearly essential in mediating the beneficial effects of current antidepressant agents.

Figure. 1. Competition Binding Analysis: Affinities of Sertraline, Citalopram, and Venlafaxine in Binding to the Serotonin Transporter[a]
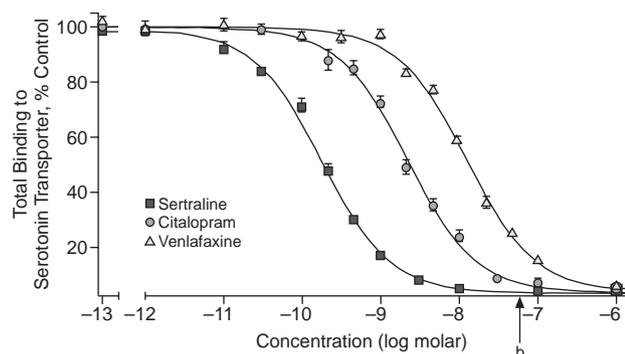
[a]Adapted with permission from Owens et al.[19]
[b]The arrow shows that although each drug has a different affinity for the serotonin transporter, there exists a concentration in which all drugs act to block the serotonin transporter.

Table 1. Potency of SSRIs: Human Monoamine Transporter Binding and Uptake Inhibition[a]

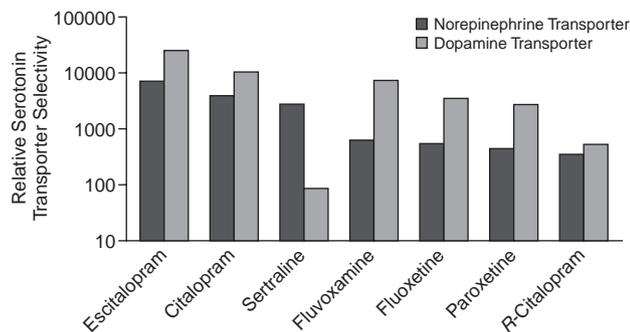| SSRI	Transporter Binding K_i (nmol/L)			Uptake Inhibition K_i (nmol/L)		
	5-HT	NE	DA	5-HT	NE	DA
Escitalopram	1.10	7841	27,410	2.5	6514	> 100,000
Citalopram	1.60	6190	16,540	9.6	5029	> 100,000
Fluoxetine	1.10	599	3764	5.7	599	5960
Fluvoxamine	2.30	1427	16,790	11	1119	32,240
Paroxetine	0.10	45	268	0.34	156	963
Sertraline	0.26	714	22	2.8	925	315

[a]Adapted with permission from Owens et al.[20]
Abbreviations: 5-HT = 5-hydroxytryptamine, DA = dopamine, NE = norepinephrine, SSRI = selective serotonin reuptake inhibitor.

AFFINITY AND SELECTIVITY OF SSRIs

Following the development of zimelidine, a number of pharmaceutical companies began clinically testing compounds that were highly selective for the presynaptic protein known as the serotonin transporter. All SSRIs bind to the serotonin transporter and inhibit the uptake of serotonin into the serotonergic neurons; through this action, continued exposure to SSRIs causes an extended increase in the synaptic availability of serotonin and enhances serotonergic function within the CNS. How this actually results in clinical improvement is not known.

The degree to which an agent binds to a particular transporter or receptor is termed its affinity and is a function of how well the 3-dimensional structure of the receptor and the drug fit together.[18] Affinity, usually defined by the inhibition constant K_i or the dissociation constant K_d, represents the concentration of drug necessary to occupy 50% of the available target sites (receptors or transporters). Affinity is constant for each drug at each particular protein target. Figure 1 demonstrates the affinities of the SSRIs sertraline and citalopram as well as the serotonin and norepinephrine uptake inhibitor venlafaxine for inhibiting binding to the serotonin transporter.[19] In the in vitro competition analysis shown, as drug concentration gradually increases (x-axis), a radiolabeled drug is progressively displaced from the common binding site on the serotonin transporter, represented by the sigmoidal curve on the semi-log scale of the figure. Depending on the concentration, the agents pictured block most of the available serotonin transporters, demonstrating their potential as efficacious antidepressant agents.

The selectivity of a particular agent for a transporter or receptor, which can be determined by comparing the af-finity of a given drug at different targets, contributes both to the therapeutic and side-effect potential of an agent. For example, high affinity for receptors not necessary to achieve therapeutic effect, such as muscarinic and histaminergic receptors, as well as for the serotonin transporter, would suggest that an agent would possess antidepressant activity but also be associated with side effects mediated by the muscarinic and histaminergic systems, such as dry mouth and sedation. Therefore, it is theorized that agents highly selective for the serotonin transporter and lacking affinity for other receptors and ligands would be preferable to those with a more broad range of effects.

Some newer antidepressants that have a high affinity for the serotonin transporter possess affinities for other sites as well, including the norepinephrine or dopamine transporters. A recent analysis of the potency of various SSRIs in binding to human monoamine transporters for serotonin, norepinephrine, and dopamine and inhibiting monoamine uptake[20] demonstrated what has long been known in that the absolute magnitude of selectivity for the serotonin transporter and other monoamine transporters varies considerably (Table 1). Escitalopram, the S-enantiomer of citalopram, is the most selective compound tested, both in terms of affinity for the serotonin transporter in both binding studies and inhibition of serotonin reuptake. By comparison, paroxetine and sertraline show moderate affinity for other transporters in addition to high affinity for the serotonin transporter.[20]

Figure 2 further illustrates the concept of selectivity as measured by the relative affinities of each of the SSRIs for the serotonin transporter compared with the norepinephrine or dopamine transporters.[20] By dividing the affinity (K_i) for the norepinephrine or dopamine transporter by the K_i for the serotonin transporter, it can be seen that all the compounds evaluated are relatively highly selective for serotonin versus norepinephrine and dopamine transporters, although paroxetine and sertraline also exhibit moderate affinity for the norepinephrine and dopamine transporters, respectively. The clinical implications of these binding profiles are the subject of ongoing study but reiterate what is already known from classic pharmacology,

Figure 2. Relative Selectivity in Binding to the Serotonin Transporter Versus the Norepinephrine or Dopamine Transporter[a]

[a]Adapted with permission from Owens et al.[20]

Figure 3. Relative Selectivity of Antidepressants for the Norepinephrine Versus Serotonin Transporter in Binding and Uptake Inhibition[a,b]

[a]Adapted with permission from Vaishnavi et al.[21]
[b]Negative numbers identify compounds that are more selective in uptake inhibition and binding for the serotonin transporter versus the norepinephrine transporter.

that selectivity is dependent upon what specific concentration (or dose) is being examined (see below).

Recent data that include the antidepressants milnacipran and duloxetine, as well as venlafaxine, citalopram, and the TCAs amitriptyline and nortriptyline, also compare uptake inhibition and binding to the serotonin transporter relative to the norepinephrine transporter (Figure 3).[21] Negative numbers identify compounds that are more selective in uptake inhibition and binding for the serotonin transporter versus the norepinephrine transporter. Positive numbers, as with nortriptyline, identify an agent that is more selective for the norepinephrine transporter than for the serotonin transporter.

Agents known to be highly selective in vitro have been shown to be less selective, and even nonselective, in vivo, particularly at higher doses,[22,23] demonstrating that selectivity of an agent for a particular transporter can be relative. As doses are escalated and free drug concentrations increase in serum and brain, even relatively selective drugs can bind, either partially or fully, to other transporters or receptors.[19]

The moderate affinity of paroxetine for the norepinephrine transporter, as well as its selectivity for the serotonin transporter, has been the subject of additional analysis. In vitro studies have demonstrated that paroxetine binds to and inhibits the human norepinephrine transporter in a concentration-dependent manner,[19,20] suggesting that at higher doses or serum concentrations paroxetine may act as a serotonin and norepinephrine reuptake inhibitor, similar to venlafaxine.[23] Using an indirect assay, Gilmor and colleagues[24] recently examined whether paroxetine inhibited the norepinephrine and serotonin transporters in vivo in an open-label study of patients with major depressive disorder, 27 of whom received paroxetine and 9 of whom were given desipramine. They observed that, at doses of 40 mg/day or more, serum paroxetine concentrations may be higher than 100 ng/mL, at which level the agent may be
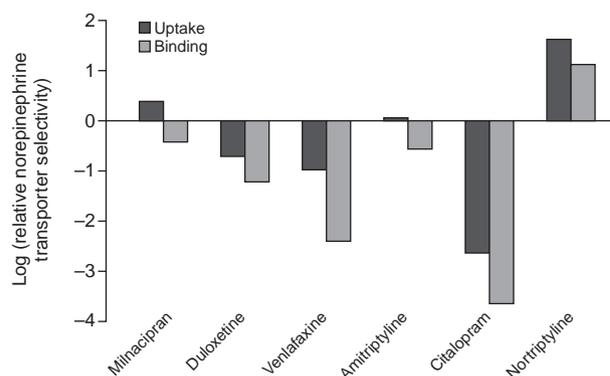
functioning as both a serotonin and norepinephrine uptake inhibitor (Figure 4).

While the serotonin and norepinephrine transporters are different proteins and have different genetic sequences, they do share similarities (homology) with many of the same amino acids found at the same locations on each of the proteins. This distinct but similar protein structure suggests that it would be difficult to make truly selective compounds. Clearly, however, that is not the case, since very small changes in the structure of a compound can lead to notable changes in selectivity, as evidenced by the S- and R-enantiomers of the SSRI citalopram (Figure 5). Moreover, escitalopram, like the parent compound citalopram, binds with high affinity and very high selectivity to the human serotonin transporter. Conversely, its mirror image, R-citalopram, is about 30-fold less potent than escitalopram.

CONCLUSION

The pathophysiology of mood disorders has yet to be elucidated. Nevertheless, chronic administration of all effective pharmacotherapeutic agents, as well as other forms of depression treatment, appear to modify monoamine transmitter function in some way. Research throughout the past century has supported the notion that the pathology of depression may involve dysfunction of monoamine neurotransmission in the central nervous system, particularly of the neurotransmitters serotonin and norepinephrine and possibly dopamine. However, this dysfunction is not simply too little or too much of these monoamines. Manipulating the actions of these monoamines by blockade of the presynaptic transporter proteins, inhibition of monoamine oxidase, or inhibition of pre- or post-synaptic receptors

Figure 4. Relationship Between Norepinephrine and Serotonin Uptake and Concentrations of Paroxetine and Desipramine in Normal Human Serum[a]

Paroxetine

Desipramine
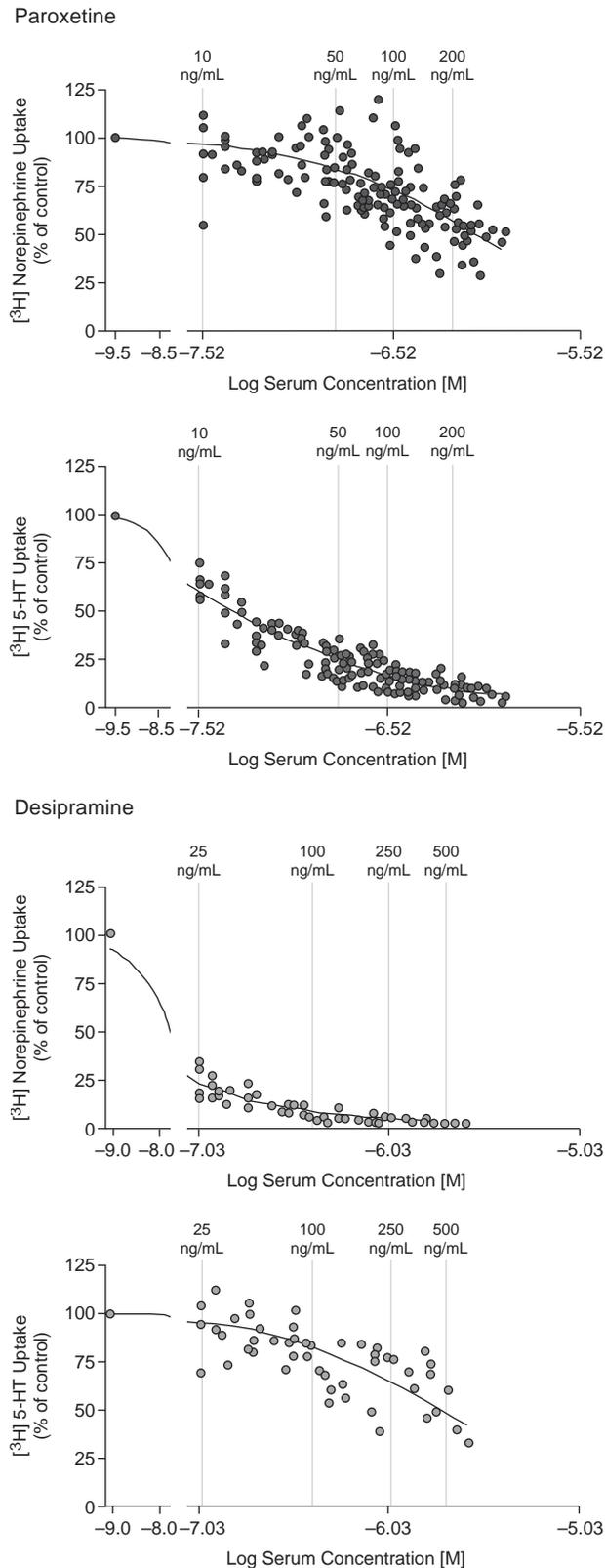
[a]Reprinted with permission from Gilmor et al.[24]

Figure 5. Molecular Structure of the *R*- and *S*-Enantiomers of the SSRI Citalopram[a]

(*S*)-Citalopram   (*R*)-Citalopram
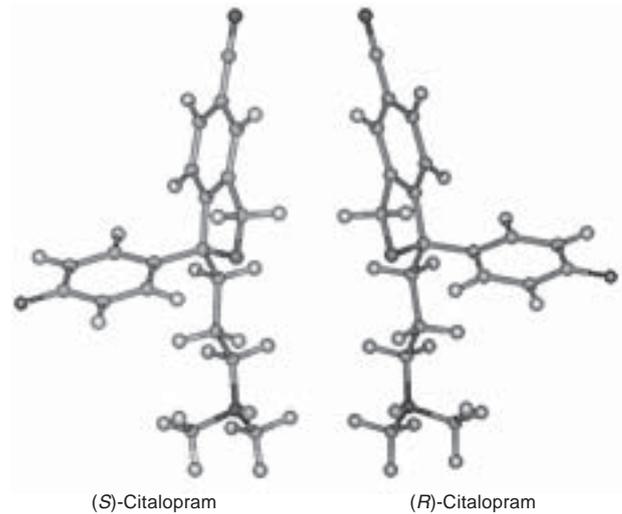
[a]Figure provided by C. Sánchez, Ph.D. (H. Lundbeck, Copenhagen, Denmark).

regulating monoamine neurotransmission have been demonstrated to treat depression successfully.

Current pharmacologic treatments generally are associated with delayed onset of response, despite effects in the CNS that occur almost immediately. Furthermore, there are differential responses among patients to individual antidepressants, with some individuals responding better to a serotonergic agent, others to a noradrenergic one, and still others to an agent that acts on both serotonergic and noradrenergic receptors or transporters. Indeed, newer agents that act on the serotonin and norepinephrine transporters have shown promising results for treatment-refractory patients,[25,26] although these data are preliminary.

Despite unanswered questions as to the biological basis of depression and optimum treatment, SSRIs have proven efficacy across a range of mood and anxiety disorders and are associated with an excellent safety profile.

Drug names: amitriptyline (Elavil and others), citalopram (Celexa and Lexapro), clomipramine (Anafranil and others), desipramine (Norpramin and others), escitalopram (Lexapro), fluoxetine (Prozac), imipramine (Tofranil, Suromontil, and others), nortriptyline (Aventyl and others), paroxetine (Paxil and others), reserpine (Diupres, Serpalan, and others), sertraline (Zoloft), venlafaxine (Effexor).

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