Antidepressants

27

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Abstract

Antidepressants, as the name implies, are drugs used to treat depression and are widely used therapeutic agents. They are also some of the most frequently encountered drugs in forensic and clinical toxicology. Antidepressants are often characterized based on when they were developed: first-generation antidepressants, second-generation antidepressants, selective serotonin reuptake inhibitors, and third-generation antidepressants. All of the antidepressants are well absorbed and reach peak serum concentrations within 2–12 h, but there is considerable first-pass metabolism with most of these drugs. They are rather lipophilic and have large volumes of distribution. In general, these drugs are extensively metabolized by cytochrome P450 isoenzymes to demethylated and hydroxylated metabolites, many of which are active. Analysis of these compounds in biological specimens requires specimen preparation either by liquid–liquid extraction or solid-phase extraction. Chromatographic separation by gas or liquid chromatography allows simultaneous analysis of most antidepressants and their major metabolites. First-generation antidepressants are associated with greater toxicity, primarily due to anticholinergic effects, central nervous system effects, and cardiovascular effects. Interpretation of postmortem concentrations of these drugs is complicated by postmortem redistribution; therefore, the use of therapeutic ranges established for antemortem serum is inappropriate.

Keywords

Antidepressants · Pharmacology · Toxicology · Analysis · Interpretation

Introduction

Depression and schizophrenia are two of the most common and most debilitating mental disorders. Depression is a mood disorder characterized by sadness, depressed mood, inactivity, loss of interest or pleasure, and a reduced ability to enjoy life. Depression in the absence of mania is referred to as unipolar disorder; in the presence of mania, it is bipolar disorder. Schizophrenia is a disorder characterized by abnormal perceptions, delusions, hallucinations, illusions, and, sometimes, bizarre behavior. Complete diagnostic criteria for depression and schizophrenia have been developed and published by the American Psychiatric Association in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5).

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According to the World health Organization, 350 million people worldwide suffer from depression. Consequently, drugs used to treat depression (antidepressants) are widely used therapeutic agents and are the focus of much research and development. They are also some of the most frequently encountered drugs in forensic and clinical toxicology. This is not surprising considering the patient population for which the drugs are designed, the frequency with which these drugs are prescribed, and their high potential for serious side effects, toxicity, and drug–drug interactions. This chapter provides an overview of the pharmacology, toxicology, and analysis of antidepressants.

Pharmacologic Actions

Antidepressants, psychotherapy, and, in some circumstances, electroconvulsive therapy, are the primary treatments for clinical depression. Various compounds classified as antidepressants are also used in the treatment of other disorders or conditions, e.g., obsessive-compulsive disorder, chronic pain, eating disorders, panic disorders, peptic ulcer disease, and childhood enuresis.

The exact mechanism of action of the antidepressants is not entirely understood, although it has been an area of intense research for the last 50 years. A thorough discussion of the topic is beyond the scope of this chapter, but it appears that an increase in monoamine transmission, especially serotonergic transmission, is an essential element.

The earliest (first-generation) antidepressants comprised the tricyclic antidepressants (TCAs) and the monoamine oxidase inhibitors (MAOIs). The TCAs inhibit the reuptake of either norepinephrine (NE), dopamine (DA), or serotonin (5-HT) to varying degrees, and the MAOIs block their metabolism; both mechanisms produce increased amounts of neurotransmitter in the synapse. These observations led to the monoamine hypothesis of depression, which held that a deficit of either NE, DA, or 5-HT at certain sites in the brain was responsible for depression. However, it was recognized early on that the mechanism of action of antidepressant drugs had to be more complicated than merely increasing the concentration of monoamine. There is a well-recognized time delay of several weeks before the therapeutic effect of the first-generation antidepressants emerges, although the inhibition of reuptake or blockage of metabolism is acute. In addition, other compounds (e.g., cocaine) that block the reuptake of neurotransmitters do not function as antidepressants. The detection and characterization of a plethora of 5-HT receptors (5-HT_{1A}, $5\text{-HT}_{1}D$, 5-HT_{2A}, 5-HT_{2C}, 5-HT_{3}, 5-HT_{4}), DA receptors (D1, D2, D3, D4, D5), and NE receptors (α_1, α_2, β_1) located on pre- and postsynaptic neurons have led to many current areas of research and to the development of new antidepressant compounds. Many of the new antidepressants do not effectively block the reuptake of NE or 5-HT or hinder their metabolism. Areas under current investigation concerning the mechanism of action of antidepressants include the direct effects of neurotransmitter binding to a variety of receptors, the subsequent downregulation of receptors, and the possibility that continued use of antidepressants produces adaptations in post-receptor signaling pathways, including regulation of neural gene expression.

Mechanism of Action

Antidepressants are often characterized as first-, second-, and third-generation antidepressants, depending on when they were developed. This discussion will address

- First-generation TCAs
- Second-generation compounds amoxapine, maprotiline, trazodone, and bupropion
- Compounds that selectively block the reuptake of 5-HT, which are referred to collectively as selective serotonin reuptake inhibitors (SSRIs)
- Third-generation compounds venlafaxine, nefazodone, mirtazapine, and duloxetine

Though the definitive mechanism leading to an antidepressant effect remains elusive, the inhi-

bition of the reuptake of 5-HT, DA, or NE is still regarded as an important action and apparently initiates the subsequent antidepressant effect for many drugs.

First-Generation Antidepressants

The structures of the TCAs are presented in Fig. 27.1. They obviously derive their name from the three-ring structure common to all members of this group. Most TCAs affect the reuptake of 5-HT and/or NE, but they are not equal in their action, and some have substantial specificity. In general, the secondary TCAs are relatively selective inhibitors of NE reuptake, while the tertiary TCAs are less selective except for clomipramine, which is a relatively selective inhibitor of 5-HT reuptake. Trimipramine has little or no effect on reuptake of monoamine. Amitriptyline, doxepin, and nortriptyline also have high affinity for and antagonize the 5-HT_{2A} receptor. The TCAs have many other pharmacologic actions that apparently do not contribute to their therapeutic effect but do contribute to the considerable side effect profiles of these drugs. These interactions include the blockade of α_1 adrenoreceptors (hypotension, dizziness, and sedation), H_1 histamine receptors (weight gain and sedation), and M_1 muscarinic receptors (dry mouth, blurred vision, constipation, and urinary retention). Sinus tachycardia and short-term memory impairment may also be a result of M_1 blockade. TCAs are also known to lower the seizure threshold.

Second-Generation Antidepressants

The structures of specific second-generation drugs are presented in Fig. 27.2. Amoxapine and maprotiline have effects on reuptake of monoamines that are similar to the secondary amine TCAs. These two drugs also have antihistamine, anticholinergic, and a1 antagonist properties similar to the TCAs. Trazodone is a weak inhibitor of 5-HT reuptake and has little effect on NE reuptake, but it is a potent antagonist of the 5-HT_{2A} and α_1 receptors. Although it is not an antihistamine, trazodone is quite sedating in vivo. Priapism has been reported to be a risk in patients taking trazodone. Bupropion is unique among the antidepressants in that it has no known effect on the serotonin system. Bupropion blocks the reuptake of NE and DA. This property probably contributes to the use of bupropion in attention-deficit disorder and as an aid to stop smoking. The adrenergic stimulation also probably accounts for the agitation, insomnia, and nausea that have been reported with bupropion. Seizures have also been encountered with bupropion, especially in former dosage units and in doses higher than recommended.

Selective Serotonin Reuptake Inhibitors

The SSRIs have become the most widely prescribed group of antidepressants in the United States; their structures are presented in Fig. 27.3. In addition to inhibiting the reuptake of 5-HT, they interact with a variety of serotonin receptors (5-HT_{1A}, 5-HT_2, and

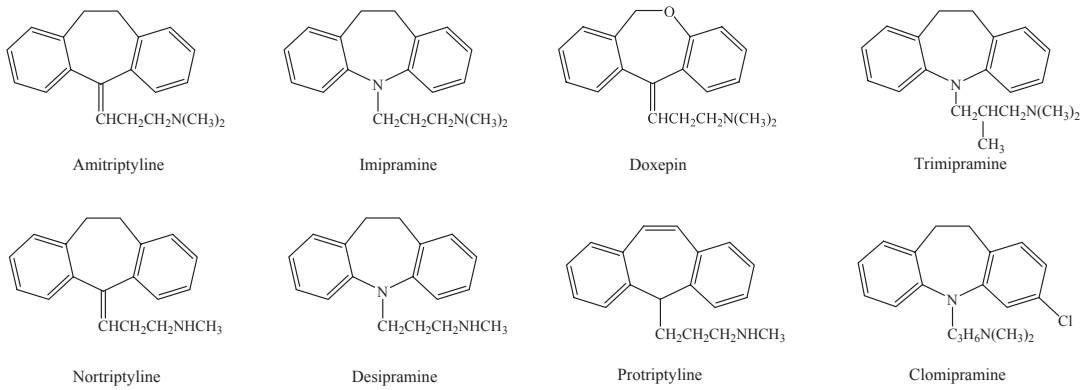

Fig. 27.1 Structures of the tricyclic antidepressants

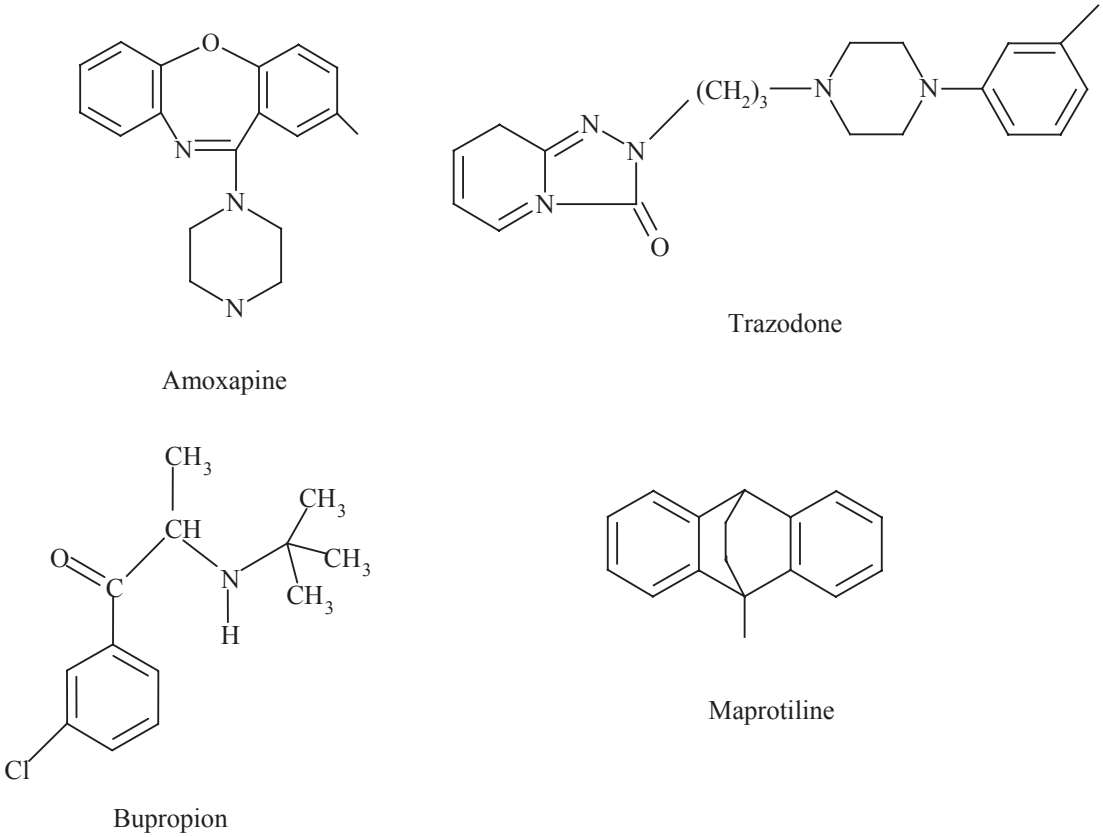

Fig. 27.2 Structures of selected second-generation antidepressants

5-HT_3). The significance of these interactions is not fully understood. These drugs lack the major adrenergic, antihistaminic, and anticholinergic side effects of the TCAs, are generally much better tolerated, and are safer. However, anxiety, sleep disturbances, sexual dysfunction, and insomnia are common side effects. These drugs also have toxicity and proven drug–drug interactions that can be fatal; these topics will be discussed in a subsequent section of this chapter.

Third-Generation Antidepressants

These drugs are a chemically and pharmacologically diverse group of compounds. Their structures are presented in Fig. 27.4.

Venlafaxine blocks the reuptake of 5-HT and NE; it is also a weak inhibitor of dopamine. At low doses, venlafaxine may function primarily as an SSRI. It lacks affinity for H_1, α_1, and M_1 receptors. Common adverse effects include headache, nausea, somnolence, dry mouth, and sexual dysfunction. Sustained hypertension is a potentially dangerous side effect.

Nefazodone is similar in structure to trazodone but has a different pharmacologic profile. It is similar to the SSRIs in blockage of 5-HT reuptake, and it interacts with the 5-HT_2 receptors. However, the 5-HT_2 receptor is blocked with nefazodone and is stimulated with the SSRIs. This may explain the improved profile of nefazodone for anxiety and insomnia as compared to SSRIs, although anxiety, dizziness, and insomnia have been reported as adverse reactions. Nefazodone also interacts with α_1 receptors and weakly inhibits the reuptake of NE.

Mirtazapine has been referred to as a "designer" antidepressant. It is an α_2 antagonist, a 5-HT_2 antagonist, a 5-HT_3 antagonist, and a

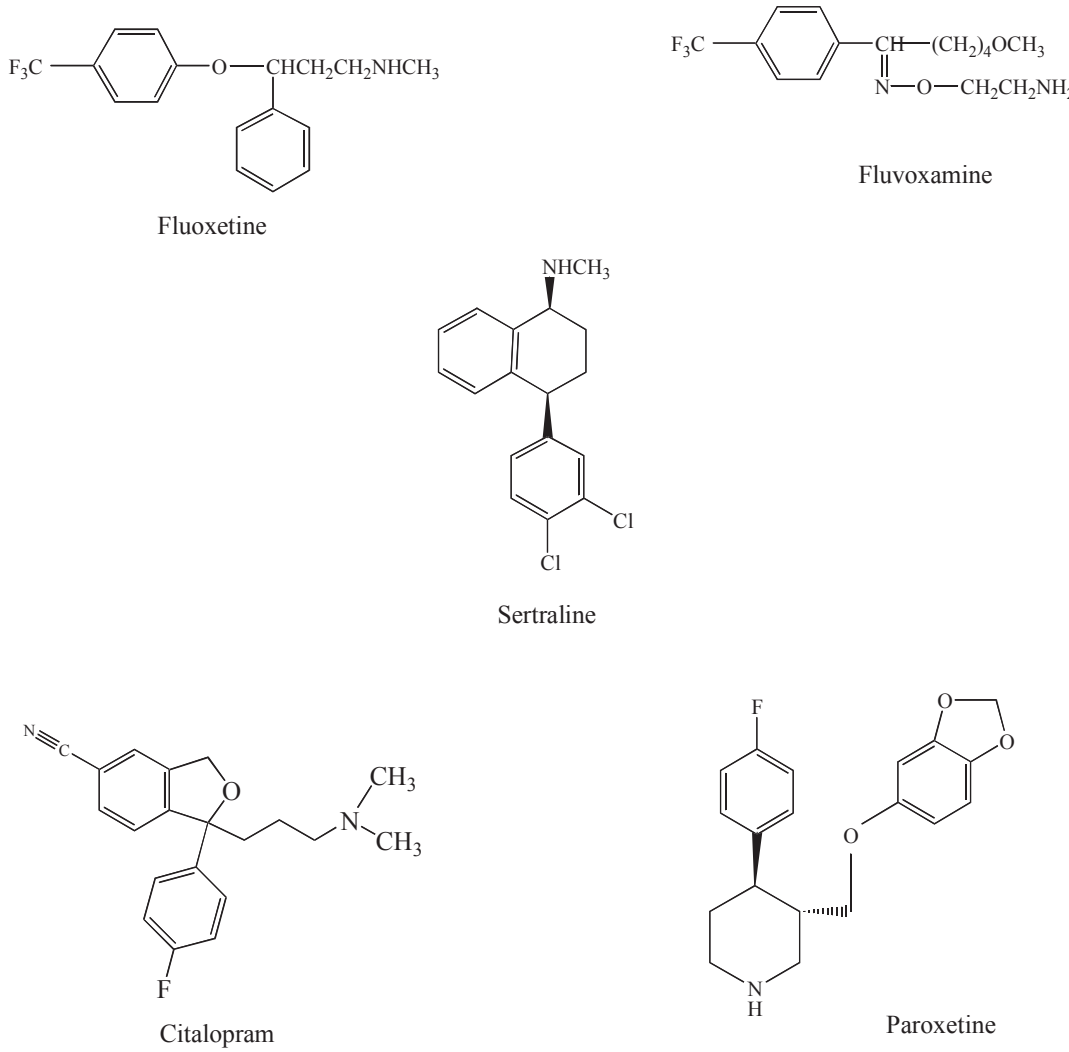

Fig. 27.3 Structures of the selective serotonin reuptake inhibitors

potent H_1 antagonist. Predictable side effects are weight gain and sedation.

Duloxetine was approved as an antidepressant in the United States in 2004. It is a potent inhibitor of both 5-HT and NE reuptake but only weakly affects DA reuptake. Common side effects include drowsiness, nausea, a slight increase in blood pressure, and a slight decrease in heart rate.

Newer Antidepressants

Several newer antidepressants are also discussed below, and their chemical structures are shown in Fig. 27.5.

Vortioxetine is an atypical antidepressant with multimodal activity. It was approved for medical use in the United States in 2013. It functions as an SSRI, a 5-HT_{1A} agonist, a 5-HT_{1B} partial agonist, and an antagonist at 5-HT_{1D}, 5-HT_7, and 5-HT_3. Vortioxetine is reported to have procognitive effects. Side effects include nausea, vomiting, diarrhea, dry mouth, headache, and abnormal dreams.

Vilazodone has dual action as an SSRI and a 5-HT_{1A} partial agonist. It was approved for medical use in the United States in 2011. Side effects include diarrhea, nausea, insomnia, and vomit-

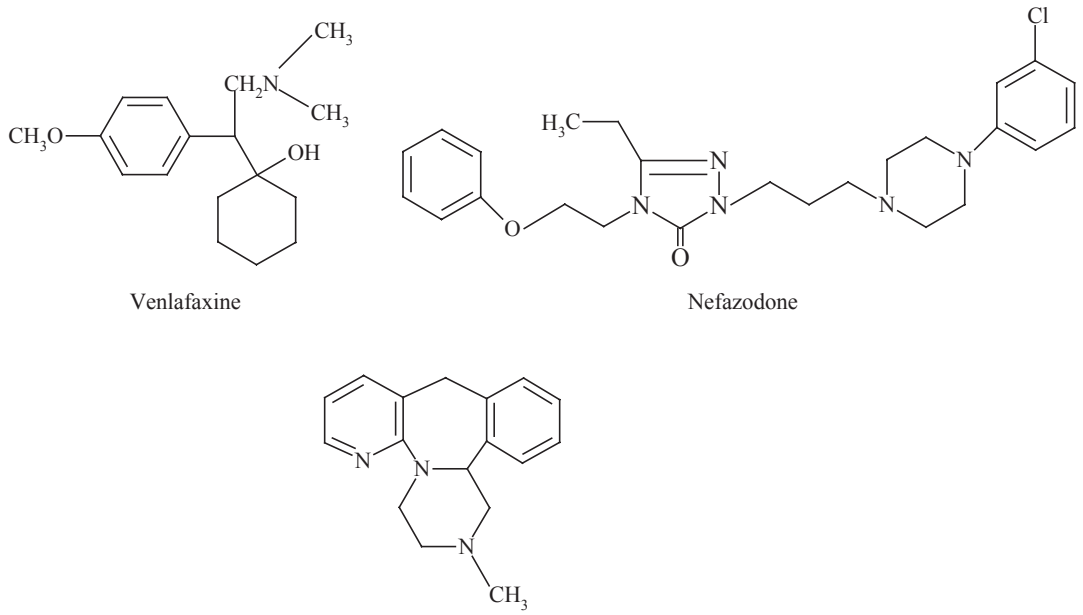

Fig. 27.4 Structure of selected third-generation antidepressants

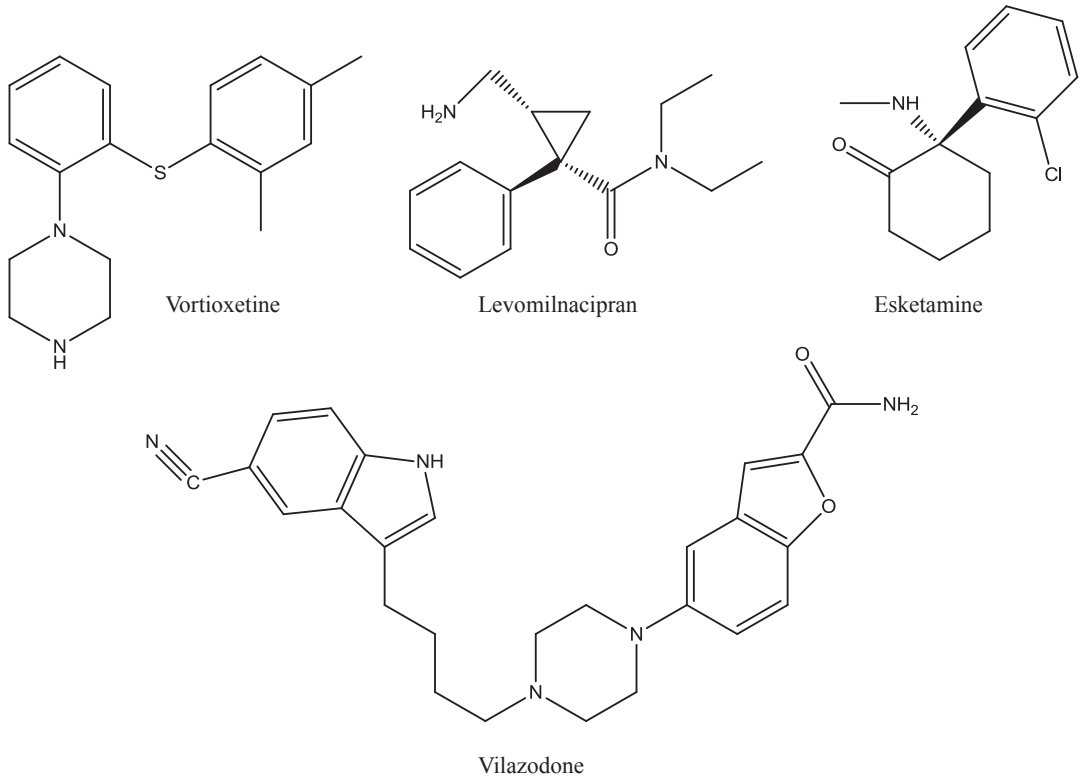

Fig. 27.5 Newer antidepressants

ing. Other adverse effects included dizziness, dry mouth, fatigue, abnormal dreams, decreased libido, arthralgia, and palpitations.

Racemic milnacipran is used to treat depression in many countries. Levomilnacipran was approved for treatment of depression in the United States in 2013. Levomilnacipran (1S, 2R-milnacipran) is the more potent of the enantiomers found in racemic milnacipran. It inhibits the reuptake of serotonin and norepinephrine and has little effect upon other receptors. It appears to be a more potent inhibitor of the NE transporter than the 5-HT transporter. Side effects include anxiety, weakness, dizziness, somnolence, headache, nausea, insomnia, tremor and orthostatic hypotension, tachycardia, and serotonin syndrome.

In 2019, a drug nasal spray formulation of esketamine was approved by the FDA for treatment-resistant major depression. The drug is the S-enantiomer of ketamine, a dissociative anesthetic. It must be used in conjunction with an oral antidepressant. Esketamine is a noncompetitive NMDA receptor antagonist. The exact mechanism of esketamine action is unknown but is under rigorous investigation. Esketamine seems to work quickly to relieve depression and then a second action is to trigger reactions that enable connections in the brain to regrow. Typical doses are 56–84 mg. Side effects require close patient monitoring and include sedation, anxiety, dissociation, abuse and misuse, cognitive impairment, ulcerative or interstitial cystitis.

Many drugs developed for other purposes are being used as adjuncts for treatment of depression. Included are **lurasidone**, aripiprazole, quetiapine, and olanzapine, and brexpiprazole.

Pharmacokinetics

All of the antidepressants are well absorbed and reach peak serum concentrations within 2–12 h, but there is considerable first-pass metabolism with most of these drugs. They are rather lipophilic and have large volumes of distribution. In general, these drugs are extensively metabolized by cytochrome P450 isoenzymes to demethylated and hydroxylated metabolites, many of which are active.

Table 27.1 lists pharmacokinetic properties and the suggested therapeutic ranges of various antidepressants. Several caveats are associated with any such compilation of data. The half-life and volume of distribution for these drugs and their active metabolites are quite variable; average or median values may not be assumed to apply to an individual. The therapeutic ranges for many of these drugs either have not been established or are controversial. The ranges listed in Table 27.1 are taken from a large number of standard references that have different study designs, patient populations, and dosing regimens. However, a growing amount of evidence indicates that a curvilinear relationship exists between serum concentration and efficacy. In many cases, therapeutic effect may be enhanced by lowering the dose of antidepressant and consequently lowering the serum concentration. For the newer antidepressants, the listed "therapeutic ranges" may be more accurately considered as the concentrations observed in early clinical trials.

There is considerable debate about the necessity of performing therapeutic drug monitoring for antidepressants. Critics of routine monitoring claim that the therapeutic ranges are so ill-defined that the expense and effort of determining the serum concentration are unwarranted. Proponents believe that, for many drugs, enough information concerning target concentrations is available to warrant the procedure and that toxicity due to increased serum concentration can be averted. There seems to be consensus that therapeutic ranges are well established for imipramine, desipramine, and nortriptyline.

The metabolism of the TCAs is illustrated in Fig. 27.6, with amitriptyline and imipramine as examples. The hydroxylated metabolites are further metabolized by glucuronidation. As is the case with amitriptyline and imipramine, the metabolism of a parent antidepressant may produce an active metabolite(s). It is common knowledge that any therapeutic monitoring program for these two drugs should include nortriptyline and desipramine; this is also true for other, less well-understood antidepressants.

Table 27.1 Selected pharmacokinetic information for antidepressants

Drug	$t_{1/2}$ (h)	V_d (L\kg)	Active metabolite	Therapeutic range (mg/L)
Amitriptyline	9–46	6.4–36	Nortriptyline	0.11–0.25[a]
Amoxapine	9–14	–	8-Hydroxy amoxapine	0.20–0.60[a]
Bupropion	10–21	27–63	Hydroxy-bupropion	0.025–0.10
Citalopram	25–35	12–16	Desmethylcitalopram	0.04–0.10
Clomipramine	15–62	9–25	Desmethylclomipramine	0.20–0.80[a]
Desipramine	12–28	24–60	–	0.115–0.25
Doxepin	9–25	9–33	Nordoxepin	0.15–0.25
Duloxetine	9–19	20–24	–	0.01–0.2
Fluoxetine	26–220	12–42	Norfluoxetine	0.20–0.90[a]
Fluvoxamine	23	25	–	0.02–0.40
Imipramine	6–28	9–23	Desipramine	0.20–0.35[a]
Maprotiline	27–50	16–32	–	0.20–0.60
Mirtazapine	13.1–33.6	4.5	N-desmethyl mirtazapine	–
Nefazodone	2–5	0.51	M-chlorophenylpiperazine hydroxynefazodone	0.30–0.50
Nortriptyline	18–56	15–23	–	0.05–0.15
Paroxetine	7–37	3–28	–	–
Protriptyline	54–198	15–31	–	0.10–0.20
Sertraline	26	25	Desmethylsertraline	–
Trazodone	6–13	0.8–1.5	M-chlorophenylpiperazine	0.80–1.60
Trimipramine	16–40	17–48	Desmethyltrimipramine	0.10–0.30
Venlafaxine	5	7.5	O-desmethyl venlafaxine	0.25–0.50[a]
Vortioxetine	66	26–34	–	0.004–0.031
Vilazodone	17–36	7–17	–	0.028–0.15
Milnacipran	7–8	3–8	–	0.039–0.157[b]
L-milnacipran	10–16	5.5–6.8	–	0.12–0.39[c]

[a]Total of antidepressant and active metabolite
[b]Trough concentration at steady state
[c]Peak plasma concentration at steady state

There is considerable indication that the hydroxylated metabolites are also active, but these compounds are infrequently incorporated into monitoring programs. In the case of amitriptyline, the 10-hydroxy compounds exist as E and Z isomers. They are difficult to determine without highly targeted procedures.

The metabolism of amoxapine and maprotiline proceeds via demethylation and hydroxylation in a manner analogous to the TCAs (Fig. 27.6). The metabolism of trazodone is depicted in Fig. 27.7. The major active metabolite is m-chlorophenylpiperazine (m-CPP), which has a reported half-life of 4 h or greater. An inactive carboxylic metabolite is also produced but is not considered in most analytical schemes. The metabolites of bupropion that are usually encountered in analytical toxicology are presented in Fig. 27.8. The hydroxybupropion ($t_{1/2}$ = 15–22 h) and threobupropion ($t_{1/2}$ = 9–27 h) are pharmacologically active; erythrobupropion ($t_{1/2}$ = 22–43 h) is inactive. Hydroxybupropion is usually present in the highest concentration after therapeutic dosing, followed by threobupropion and bupropion. Bupropion is reported to be unstable in biological specimens. Suspected cases should be analyzed as quickly as possible or the specimens should be frozen if immediate analysis is not possible.

The SSRIs are extensively metabolized, mostly to inactive metabolites, with very little unchanged drug excreted in the urine. The major metabolic route for the production of active metabolites is demethylation. Fluoxetine, sertraline, and citalopram are metabolized into norfluoxetine, desmethylsertraline, and desmethylcitalopram, respectively. The metabolism of fluoxetine is illustrated in Fig. 27.9. Fluvoxamine and paroxetine have no demethylated or other active metabolites. The half-life of norfluoxetine is quite long: 7–15 days for short-term administration and up to 21 days for patients who have been taking the drug for extended peri-

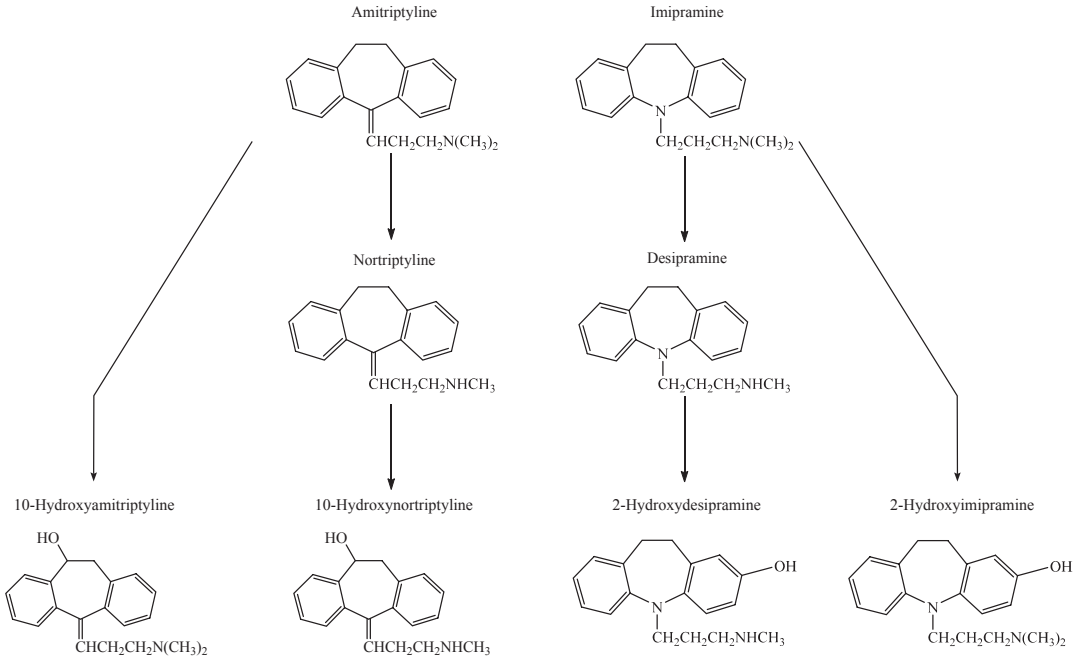

Fig. 27.6 Major metabolic pathways for amitriptyline and imipramine

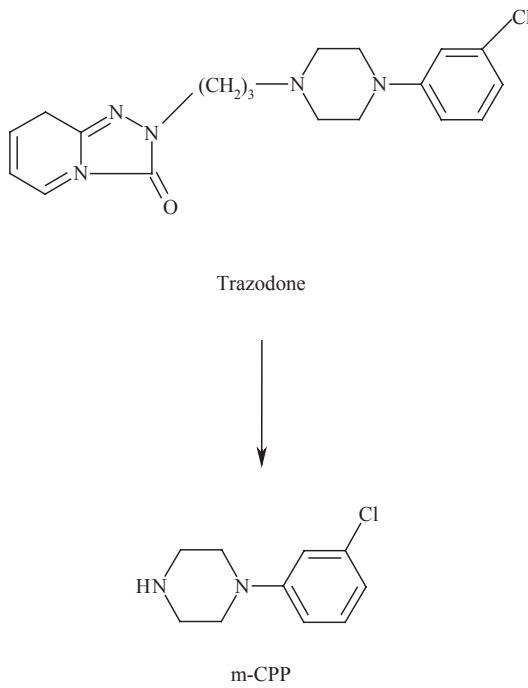

Fig. 27.7 Selected metabolites of trazodone

ods. The extremely long half-life of fluoxetine and norfluoxetine is an important consideration when dose adjustments of fluoxetine are attempted, especially if fluoxetine is discontinued and another antidepressant initiated. Similarly, the half-life of desmethylsertraline is 3–10 days. Therapeutic ranges for the SSRIs have not been established with certainty. In clinical dosing, the total fluoxetine and norfluoxetine concentration is usually less than 1.0 mg/L with a drug/metabolite ratio near unity. Sertraline concentrations are usually 0.1–0.2 mg/L and desmethylsertraline 0.15–0.3 mg/L. Paroxetine and fluvoxamine steady-state concentrations are usually less than 0.2 mg/L. Steady-state citalopram concentrations range from 0.04 to 0.1 mg/L.

The metabolism of venlafaxine is presented in Fig. 27.10. O-desmethylvenlafaxine (ODV) is the major metabolite; it is active and has pharmacologic properties similar to venlafaxine. ODV has a half-life of 11 h and a volume of distribution of 5.7 L/kg. N-desmethylvenlafaxine may have some NE and 5-HT reuptake inhibi-

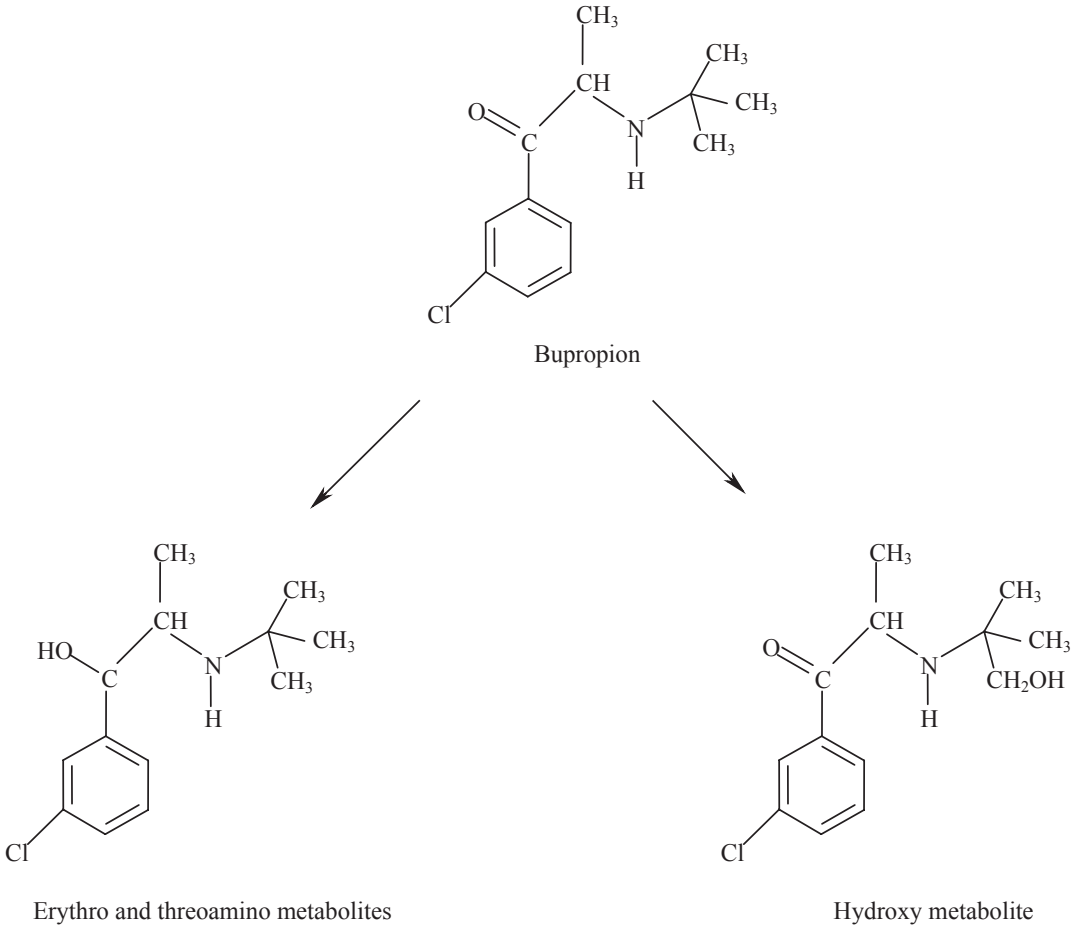

Fig. 27.8 The metabolic pathway for bupropion

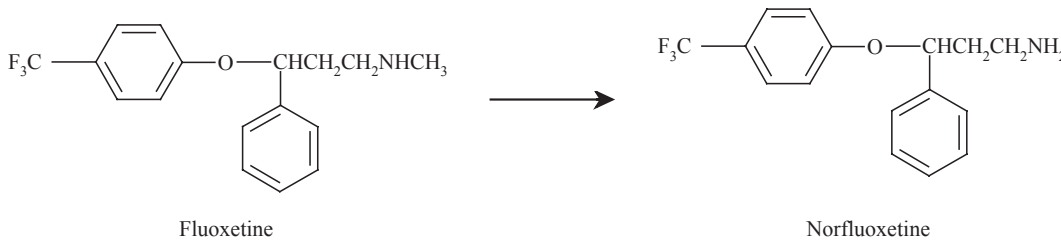

Fig. 27.9 The major metabolic pathway for fluoxetine

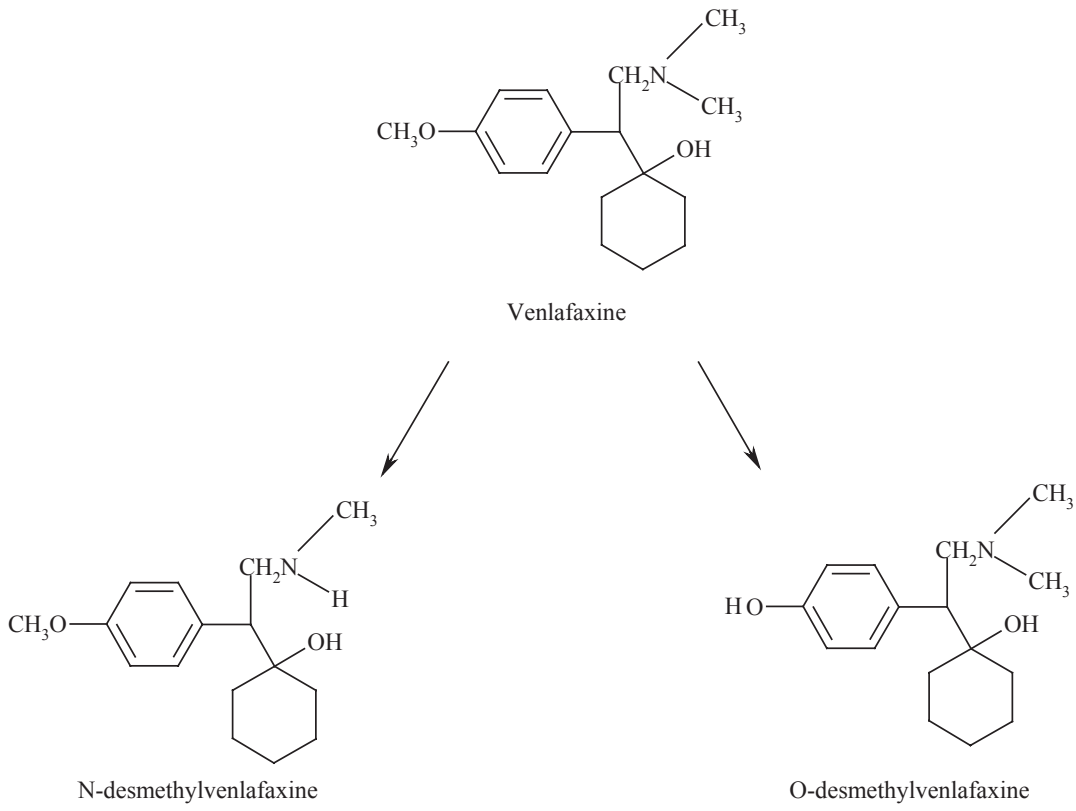

Fig. 27.10 The metabolic pathway for venlafaxine

tion, but it is a minor metabolite. Concentrations of venlafaxine and ODV at steady state have been reported to be near 0.15 and 0.4 mg/L, respectively. Venlafaxine and ODV are approximately 30% bound to plasma proteins; this characteristic is unique among the antidepressants discussed in this chapter. All other antidepressants are extensively bound, usually 90% or more.

Nefazodone is metabolized to three active metabolites, hydroxynefazodone, triazoledione, and m-CPP, as illustrated by Fig. 27.11. The half-lives of hydroxynefazodone, triazoledione, and m-CPP are on the order of 2.5–10.5, 7–12, and 5–9 h, respectively. The concentrations after nefazodone administration are quite variable. Average maximum serum concentrations (ng/mL) after a single 100-mg dose have been reported within these ranges: nefazodone, 0.25–0.4 mg/L; hydroxynefazodone, 0.09–0.11 mg/L; triazoledione, 0.58–0.71 mg/L; and m-CPP, 0.01–0.025 mg/L. When two 300-mg tablets are taken daily, average maximum concentrations have been reported within these ranges: nefazodone, 2.8–3.86 mg/L; hydroxynefazodone 0.8–1.2 mg/L; and m-CPP, 0.07–0.11 mg/L. Nefazodone exhibits nonlinear pharmacokinetics, resulting in greater-than-expected serum concentrations with increasing doses. Plasma concentrations per dose are greater in the elderly, especially elderly women.

Mirtazapine is metabolized to the active metabolite N-desmethylmirtazapine, as illustrated in Fig. 27.12. The N-desmethyl metabolite has pharmacologic activity of one-third to one-fourth that of mirtazapine. Inactive N-oxide and 8-hydroxy metabolites have also been

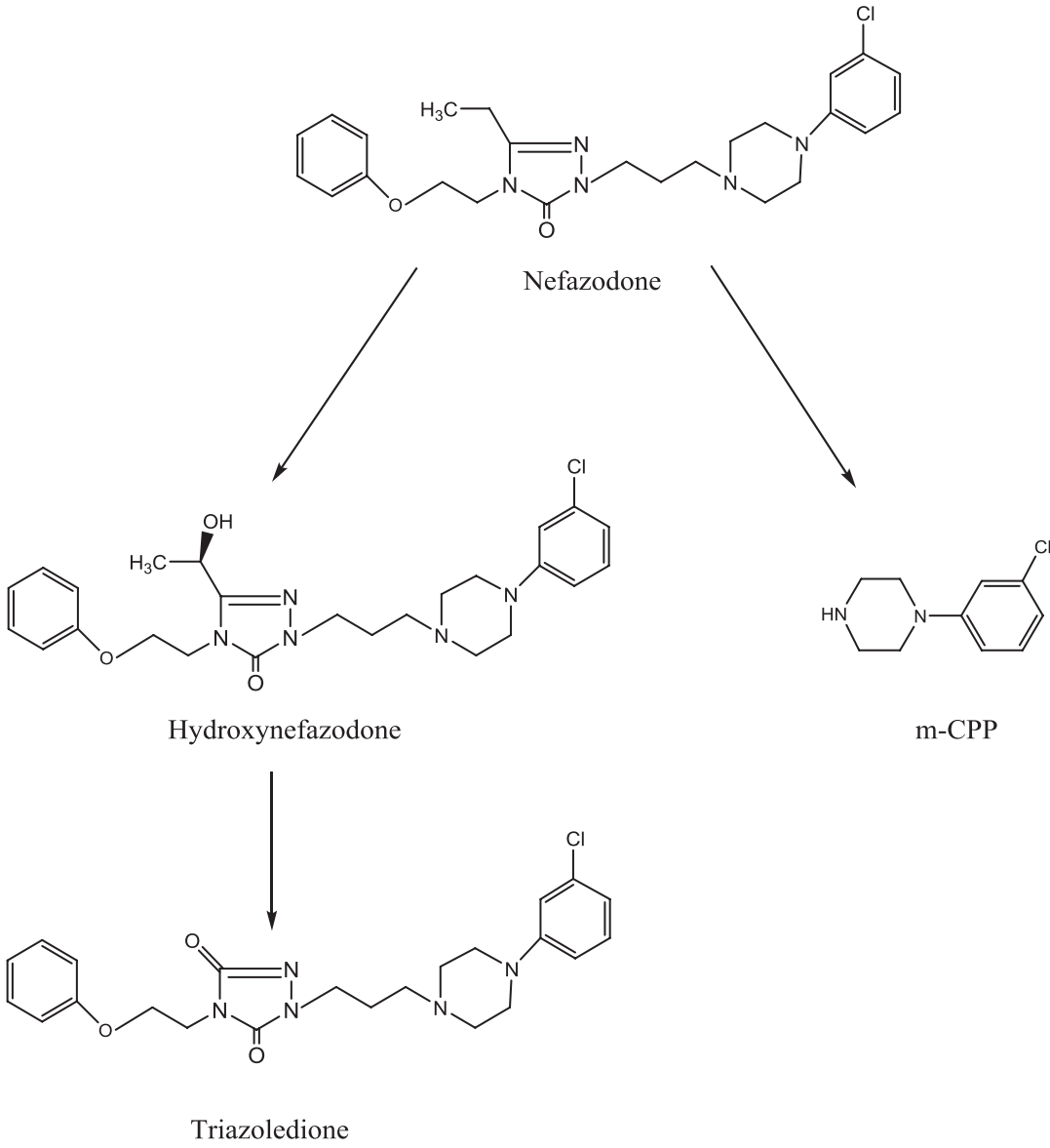

Fig. 27.11 The metabolic pathway for nefazodone

reported. Little pharmacokinetic information has been published to date concerning the metabolites of mirtazapine. The drug itself displays linear kinetics over the usual therapeutic dosing range. Maximum steady-state plasma concentrations are reached within 2–3 h after dose. After a once-per-day 15-mg dose and a once per day 75-mg dose, the concentration of mirtazapine is approximately 0.03 mg/L and 0.15 mg/L, respectively.

Duloxetine is well absorbed orally with peak plasma concentrations occurring 6–10 h after use. Phase I metabolism involves hydroxylation. The primary metabolite in plasma is the glucuronide conjugate of 4-hydroxyduloxetine. Two other conjugated metabolites, 4,6-dihydroxyduloxetine

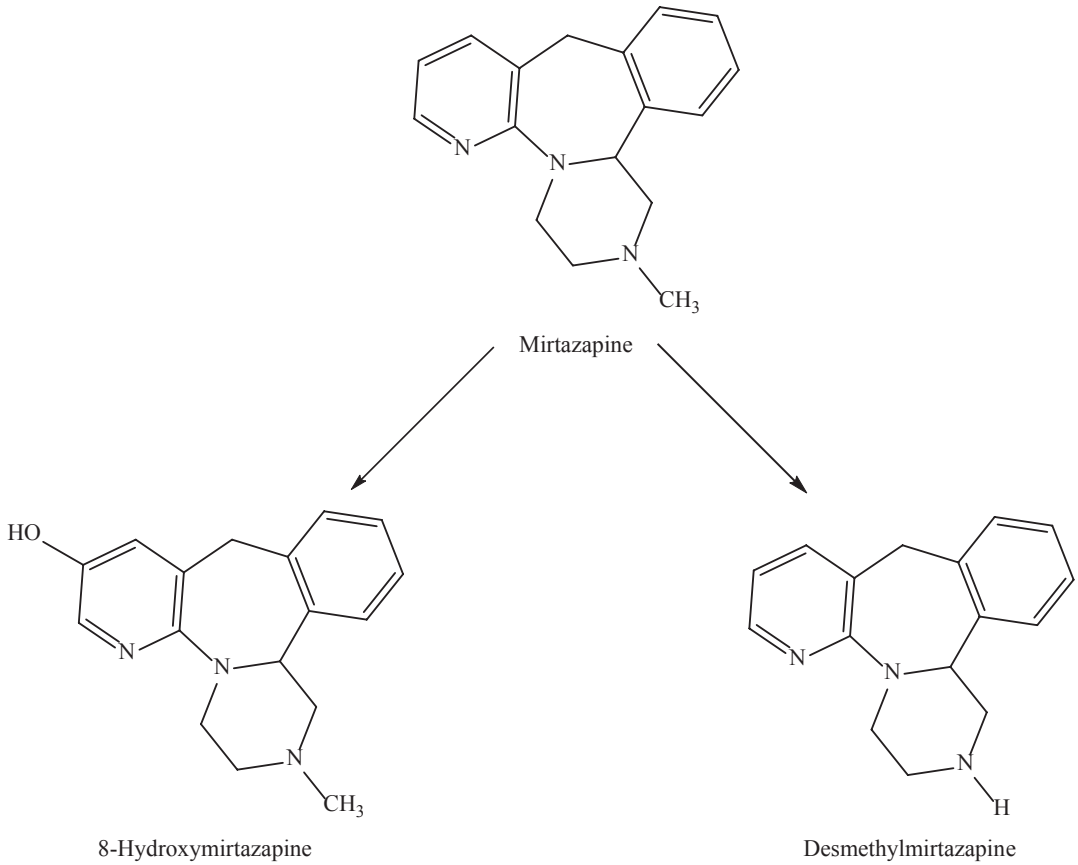

Fig. 27.12 The metabolic pathway for mirtazapine

sulfate and 6-hydroxy-5-methoxyduloxetine sulfate, have also been identified in plasma. The conjugated metabolites form rapidly and are inactive.

Vortioxetine is well absorbed with a bioavailability of 75%; peak plasma occurs in 7–11 h. The drug is extensively metabolized to inactive metabolites. Only traces are eliminated as free drug in the urine.

Vilazodone is well absorbed with a bioavailability of 72% with food; bioavailability is considerably less without food. There are no know active metabolites of vilazodone. Only 1% of the dose excreted in the urine as free drug. CYP3A4 is primarily responsible for its metabolism among CYP pathways, with minor contributions from CYP2C19 and CYP2D6.

Milnacipran is rapidly absorbed with a bioavailability of 85–90% with peak concentration 2–4 h post-dose. It is primarily metabolized to oxidized and conjugated metabolites. A significant portion of the dose, 50–60%, is excreted unchanged in the urine.

Levomilnacipran is absorbed from its extended-release capsule with a bioavailability of 80–90% with peak plasma levels occurring in 6–8 h. It is metabolized to inactive metabolites. Approximately 60% of the dose is excreted in the urine as unchanged drug.

Esketamine as a nasal spray has a bioavailability of 48%. The time to reach maximum esketamine plasma concentration is 20–40 min after the last nasal spray of a treatment session. No accumulation of esketamine in plasma was observed following twice-a-week administration. The drug is metabolized to the active metabolite noresketamine. Plasma concentrations on the fourth day of treatment taken 2 h after a 56-mg dose ranged from 0.014 to 0.142 mg/L. Less than 1% of a dose of nasal esketamine is excreted as unchanged drug in urine. The mean steady-state volume of distribution of esketamine administered by the intravenous route is 709 L. After C_{max} was reached following intranasal administration, the decline in plasma esketamine concentrations was biphasic, with rapid decline for the initial 2–4 h and a mean terminal half-life that ranged from 7 to 12 h. The elimination of the major metabolite, noresketamine, from plasma is slower than esketamine.

Analysis

Specimen Pretreatment

An examination of the structure of the antidepressants indicates that they are organic bases with moderate pK_a and sufficient lipophilic character to make them amenable to a variety of extraction techniques. Although single-step liquid–liquid extractions have been used with success, double or back extractions are more common in forensic toxicology *for gas chromatographic procedures*. One common procedure uses chlorobutane as the extraction solvent and is prototypical of those schemes that use back extraction. Common modifications to this procedure include addition of polar compounds to the extraction solvent and substitution of heptane/isoamyl alcohol or other nonchlorinated solvent mixtures for chlorobutane. Solid-phase extraction is also widely utilized for this class of drug and can be adapted for use with a variety of biological specimens. Specimen preparation is discussed in more detail in Chap. 9.

Chromatographic Separation

Gas chromatography (GC) is widely used for separating antidepressants in biological specimens. Columns are typically fused silica capillary columns with bonded nonpolar to intermediate polarity methyl silicone liquid phases (0–50% phenyl); usual column dimensions are as follows—length 10–30 m, internal diameter 0.20–0.53 mm, and film thickness 0.25–1.5 μm. Most of the antidepressants can be detected in routine temperature programmed analyses.

Table 27.2 presents the relative retention times (relative to amitriptyline) of the common antidepressants on a 5% phenyl-methyl silicone column. The retention time of amitriptyline under these con-

Table 27.2 Relative retention time (RRT) of antidepressants

RRT	Compound
0.43	Bupropion
0.52	Erythrobupropion
0.53	Threoaminobupropion
0.55	m-CPP
0.65	Hydroxybupropion
0.67	Norfluoxetine
0.69	Fluoxetine
0.70	Fluvoxamine
0.89	Venlafaxine
0.92	N-desmethylvenlafaxine
0.95	O-desmethylvenlafaxine
1.00	Amitriptyline (RT = 10.25 min)
1.01	cis-Doxepin
1.02	Imipramine
1.02	Nortriptyline
1.03	Trimipramine
1.03	trans-Doxepin
1.04	Mirtazapine
1.04	Desipramine
1.05	Protriptyline
1.05	Nordoxepin
1.13	Desmethylsertraline
1.14	Sertraline
1.14	Citalopram
1.14	Clomipramine
1.15	Desmethylcitalopram
1.16	Desmethylclomipramine
1.20	Duloxetine
1.30	Paroxetine
1.85	Trazodone
2.67	Nefazodone

ditions is 10.25 min. The detection of trazodone and especially nefazodone requires a high elution temperature and may persuade some analysts to use an alternate technique to detect these analytes.

One does not usually encounter chromatographic difficulties with the antidepressant drugs in overdose quantities. However, with low concentrations or in certain chromatographic systems, the secondary amines or hydroxylated metabolites may have asymmetrical peak shapes. This can be overcome by preparing acyl, fluoracyl, or silane derivatives. The use of derivatives may also allow the separation of closely eluting pairs of antidepressants or other drugs. Derivatization is discussed in more detail in Chap. 12.

Liquid chromatography (LC)-based separations are also an attractive separation technique for the analysis of antidepressants due to the polarity of the secondary amines and hydroxy metabolites. LC/MS/MS is widely utilized, does not require derivatization, and offers excellent sensitivity. For trazodone and nefazodone and their metabolites, methods are available that obviate the problems associated with their analysis by GC. Numerous stationary and mobile phases have been described for the various classes of antidepressants. Some antidepressants are not readily amenable to analysis by gas chromatography; therefore, LC-based methods are gaining popularity. Chromatographic separations and mass spectrometry are discussed in more detail in Chaps. 11 and 14. It should be noted that many chromatographic methods do not separate stereoisomers of antidepressants.

Toxicity and Postmortem Findings

The subject of antidepressant toxicity is evolving. New compounds are being introduced at a rapid rate. As a group, the newer drugs exhibit less inherent toxicity than do their predecessors; however, many have properties that can lead to toxic or fatal drug–drug interactions. The ability of many antidepressants to affect hepatic metabolism is an important factor in understanding the potential toxicity of this class of therapeutic agents. These concepts figure significantly in the following discussion.

The TCAs are compounds with well-known toxicity. They are among the leading causes of drug-related deaths throughout the world. There is no evidence of significant differences in toxicity among the TCAs. Amoxapine and maprotiline are so similar to TCAs in their toxicity that they are included in this discussion. The major toxicity associated with overdose of these compounds is due to anticholinergic effects, central nervous system effects, and cardiovascular effects. Effects include flushing, mydriasis, delirium, confusion, lethargy, fever, seizures, tachycardia, coma, and, most important, cardiac arrhythmia. Hypertension can occur early in the toxicity due to an anticholinergic effect; hypotension, probably due in part to alpha-adrenergic blockade, can also occur and can be a major contributor to morbidity. Seizures and cardiac arrhythmias are the most likely conditions to cause death in TCA overdose. Although any type of arrhythmia may be observed, the prolongation of the QRS interval in the electrocardiogram is often a diagnostic tool in overdose by TCAs. In the living patient, concentrations of TCA and active metabolite above 0.45 mg/L have been associated with toxicity. Concentrations of TCAs and their active metabolite in excess of 1.0 mg/L are often associated with life-threatening toxicity. In contrast, concentrations in postmortem blood are often much higher.

In postmortem specimens, the interpretation of postmortem blood concentrations is not as simple as applying the concentrations in Table 27.1 to the postmortem blood. Blood concentrations in postmortem specimens are much higher than expected from clinical data and in comparison to concentrations in specimens taken near or at the time of death. This is a result of the well-established concept of postmortem redistribution. The premise of postmortem redistribution is that drug concentrations are not static after death; they tend to rise, especially for basic drugs with high volumes of distribution. It is common for TCA blood concentrations to rise by a factor of 2–8 during the postmortem interval from death to specimen collection. Typically, concentrations of TCAs rise faster and to higher concentrations in blood specimens from the central cavity as compared to more peripheral sites.

Several implications of this are obvious. The exact dose taken by an individual cannot be estimated by a pharmacokinetic calculation that depends on the concentration measured in a postmortem blood specimen, especially if obtained from the central cavity. Postmortem redistribution also plays a role in defining what constitutes a toxic concentration in postmortem specimens. A concentration of TCA plus metabolite of 1.0 mg/L in clinical specimens would be considered potentially toxic. The same concentration in postmortem specimens is often observed in cases where it is clear that the drugs played no role in the death. Postmortem blood concentrations of 2.0 mg/L are considered to be potentially toxic; however, in the absence of clear and convincing evidence of the role of a TCA in death, interpretations of concentrations in this range that are based solely upon the analysis of a blood specimen should be undertaken with extreme caution.

The analysis of peripheral blood specimens and tissues can often provide the necessary information to successfully interpret a case. A liver specimen is a most useful complement to blood specimens in the interpretation of TCA-related cases. In acute TCA overdose cases, the concentration of drug and metabolite is much higher in the liver than in the blood. Liver concentrations in cases related to TCA toxicity can be quite high and are typically greater than 35 mg/kg. The drug-to-metabolite ratio is typically greater than unity. In certain situations, toxic concentrations of TCAs, and especially their active metabolites, may arise from chronic dosing because of a genetic deficiency of metabolizing enzyme or because of enzyme inhibition by coadministered medications. If the TCA in question is a tertiary amine, and a high desmethyl metabolite-to-drug ratio is observed, the toxicologist should be alerted to the possibility of a chronic poisoning. For secondary amine TCAs and other compounds, which have no routinely detected metabolites, the problem is even more vexing. If the circumstances surrounding a death are not clear, it is imperative to consider all possibilities before the manner of death is determined. Enzyme inhibition will be addressed in more detail in the discussion of toxicity of the SSRIs.

Trazodone appears to be safer in overdose situations than TCAs. Symptoms observed after trazodone overdose include drowsiness, vomiting, respiratory arrest, seizures, and EKG changes. Most reported trazodone-related deaths have involved trazodone and other medications. The concentration of trazodone in reported fatal overdose cases has been 15–30 mg/L in blood and 50–80 mg/kg in liver. These blood values overlap with those reported for nonfatal outcomes; however, concentrations of this magnitude clearly indicate an overdose situation. Trazodone has been reported to be much less susceptible to postmortem redistribution than the TCAs. In contrast to therapeutic cases, significant amounts of trazodone may be detected in the urine in overdose cases.

Symptoms related to bupropion overdose include hallucinations, tachycardia, and seizures, with seizures being the most significant event. The concentrations of bupropion and metabolites in reported overdose cases have been in the ranges: bupropion, 4 mg/L or greater; hydroxybupropion, 3–5.1 mg/L; threoamino metabolite, 4.6–11.6 mg/L; and erythron-metabolite, <1 mg/L. For those cases in which liver values were reported, bupropion concentrations were 1–14 mg/kg. As mentioned previously, bupropion is unstable in biological specimens; this must be taken into account not only during the analysis but also in the interpretation of analytical results.

The SSRIs have become the most widely prescribed group of antidepressants. Their efficacy in treating other disorders such as obsessive-compulsive disorder and bulimia nervosa have added to their popularity. They exhibit fewer troublesome side effects and are better tolerated than the first-generation antidepressants. Moreover, they are safer in overdose situations, primarily because they do not demonstrate the cardiovascular toxicity associated with the TCAs. Unfortunately, their relative safety in overdoses compared to TCAs has led many to believe that there is little to no potential for a fatal outcome with these drugs. This is not the case. These drugs can cause serious toxicity, especially when taken with serotonin-enhancing drugs, and they can affect the metabolism and clearance of a variety of drugs.

All of the SSRIs exhibit similar toxicity. They have been noted to cause nausea, vomiting, mydriasis, tachycardia, tremor, seizures, and coma when taken in overdose. Serotonin syndrome, a potentially fatal condition caused by a sudden systemic excess of serotonin, has been reported after SSRI ingestion. Symptoms of serotonin syndrome include hyperthermia, diaphoresis, excitement or confusion, shivering, tremors, hypotension, and seizure. This condition can be caused by the ingestion of SSRIs alone but occurs more often when SSRIs are ingested with other drugs that have serotonergic-enhancing properties. Serotonin syndrome is commonly seen with MAOIs, but it has been reported to occur with TCAs, tramadol, administration of more than one SSRI, lithium, dextromethorphan, and others.

The development of serotonin syndrome is often delayed as much as 12 h after ingestion. The long half-life of some of the SSRIs, such as fluoxetine, makes the development of a serotonin syndrome possible for long periods after the drug is discontinued. Another major complication of all SSRIs is their effect on the hepatic cytochrome P450 (CYP) isoenzymes. These enzymes are involved in the metabolism of many drugs. The systems primarily involved in drug metabolism are CYP 1A2, 2C, 2D6, and 3A4. The most studied of the isoenzymes is CYP2D6. This isoenzyme exhibits polymorphism; a percentage of the population (5–10% for Caucasians, other races vary) lacks it entirely or has less than normal amounts. These individuals (poor metabolizers) are in contrast to those with normally functioning CYP2D6 (extensive metabolizers). When a compound inhibits CYP2D6, an extensive metabolizer can functionally become a poor metabolizer. The CYP2D6 isoenzyme catalyzes many important hydroxylation reactions, including hydroxylation of antidepressants, antipsychotics, analgesics, and cardiovascular drugs, among others. When CYP2D6 is inhibited, it can strongly affect the concentration and clearance of any drug dependent on it for metabolism. Clinically significant interactions arising from CYP2D6 inhibition have been reported for imipramine, methadone, alprazolam, and haloperidol, among others. The other isoenzymes primarily involved in drug metabolism do not naturally exhibit polymorphism to the degree of CYP2D6, but they can be inhibited by drugs or drug metabolites. It is not necessary for a drug to be a substrate for an isoenzyme to cause inhibition. The SSRIs vary in the isoenzymes that they inhibit and in the magnitude of their inhibition. Pharmacogenomics is discussed in more detail in Chap. 36. However, it is now clear that significant drug–drug interactions can occur with the ingestion of SSRIs and these interactions may have significance in forensic toxicology.

There are more data in the literature about fluoxetine and norfluoxetine concentrations after self-poisoning than for the other SSRIs. In one clinical study of 87 patients who had taken overdose quantities of fluoxetine, serum concentrations, when measured, were 0.23–1.39 mg/L of total fluoxetine (fluoxetine + norfluoxetine); none of the 87 patients died. A postmortem case in which fluoxetine is the only ingested agent is rare, and only a few are found in the literature. In non-fluoxetine-related postmortem cases, the total fluoxetine concentration is typically less than 2 mg/L, with a fluoxetine/norfluoxetine ratio less than or near 1.0. Liver total fluoxetine in non-fluoxetine-related cases is typically less than 20–50 mg/kg, and the fluoxetine concentration is usually less than the norfluoxetine concentration. Fluoxetine exhibits considerable postmortem redistribution.

Sertraline concentrations averaged 0.25 mg/L in a series of nonfatal overdose cases. The concentration of sertraline in non-sertraline-related deaths is generally less than 0.8 mg/L in the blood and less than 20 mg/kg in the liver. Desmethylsertraline is generally less than 1.5 mg/L in the blood and less than 50 mg/kg in the liver; a majority of the liver values are expected to be less than 20 mg/kg. One unique aspect to therapeutic sertraline use is that very low or even non-detectable concentrations of parent drug and metabolite are found in urine specimens. This is contrasted to other antidepressants where urine concentrations usually exceed blood concentrations. Therefore, blood or bile specimens are better postmortem specimens to screen for the use of sertraline than is urine. In addition, several groups of investigators have

reported a lack of significant differences in postmortem heart and femoral blood concentrations in sertraline cases.

Therapeutic use of paroxetine and citalopram is indicated when the postmortem heart blood concentration is <1.0 mg/L. Concentrations of these drugs in intoxication cases are generally several times higher than this. Like most other antidepressants, the liver concentration of these drugs is generally an order of magnitude higher than the blood concentration.

Of the third-generation antidepressants, venlafaxine has the most data concerning its toxicity. Overdose symptoms include tachycardia, convulsions, somnolence, and coma. The concentrations of venlafaxine in overdose deaths have varied widely. Concentrations of 6.6–89.7 mg/L have been reported for venlafaxine and 3.44–50 mg/L for O-desmethylvenlafaxine (ODV). The ratio of venlafaxine to ODV was not consistent in these cases.

Symptoms reported as a result of nefazodone overdose include nausea, vomiting, and somnolence. Because nefazodone inhibits the reuptake of 5-HT, serotonin syndrome can be produced by the coadministration of a MAOI or another serotonin-enhancing drug. Such reactions have been reported for nefazodone and paroxetine. Nefazodone is a weak inhibitor of CYP2D6 but is a potent inhibitor of CYP3A4. It has the potential to increase the concentration of drugs metabolized by this enzyme. Very little information is available concerning the concentration of nefazodone in overdose situations. No fatal cases have been reported in which nefazodone was thought to be sole cause of death.

Symptoms of mirtazapine toxicity include drowsiness, disorientation, tachycardia, and impaired memory. A number of postmortem studies involving mirtazapine indicate that blood concentrations <0.5 mg/L are associated with therapeutic use. Postmortem concentration in overdose cases were generally between 1.0 and 3.0 mg/L.

In cases where death was due was due to duloxetine and other drug(s), duloxetine concentrations ranged from 0.32 to 2.5 mg/L. Deaths due to duloxetine have occurred at concentrations of 6.1 and 7.5 mg/L.

Patients who accidently or intentionally consumed 40–75 mg of vortioxetine had increased rates of nausea, dizziness, abdominal discomfort, generalized pruritus (skin irritation), somnolence, and flushing. Little information is available concerning the toxicity of vortioxetine in overdose cases, especially in death cases.

Little is known about vilazodone concentrations in overdose situations. During clinical trials overdose with serotonin syndrome, lethargy, restlessness, hallucinations, and disorientation were noted. A concentration of 0.36 mg/L was observed in a pediatric patient who survived an overdose.

Milnacipran concentrations in two nonfatal overdose cases were 3.0 and 8.4 mg/L. Two deaths due to milnacipran had blood concentrations of 22 and 40 mg/L.

There have been no reported deaths due to esketamine. Deaths due to racemic ketamine have been reported from 1.8 to 27 mg/L. Esketamine is more potent than racemic ketamine.

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