CHAPTER II

BACKGROUND

DEPRESSION

1. Definition

Depression is a poorly defined entity. In 1959, Lehmann point out, depression may refer to a symptom, a syndrome, or a nosological entity. Depressive syndrome is often defined as consisting of both primary and secondary symptoms. The primary symptoms consist of a despairing emotional state and the depressive mood. The secondary symptoms vary and are less regularly found. They may include such things as social withdrawal, psychomotor retardation, anorexia, weight loss, and sleep disturbances (McKinney and Bunney, 1969) . According to the American Psychiatric Association's forth edition of the diagnostic and Statistical Manual of Mental Disorder, (DMS-IV), critical variables in diagnosis are list in Table 1. The fundamental point in diagnosis and understanding major depression is that it is a syndrome. It is not just low mood, but rather a cluster of signs and symptoms termed a depressive episode (Preskorn, 1999).

Depression affects million of people of all ages. It is a common finding in primary care practice, with a 6 month prevalence of 5.8 percent and a lifetime prevalence of 17 percent in the United State (Majeroni and Hess, 1998).

Table 1. DSM-IV Diagnostic Criteria for a Major Depressive Episode (Preskorn, 1999)

- Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure (*Note*: do not include symptoms that are clearly due to a general medical condition, or mood-incongruent delusions or hallucinations):
    - Depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels said or empty) or observation made by others (e.g., appears tearful). *Note*: In children and adolescents, can be irritable mood
    - Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation made by others)
    - Significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day. Note: In children, consider failure to make expected weigh gains
    - Insomnia or hypersomnia nearly every day
    - Psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down)
    - Fatigue or loss of energy nearly every day
    - Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick)

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- Diminished ability to think or concentrate: or indecisiveness, nearly every day (either by subjective account or as observed by others)
- Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide

- The symptoms do not meet criteria for a mixed episode
- The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning
- The symptoms are not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition (e.g., hypothyroidism)
- The symptoms are not better accounted for by bereavement, i.e., after the loss of a loved one, they symptom persist for longer than 2 months or are characterized by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms, or psychomotor retardation

2. Etiology

The cause of depression and the mechanisms of action of antidepressants are not clear. The monoamine theory of depression postulates that the symptoms of the illness arise from a deficit in the availability of serotonin and/or noradrenaline (Leonard, 1995). There are serious problems with this theory, but it has not been replaced with a better one (Neal, 1994). More recently, interest has been focussed on receptor regulation in the brain. Studies in blood platelets of depressed patient, frontal cortex of suicide or depressed patients, increasing 5-HT_2 and beta-adrenergic receptor (Stanley and Mann, 1983; Mann, Stanley, McBride, and McEwen, 1986; Aurora and Meltzer, 1989). In addition, patients with major depressive disorder may be related to 5-HT_{1A} receptors which these receptors may be desensitized (Stahl,1994). Down regulation of β -adrenergic and 5-HT receptor not only by different type of antidepressant drugs (TCAs, MAOIs, and some second generation antidepressant), but also by electroconvulsive therapy, which also has antidepressant action effects in many patients (Charney, Menkes, and Heninger, 1981; Coven *et al*, 1990, Sulser, 1984; Okada, Tokumitsu, and Ui,1986). For mechanism of action, receptor regulation may relate to the slow onset of antidepressant action of all antidepressant drugs which require the time for changes in central 5-HT or catecholamines pathway (Khanna, 1967)

3. Neurotransmitter and neural pathway implicated in depression

3.1 Transmitter release and uptake

Transmitter must be synthesized and stored in the presynaptic structure. Synthesis can take place both in the soma, where upon either the transmitter or a precursor will be transported down the axon to the terminal, or in the presynaptic terminal in such a manner that they can be released by the nerve impulse. It is commonly believed that transmitter is stored in membrane-bound vesicle and that

release of its by the nerve impulse is effected by exocytosis (fusion between membrane of vesicle which contain transmitters with axolemma of active zone). The transmitter release is a graded function and is proportional to the depolarization applied to a nerve terminal. In addition, Ca^{++} is required for transmitter release. An increase Ca^{++} permeability and intracellular Ca^{++} that is critical are widely believed that causes action potential. Apparently not all of the transmitter in a nerve terminal is uniformly accessible for release by a nerve impulse. The degree to which there is a physical differentiation of different pools of transmitter in synaptic terminals is not yet establishes (Goldfarb and wilk, 1976).

Neurotransmitter is efficiently cleared from the extracellular space by transporter proteins localized in the plasma membrane of presynaptic terminals. Transmitter reuptake is believed to have three important consequences.

(1). Levels of transmitter in the synapse are reduced faster than can be achieved by simple diffusion, allowing for improved temporal discrimination of consecutive release events.

(2). The effects of released transmitter are constrained to a smaller area, permitting dense packing of chemically identical but functionally distinct synapses. And

(3). Transmitter can be recycled for another round of release once it is transported back across the presynaptic membrane and into synaptic vesicles.

The common properties recognized for NE, DA, and 5-HT transport were an absolute dependence on extracellular Na^+, a feature known to be characteristic of Na^+/cotransport processes where energy for inward solute transfer is coupled to the energetically favorable influx of Na^+ down its concentration gradient. In addition, extracellular Cl^- is absolutely necessary for NE, DA, and 5-HT transport, although the halide specificity appears less rigid than the Na^+ requirement; other anions such as NO_2^- and Br^- are capable of substituting, at least partially for Cl^-. Serotonin (5-HT) and Norepinephrine (NE) uptake process, for example, it has been proposed in which Na^+, Cl^-, and a protonated 5-HT or NE molecule bind to the transporter, forming a complex

which the undergoes a conformational change to release the neurotransmitter and the ions into the cytoplasm. Subsequently K^+ which promote the reorientation of the unload carrier for another transport cycle. Thus, intracellular K^+ accelerates transmitter influx by facilitating a conformational change required for external exposure of unoccupied transmitter binding sites on the unloaded transporter. Serotonin transporter and Noradrenaline transporter appear to differ in the role of intracellular K^+ in transport (Barker and Blakely, 1995). (Figure 1)

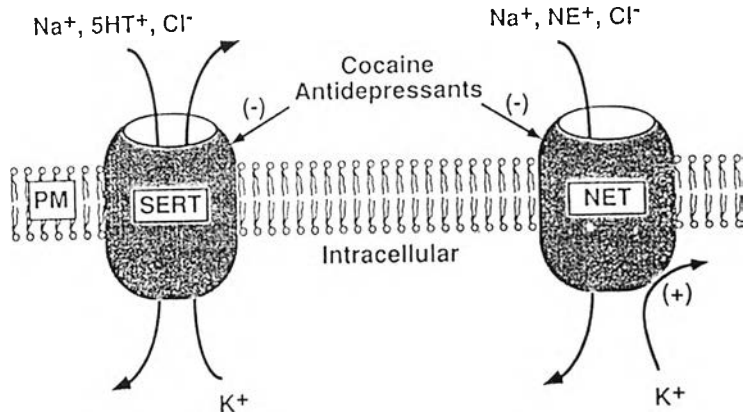


Figure 1. General model of ion-coupled NE and 5-HT uptake. Left: Proposed mechanism for 5-HT transporter(SERT) where uptake of 5-HT is dependent upon cotransport of Na^+ and Cl^- and countertransport of K^+. Right: NE transporter(NET) model showing Na^+ and Cl^- -dependent NE uptake with intracellular K^+ stimulation of NE uptake, but no associated K^+ efflux. PM=plasma membrane (Barker and Blakely, 1995)

3.2 Neurotransmitter transporters

The neurotransmitter transporter protein that carry out NE, DA, and 5-HT clearance is one of the most important set of macromolecules that regulate monoamine signaling. It localizes in the plasma membranes of presynaptic terminals (Figure 2). Changes in 5-HT, NA, or DA uptake sites have long been associated with major affective disorder, particularly depression.

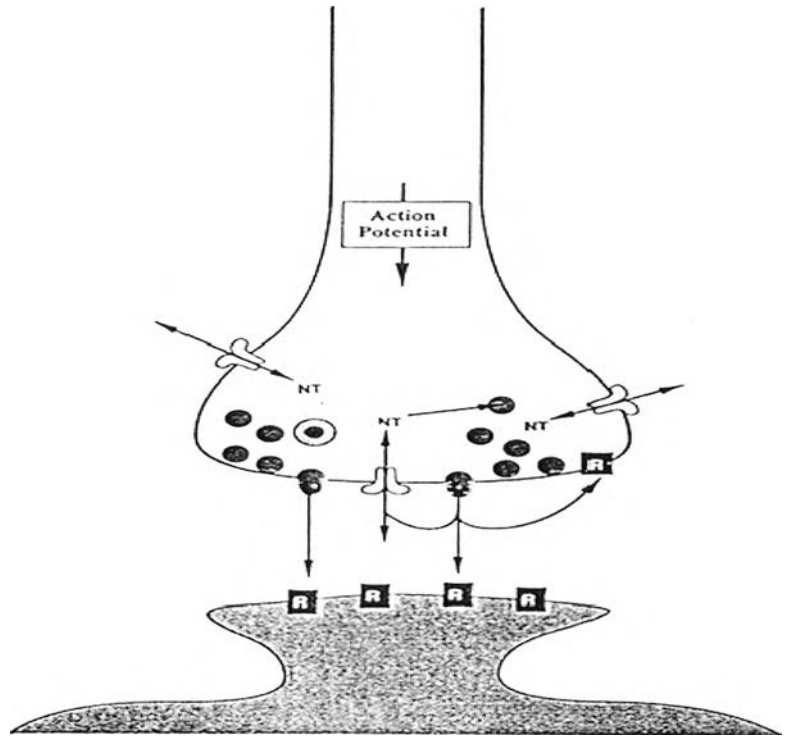


Figure 2. Schematic representation of a synapse showing vesicular release of biogenic amines and transmitter reuptake by presynaptic transporters. Transmitter movements by transporters are given bi-directional arrows to indicate a capacity for both influx and efflux. R=receptor; NT=neurotransmitter (Barker and Blakely, 1995)

NE transporter (NETs), DA transporter (DATs), and 5-HT transporter (SERTs) are members of a large family of Na^{+}/Cl^{-} dependent. Protein purification strategies have achieved only limited success in the elucidation of neurotransmitter transporter structure; therefore, to clone transporter cDNAs in the absence of protein – derived sequence information. DATs is an N-linked glycoprotein containing N-acetylglucosamine and terminal sialic acid residues. It has a molecular weight between 58,000 and 80,000 Da and the deduced primary (615-or 620) amino acids sequences. The most highly conserved sequences are thought to encompass the 12 hydrophobic (membrane spanning) domain and flanking amino acids, with the predicted intracellular

amino and carboxyl terminal and extracellular loops exhibiting less sequence similarly. An aspartate and serine residues conserved within certain transmembrane domains of the DATs. Replacement of an aspartate in transmembrane domain1 resulted in a dramatically reduced uptake of DA and reduced affinity for the cocaine analogue CFT. Mutations of several serines in transmembrane domain7 (but not 8) also substantially altered these parameter. An aspartate residue of transporters may interact with the amine group of DA while the serines may interact with its catechol group (Bannon, Granneman, and Kapatos, 1995; Lew et al, 1991). DATs, SERTs and NETs are the pharmacological target of a variety of therapeutic antidepressant. Tricyclic antidepressant sensitivity is shared by NETs and SERTs, but not by DA transporters. Tertiary amine tricyclic are more potent at SERTs as compared to the NETs-preferring secondary amine tricyclics. Other potent NETs antagonists include nomifensin. Highly selective antagonists for SERTs such as fluoxetine and paroxetine. For DATs antagonist bupropion, amineptine, and nomifensine. The primary sequence of the human NETs cDNA predicts a highly hydrophobic 617- amino acid polypeptide of ~ 67,000 Da with 12 potential transmembrane domains (Figure 3)

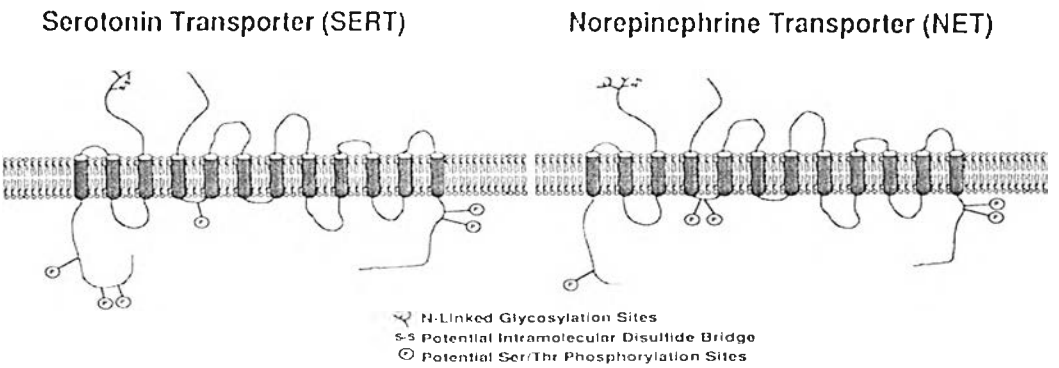


Figure 3. Proposed transmembrane topology and structural features of SERT and NET subunits. Note the 12 proposed transmembrane domains (TMDs), the large extracellular loop between TMD3 and TMD4 bearing multiple N-linked glycosylation sites, cytoplasmic $-NH_2$ and –COOH tails, and sites for potential intermolecular disulfide bridge formation and phosphorylation. (Barker and Blakely, 1995)

3.3 Norepinephrine

Norepinephrine is synthesized from tyrosine. In mammals, tyrosine can be derived from dietary phenylalanine by the enzyme (phenylalanine hydroxylase), dietary soures provide sufficient tyrosine for the biosynthesis of the catecholamines. The term catecholamine is usually used to describe the endogenous compounds dopamine (dihydroxyphenylethylamine), norepinephrine, and epinephrine. Catecholamines are formed in brain, adrenal chromaffin cells, and sympathetic nerves. In general, the processes regulating catecholamine synthesis are the same in the various tissues.

The amino acid L-tyrosine is converted into 3,4-dihydroxyphenylalanine (L-dopa) by the enzyme tyrosine hydroxylase (TH), which is the rate-limiting step. The L-dopa is almost immediately metabolized to dopamine (DA) by L-aromatic amino acid decarboxylase (AADC). In dopamine-containing neurons, this is the final step in transmitter synthesis. However, in neurons using norepinephrine as transmitters, dopamine is oxidized by dopamine β-hydroxylase (DBH) to yield norepinephrine (NE). Because DBH is present in vesicles rather than the cytosol, the process that DA is converted to NE is in vesicle presynaptically and NE is stored in vesicles. By the reason that, noradrenergic neurons contain three enzymes (TH, AADC, and DBH) that sequentially metabolize tyrosine to norepinephrine and thus norepinephrine cannot be further metabolized to epinephrine because it lack an enzyme, phenylethanolamine N-methyltransferase (PNMT) which convert norepinephrine into epinephrine (Dinan,1996; Deutch and Roth, 1999). (Figure 4)

Norepinephrine in the brain is metabolized predominantly to the glycol 3-methoxy-4-hydroxyphenyl glycol (MHPG), whereas the major urinary (peripheral) metabolite of norepinephrine is the acid 3-methoxy-4-hydroxymandelic acid (VMA) (Goldfarb and Wilk, 1976).

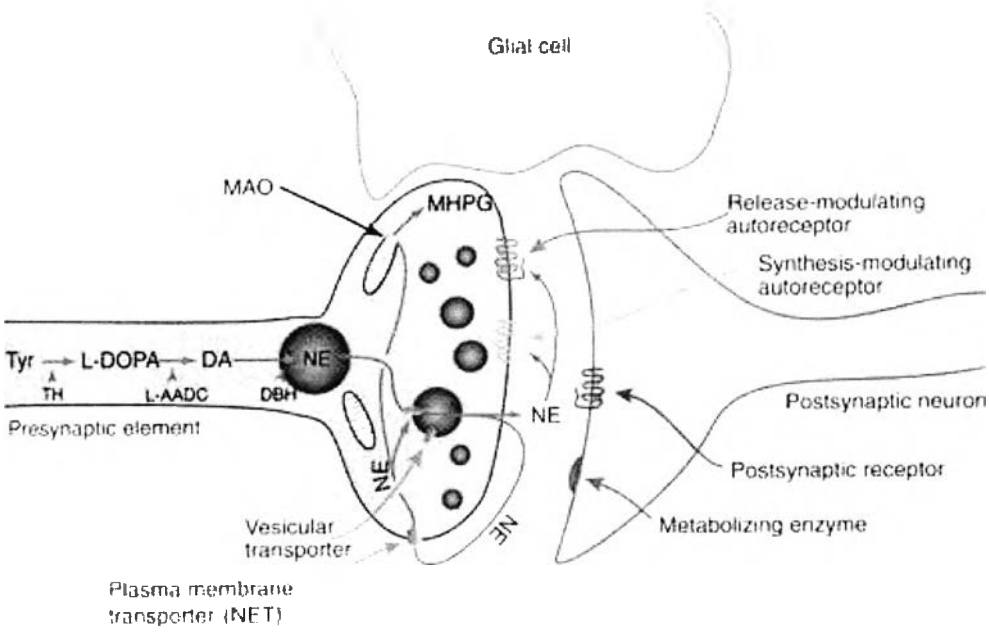


Figure 4. Diagrams of a norepinephrine-containing neuron showing synthesis, storage, release and inactivation of the synaptic transmitter (Deutch and Roth, 1999).

3.3.1 Norepinephrine pathway

The cell bodies of the norepinephrine-containing neurons in the brain are located in the locus ceruleus and other nuclei in the pons and medulla. From the locus ceruleus, the axons of the noradrenergic neurons form the locus ceruleus system. They descend into the spinal cord, enter the cerebellum, and ascend to innervate the paraventricular, supraoptic and periventricular nuclei of hypothalamus, the thalamus,the basal telencephalon and the entire neocortex. From cell bodies in the dorsal motor nucleus of the vague, the nucleus of the tractus solitaries, and areas in the dorsal and lateral tegmentum, the axons of the noradrenergic system form a lateral tegmental system that project to the spinal cord, the brain stem, all of the hypothalamus and the basal telencephalon (Ganong,1997) (Figure 5).

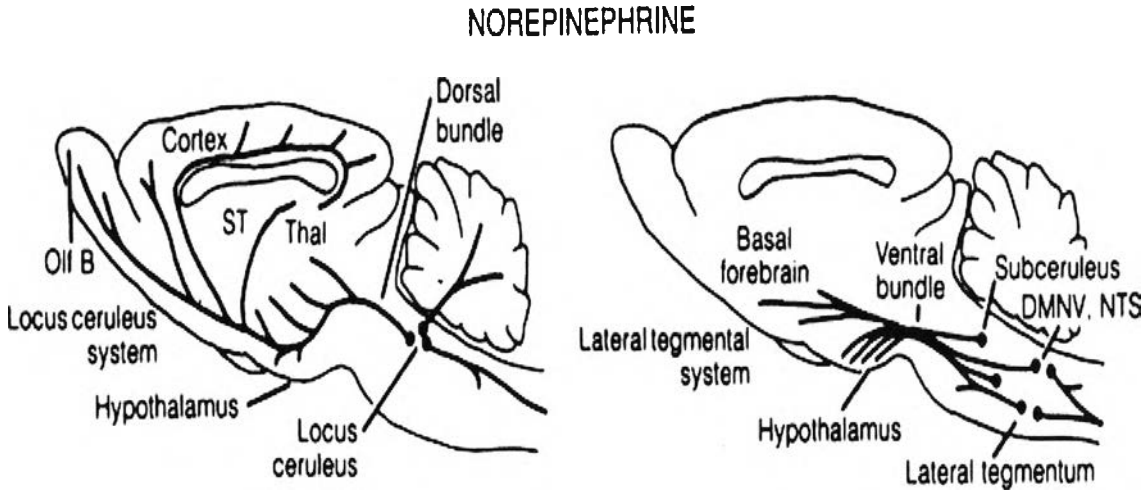


Figure 5. Schematic illustrating some of the major collection of noradrenergic neuron and their projection areas in rat brain. The pathways in humans appear to be similar. The two principal noradrenergic systems (locus ceruleus and lateral tegmental) are shown separately. Olf B, olfactory bulb; Thal, thalamus; ST, stria terminalis; NTS, nucleus of tractus solitarius (Ganong, 1997).

3.3.2 Norepinephrine receptor subtype

There are many ligands and many subtypes of receptors for each ligand. Many are receptors that act via G proteins and protein kinases to produce their effect. Other are ion channels. The receptors for some neurotransmitter are listed in Table 2.

Norepinephrine acts on a variety of adrenoceptors (β-adrenoceptor and α-adrenoceptor), both pre- and post-synaptic designated β1, β2, β3, α1A, α1B, α2A, α2B,and α2C. The presynaptic receptor, or autoreceptors, often inhibit further secretion of the ligand providing feedback control. For example, activation of α2-receptor decreases intracellular cyclic AMP concentrations, but there is evidence that the G-protein activated by α2 presynaptic receptors acts directly on Ca^{2++} channels to

inhibit norepinephrine release by decreasing the Ca^{2++} increase. Drugs that increase extracellular norepinephrine levels in the brain elevate mood, and drugs that decrease extracellular norepinephrine levels cause depression.

Table 2. Mechanism of action of some biogenic amines neurotransmitters (Ganong,1997)

Transmitter	Receptor	Second Messenger	Net Channel Effects
Dopamine	D_1, D_5	↑Cyclic AMP	
	D_2	↓Cyclic AMP	↑K^+, ↓Ca^{2+}
	D_3, D_4	↓Cyclic AMP	
Norepinephrine[1]	α_1	↑IP_3, DAG	↓K^+
	α_2	↓Cyclic AMP	↑K^+, ↓Ca^{2+}
	β_1	↑Cyclic AMP	
	β_2	↑Cyclic AMP	
	β_3	↑Cyclic AMP	
5HT[2]	$5HT_{1A}$	↓Cyclic AMP	↑K^+
	$5HT_{1B}$	↓Cyclic AMP	
	$5HT_{1D}$	↓Cyclic AMP	↓K^+
	$5HT_{2A}$	↑IP_3, DAG	↓K^+
	$5HT_{2C}$	↑IP_3, DAG	
	$5HT_3$	———	↑Na^+
	$5HT_4$	↑Cyclic AMP	

[1] Three subtypes of α_1 (α_{1A}, α_{1B}, α_{1D}) and three subtypes of α_2 (α_{2A}, α_{2B}, α_{2C}) receptors have been cloned.
[2] $5HT_{1E}$, $5HT_{1F}$, $5HT_{2B}$, $5HT_{5A}$, $5HT_{5B}$, $5HT_6$, and $5HT_7$ receptors also cloned.
[3] Eleven subtypes identified; all decrease cAMP or increase IP_3 and DAG, except one, which increases cAMP.

3.4 Dopamine

Dopamine is the neurotransmitter that is converted to NE. In some brain area, the synthesis of catecholamine is ended at Dopamine. Dopamine is released or deactivated by active reuptake mechanism into presynaptic vesicles. The major metabolites of brain dopamine are homovanillic acid and dihydroxyphenyulacetic acid.

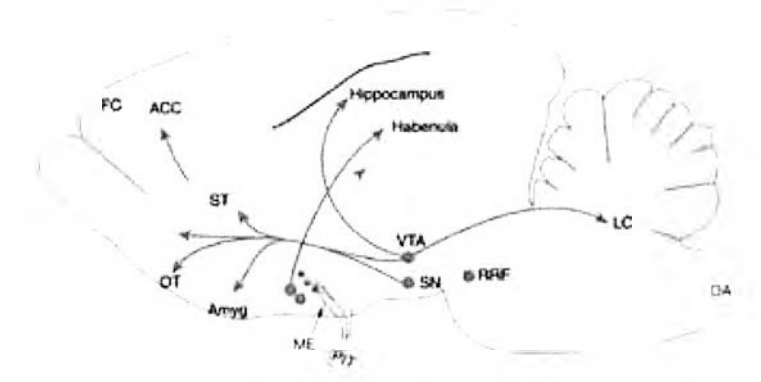
3.4.1 Dopamine pathway

The greatest number of dopamine-containing cells are in the brainstem at mesencephalic levels. There are two main groups of dopaminergic cells. The larger nigrostriatal pathway is known as the nigrostriatal system. It is involved primarily in

extrapyramidal motor functions. Its cell bodies are in the pars compacta of the substantia nigra, which projects to the striatum. The second major grouping of dopaminergic fibers is known as the mesocorticolimbic system. It supports a variety of behavioral functions related to motivation and reward. It has been known that this system is involved in converting emotion into motivated action and control movement. The cell bodies are located in the ventral tegmental area and project to the forebrain, largely to nucleus accumbens, olfactory tubercle, septal area, amygdala, and frontal cortex.

There are also several groups of dopamine cell bodies in the hypothalamus, including those in the arcuate nucleus (which give rise to the dopamine innervation of the median eminence), the periventricular dopamine neurons (which project to the spinal cord and medial thalamus among other sites), the preoptic area dopamine neurons, and dopamine cells in the zona incerta (Willner, 1995; Deutch and Roth, 1999) Figure 6. Behavioral evidence indicated that chronic treatment with antidepressants results in the potentiation of those responses to dopamine agonists that are considered to be mediated by the mesocorticolimbic dopamine system (de Montis, 1990).

Dopamine



Serotonin

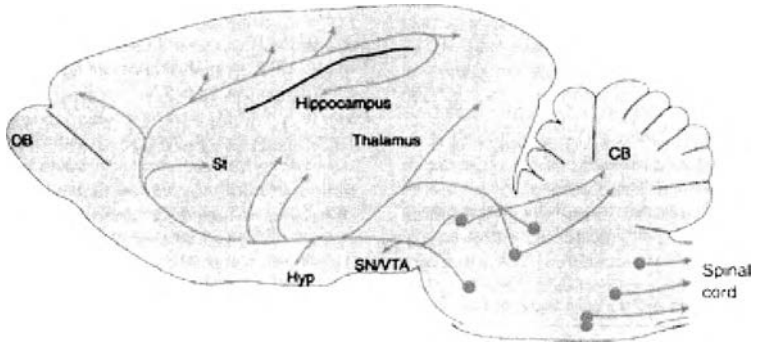


Figure 6. Illustration the dopaminergic (top panel) and serotonergic (bottom panel) cell bodies (circles) and their projection areas. Abbreviations: LC, locus coeruleus; SN, substantia nigra; OT, optic tract; ST, striatum; FC, frontal cortex; VTA, ventral tegmental area; Amyg, amygdala; RRF, retrarubral field; ME, medial eminence; PIT, pituitary (Deuth and Roth, 1999).

3.4.2 Dopamine receptor subtype

Dopamine receptors were at categorized to at least five subtypes D1, D2, D3, D4, and D5. Each molecular subtype has a unique regional distribution in the brain. They can be divided pharmacologically similar into two families that are often referred to as D1-like (The D1A and D2B or D5) and D2-like dopamine receptor (D2, D3 and D4). These two receptor exert their biological actions by coupling to G protein complexes. The D1 receptors interact with G-protein (Gs) resulting in the stimulation of adenylate cyclase and increase the synthesis of cyclic AMP. The D2-like receptor interacts with G-protein (Gi)

complexes to inhibit intracellular cyclic AMP production. D1-like dopamine receptors have intron-less genes, are expressed as proteins having a relatively short third intracellular loop and a relatively long carboxy tail, and show high affinity for phenyl-tetrahydrobenzazepines shch as SCH 23390 (antagonist) and SKF 38393 (agonist). D2-like dopamine receptors are genes having multiple introns, are expressed as proteins having a long third intracellular loop and a short carboxy tail, and show high affinity for butyrophenones (e.g.,spiperone) and benzamides (e.g., sulpiride). D1-like receptors are mainly located at neurons post-synaptic terminals and D2-like receptors are expressed both as autoreceptor on dopamine neurons and terminals, and as postsynaptic receptor on target cells (Smith, Nichols, Mailman, and Lawler, 1997; Seeman, 1995; de Montis , 1990).

3.5 Serotonin

Serotonin (5-hydroxytryptamine, 5-HT) is an indole derivative. It is derived from the amino acid tryptophan. This amino acid is concerted to 5-hydroxytryptophan (5-HTP) by tryptophan hydroxylase and then to 5-HT (Figure 7). In the CNS, serotonin is the final product of this synthetic pathway, with no subsequent enzymes generating other transmitters. However, in the pineal gland, serotonin in metabolized further to the hormone melatonin. The main metabolite of 5-HT in man is 5-hydroxyindoleacetic acid

(5-HIAA). Serotonin is found in many cells that are not neurons, such as platelets, mast cells, and enterochromaffin cells. Serotonin is involved in numerous physiological events. At the peripheral level, it affects smooth muscle fibers, causing constriction or relaxation, and thus exerts a major effect on the vascular bed and the digestive tract. Serotonin to some extent affects various functions of the CNS.

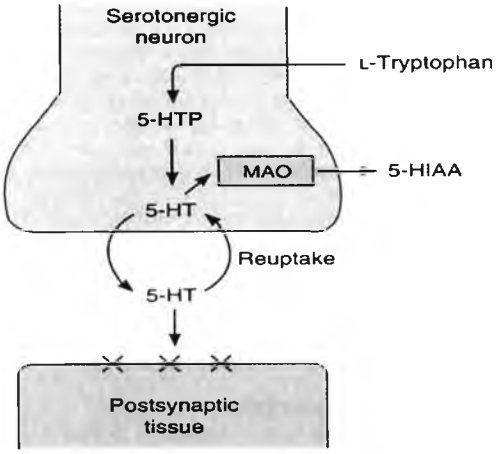


Figure 7. Biochemical events at serotonergic synapses. 5-HTP, 5-hydroxytryptophan; 5-HT, 5-hydroxytrytamine (serotonin); 5-HIAA, 5-hydroxyindoleacetic acid; X, serotonin receptor.

3.5.1 Serotonin pathway

The serotonergic system is known to modulate temperature, sleep, appetite, sexual behaviour and of course, mood. Serotonin is thought to play a role in various type of pathological condition: psychiatric disorders such as anxiety, depression, aggressiveness, panic, obsessive-compulsive disorders, schizophrenia, suicidal behavior and autism; neurodegenerative disorder such as Alzheimer's disease, Parkinsonism, and Huntington's chorea, migraine, emesis and alcoholism (Zifa and Fillion, 1992). Decreased serotonergic neurotransmission has been proposed to play a key role in the etiology of depression (Schloss and Williams, 1998). The serotonergic

neurons of the CNS have diverse rostral and caudal projections, with virtually all areas of the brain receiving serotonergic inputs (Figure 6). Serotonin-containing cell bodies in the ventral medulla and caudal pons provide descending projections to the dorsal horn of the spinal cord. The spinal cord also receives serotonergic afferents from the rostral serotonergic neurons at the postmesencephalic juncture. Serotonergic neurons in the pontine dorsal and medial raphe provide extensive serotonergic innervations of the cortex, thalamus, hypothalamus, limbic regions, and midbrain (Goldfarb and Wilk,1976; Deutch and Roth, 1999).

3.5.2 Serotonin receptor subtype

It is now known that 5-HT acts through a complex receptor system. Multiple 5-HT receptors have been divided into 5-HT_1 (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E} , 5-HT_{1F}), 5-HT_2(5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}), 5-HT_3(5-HT_{3A}, 5-HT_{3B}), 5-HT_4(5-HT_{4A}, 5-HT_{4B}), 5-HT_5 (5-HT_{5A}, 5-HT_{5B}), 5-HT_6, 5-HT_7(5-H_{7A}, 5-HT_{7B}, 5-HT_{7C}, 5-HT_{7D}) (Goodwin, 1996). In Figure 8 show the growing family of serotonin. Clinical relevance of serotonin receptor subtype was illustrated (Table 3). 5-HT receptor subtype, especially5-HT_{1A} and 5-HT_2 play a role not only in the illness of depression, but also in the treatment of depression (Perouta, 1995).

The 5-HT receptors are classify on the basis of their structural homology, which had been established by molecular biology, and their predominant transduction system. Serotonergic receptors are distinguished by the activity of which are coupled to a G-protein, from those directly linked to an ionic channel. The 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_4, 5-HT_6, and 5-HT_7 receptors were associated with adenylyl cyclase whereas the HT_{1C} and 5-HT_2 receptors were associated with PLC. The 5-HT_3 receptor appears to be directly linked to the opening and closing of an ionic channel that is not selective for monovalent cations (K^+ and Na^+) and which is independent of a G protein. Its activation induces a rapid depolarization (Zifa and Fillion, 1992; Dinan T.G, 1992). Brain serotonin receptors are the targets in the treatment of depression 5-HT 1A agonist (e.g.,

buspirone, ipsapirone), or 5-HT2 antagonist (e.g., mianserine, nefazodone, mirtazapine) are often effective in treatment of depression (Peroutka, 1995).

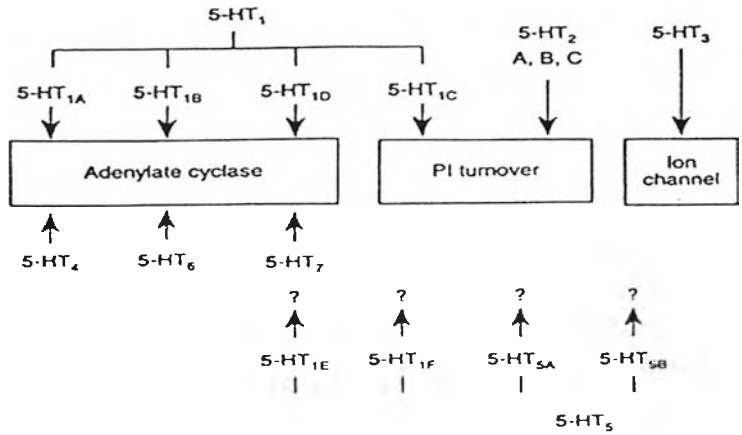


Figure 8. A diagram shows the growing family of serotonin (5-HT) receptors (Goodwin, 1996)

Table 3. Summary of clinical indications for serotonin receptor agents (Peroutka,1995)

Disease	Possible serotonin receptor(s) involved
Anxiety	5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_{3}
Depression	5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, Serotonin transporters
Migraine(acute)	5-HT_{1B}, 5-HT_{1D}
Migraine(prophylactic)	5-HT_{2A}, 5-HT_{2C}
Psychosis	5-HT_{2}
Nausea	5-HT_{3}
Gastroparesis	5-HT_{4}
Obsessive-compulsive Disorder	Serotonin transporter
Panic attacks	Serotonin transporter

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ANTIDEPRESSANT DRUGS

1. The first generation antidepressant

There are two main groups of antidepressant drugs, the tricyclic antidepressants (TCAs) and the monoamine oxidase inhibitors (MAOIs), which were both introduced into the clinical therapy of affective disorders in the late 1950s and are still used for the treatment of major depression. However, both of these groups have numerous side effects, and both are quite slow in onset of their antidepressant action

1.1 Tricyclic Antidepressants

Tricyclic antidepressants are generally categorized as tertiary or secondary amines, based on the degree of chemical substitution that exists on the terminal nitrogen of the side chain

Desipramine HCl(Norpramin), Nortriptyline HCl(Pamelor,Aventyl), Protriptyline HCl(Vivactil) are classified as secondary amines. Imipramine(Tofranil), Amitriptyline(Elavil, Endep), Trimipramine(Surmontil), Doxepin(Semeqiam) are categorizes as tertiary amines. Desipramine and nortriptyline are major metabolites of imipramine and amitriptyline, respectively. Tricyclic antidepressants are potent inhibitors of the neuronal re-uptake of NA and 5-HT. Generally, tricyclic secondary amines are more potent than the corresponding tertiary amines in inhibiting NA reuptake. On the other hand, tricyclic tertiary amines are more potent inhibitors of 5-HT re-uptake than the corresponding secondary amines. (Khanna, 1967)

The precise molecular mechanism responsible for the antidepressant action of the Tricyclic antidepressants (TCAs) is unknown. The action of drugs could alter the normal physiology of neurotransmission at norepinephrine and serotonin synapses of the brain. These resulted from an inhibitory effect of the TCA drugs on

neuronal membrane transport mechanisms (neuronal reuptake) present at the nerve terminal; reuptake serves to terminate biological activity of these amine transmitter substances (Azzaro and Ward, 1967)

The tricyclic antidepressants possess strong anticholinergic properties. They are also potent antihistamines. Cardiovascular effects are numerous and can be life threatening related in part to their anticholinergic property and to inhibition of catecholamine uptake. Tricyclic antidepressants also have direct toxic effects on cardiac muscle and may contribute to cardiomyopathies. The most common of the cardiovascular effects include orthostatic hypotension and/or tachycardia. Tachycardia results from actions of these agents on the autonomic nervous system. Orthostatic hypotension is caused by a combination of the weak antagonist properties of these agents at α1-adrenoceptors in vascular smooth muscle and ablunting of the carotid sinus reflex. Most antidepressants, including the TCA agents, cause a reduction in the seizure threshold. Therefore patients with a history of seizures must be dosed cautiously. Concurrent administration of TCA agents and sympathomimetic amines can augment the amine presser effects to the point of a hypertensive crisis.

1.2 Monoamine oxidase inhibitors (MAOIs)

Drugs that inhibit monoamine oxidase have proven useful in the treatment of depression. Classical MAO Inhibitors such as phenelzine(Nardil), isocarboxazid(Marplan), and tranylcypromine(Parnate) are a series of hydrazine and nonhydrazine that exhibited antidepressant properties. They are irreversible, nonselective inhibitors of MAO-A and MAO-B, However, their use has been severely restricted by its life-threatening toxicity such as hepatotoxicity and hypertensive crisis. These agents are generally reserved for use when the other antidepressant drug or when electroconvulsive therapy (ECT) is inappropriate, or in atypical depression.

Hepatotoxicity is especially likely to occur with isocarboxazid or phenelzine since hydrazine compounds can cause damage to hepatic parenchymal

cells. Hypertensive crisis can occur when patients receive MAOI and sympathomimetic amines, either as medication or in the diet (tyramine). Normally, these substances are rapidly metabolized by MAO-A present within the cells of the intestinal wall and MAO-A and MAO-B in the liver parenchyma. If each isozyme of MAO is inhibited, circulating levels of tyramine will be elevated. Tyramine is then free to interact with the sympathetic, noradrenergic nerve terminals innervating smooth muscle cells of the blood vessel to act inhibition of synaptic MAO, this norepinephrine-releasing action of tyramine can cause an elevation in blood pressure, leading to a hypertensive crisis.

Severe, sometimes fatal, interactions between nonselective irreversible MAO Inhibitors and pethidine(meperidine) and dextromethophan which have weak serotonergic properties have been reported about serotonin syndrome. (Rudorfer et al,1994) The serotonin syndrome is a condition of CNS excitabilidty induced when combinations of drugs with serotonergic activity are administered. It is characterised by myoclonus, agitation, hyperreflexia, mental status changes, diaphoresis and fever (Lejoyenx et al, 1994)

MAO enzyme are widely distributed in the periphery and central nervous system. They are located in the outer membranes of mitochondria in both neuronal and non-neuronal cells. MAO enzymes are responsible for oxidative deamination of endogenous monoamines such as adrenaline, noradrenaline, dopamine and serotonin as well as endogenous and exogenous xenobiotic amines (tyramine, octopamine, 2-phenylethylamine and tryptamine) in the CNS and the periphery. Two functional forms of MAO exist: MAO-A and MAO-B, which are distinguished by differences in substrate affinity, inhibitor specificity and tissue distribution. MAO-A preferentially deaminates adrenaline, noradrenaline and serotonin, whereas MAO-B is more selective for 2-phenylethylamine and benzylamine. Tyramine and dopamine are substrates for both isozymes (Fulton and Benfield , 1996; Fitton, et al. 1992)

MAO Inhibitors induce adaptive changes in CNS synaptic physiology over a period of 2 to 3 weeks. These changes result in both a down-regulation of synaptic transmission mediated through noradrenergic $\alpha 1$, β-adrenoceptors and an up-regulation or enhancement of synaptic transmission at serotonin synapses (at 5-HT1A receptor). This action on serotonin neurotransmission is the regulation of the firing rate of serotonin-containing neurons of the forebrain. Accordingly, these neurons fire at elevated state, releasing large quantities of serotonin into the synapse; this serotonin is protected from degradation by the inhibition of synaptic MAO-A.

2. Second generation antidepressants

Table 4. Some second generation antidepressants (Leonard,1993)

Drug class	Examples
1. Selective and reversible monoamine oxidase A inhibitors	Cimoxatone, Moclobemide, Brofaromine Befloxatone
2. Selective serotonin reuptake inhibitors	Fluvoxamine, Paroxetine, Sertaline Fluoxetine, Citalopram
4. Dopaminergic drugs	Amineptine, Minaprine
5. NE/5HT reuptake inhibitors	Venlafaxine
6. NE/DA reuptake inhibitors	Nomifensine
7. 5-HT1A partial agonists	Gepirone, Ipsapirine
8. Specific serotonin and adrenergic receptor blocker	Mianserine, Mirtazapine
9. 5-HT2A receptor blockage and weak 5-HT reuptake inhibition	Nefazodone, Trazodone

2.1 Reversible Inhibitors of Monoamine Oxidase type A (RIMAs)

This class including Moclobemide, Brofaromine, Befloxatone, Cimoxatone. The Reversible inhibitors of monoamine oxidase type-A agents are distinguished from the classical monoamine oxidase inhibitors (MAOIs) by their selectivity and reversibility (Lotufo-Neto et al,1999). The selective inhibitors bind to and block only one of the two isozyme, MAO-A or MAO-B. The inhibition of MAO-A cause the rise of Noradreanline, Dopamine, and 5-Hydroxytryptamine (5-HT) in the synaptic cleft, of MAO-B only of Dopamine. The new inhibitors diminish also to some extent the reuptake of monoamines . The Drugs only weakly potentiates the presser response induced by tyramine or indirectly acting sympathomimetics such as ephedrine, pseudoephedrine therefore hypertensive crises are quite rare. Moclobemide is reported to be a potent inhibitor of cytochrome P450 (CYP) 2D6, the primary enzyme involved in the metabolism of TCAs. Dosages of 300-450 mg/day may be initiated at full therapeutic. The maximum recommended dosage is 600 mg/day (Fulton and Benfield, 1996)

2.2 Selective serotonin reuptake inhibitors (SSRI)

Fluoxetine(Prozac) , Fluvoxamine(Luvox) , Paroxetine(Paxil) , Sertraline (Zoloft), and Citalopram(Celexa) are the member of this class of drugs. They work by increasing levels of serotonin in the brain. Because they act on serotonin specifically, they have favorable safety profiles and are milder side effects than those of the older classes of antidepressants. The most common side effects are nausea and gastrointestinal problems (Majeroni and Hess,1998). Drug interaction of SSRIs with monoamine oxidase inhibitors (MAOIs) has resulted in many adverse events, including the serotonin syndrome. SSRIs inhibit the cytochrome P-450 2D6 and other enzyme system in the liver, resulting in altered metabolism of a number other drugs. Paroxetine, Fluoxetine, and Fluvoxamine are the most potent inhibitors. Sertraline and Citalopram are less active. (Sheldon, 1999)

2.3 5-HT 2A receptor blockade and weak 5-HT reuptake inhibition

This class includes both nefazodone and its precursor, trazodone. Nefazodone is a structural analogue of trazodone and was designed with the goal of producing a better antidepressant than trazodone. Specifically. Nefazodone is a less potent antihistamine than trazodone. The antihistaminergic properties of trazodone are in part responsible for the use of it as a nonhabit-forming sleep aid rather than as an antidepressant. (Sheldon, 1999)

2.3.1 Trazodone

Trazodone HCl(Desyrel) was introduced in the early 1980s as a second generation antidepressant medication. It is very sedating, which can be beneficial for patients suffering from insomnia caused by their depression. It also has weak anticholinergic and cardiovascular effects.

2.3.2 Nefazodone

Nefazodone HCl(Serzone) is a phenylpiperazine antidepressant with a novel dual mechanism of action, acting as an antagonist at postsynaptic 5-HT2A receptors and as an inhibitor of serotonin and noradrenaline reuptake. Adrenergic activity is only slight, and there is virtually nc cardiotoxicity (Majeroni and Hess, 1998). As with most clinically active antidepressant drugs, long term administration of nefazodone down-regulates cortical 5-HT2A receptor. These actions are believed to modulate serotonergic neurotransmission through postsynaptic receptors, particularly 5- HT1A receptors.

Nefazodone is extensively metabolized in the liver to 4 metabolites: hydroxynefazodone, mCPP, a triazoledione metabolite and p-hydroxynefazodone. The

specific isoenzyme involved in the metabolism of nefazodone is most likely cytochrome P450 (CYP) 3A4; in vitro and in vivo data show that nefazodone inhibit the CYP 3A4 isoenzyme. The CYP 3A4 isoenzyme mediate the biotranformation of many drugs, including a number of psychotropic, cardiac, analgesic, hormonal, immunosuppressant, antineoplastic and antihistamine. (Von Moltke et al., 1995) Therefore, caution is advise whenever nefazodone is coadminister with any agents know to be metabolised by this isoenzyme. In particular, concomitant use of nefazodone and either terfenadine, astemizole or cisapride is contraindicate because of the increase potential for cardiac toxicity associated with increase plasma concentration of these drugs. Starting dosage for nefazodone in the treatment major depression is 100-200 mg/day, administered in 2 divided dose. An adequate therapeutic response is most likely to occur with dosages of between 300 and 600 mg/day (Davis, Whittington, Ruth and Bryson, 1997)

2.4 Specfic serotonin and adrenergic receptor blockers

2.4.1 Mirtazapine

Mirtazapine (Remeron) does not block the uptake pump for any of the biogenic amine neurotransmitters (ie, 5-HT, NE, and DA). Instead, mirtazapine blocks histamine-1 receptor (its most potent action) and specific serotonergic and adrenergic receptors: the 5-HT2A, 5-HT2C, 5-HT3 and alpha-2-adrenergic receptors.

Mirtazapine is a selective antagonist at α2-adrenergic auto-and heteroreceptors, which are involved in regulation of neuronal noradrenaline and serotonin release. Mirtazapine enhances noradrenergic neurotransmission via 2 synergistic mechanisms: by increasing raphe cell firing and by facilitating serotonin release. Mirtazapine-induced stimulation of serotonergic raphe cell firing results from enhanced noradrenergic transmission and is mediated via α1 adrenoceptors on the serotonergic cell body. The mirtazapine mediated increase in serotonin release is attributable to its blockage of inhibitory α2-adrenergic heteroreceptors located on the

serotonergic nerve terminal. The net effect of mirtazapine is therefore a combination of enhanced serotonergic cell firing and serotonin release. This results in specific potentiation of 5-HT1A- mediate neurotransmission in particular, because mirtazapine antagonises those effects mediated via 5-HT2 and 5-HT3 receptor

Serotonergic effects are mediated by a variety of serotonin receptor subtype. Stimulation of 5-HT1 receptor, especially the 5-HT1A receptor, is probably responsible for antidepressant effects. Stimulation of 5-HT2 receptor increase wakefulness, while blockade of these receptors increase deep sleep. Stimulation of 5-HT3 receptor is associated with the appearance of adverse effects such as anxiety and neasea.

Mirtazapine does not inhibit monoamine re-uptake and is inactive in classical tests predictive of antidepressant (antagonism of reserpine-induced hypothermia, Persolt test, and muricidal behaviour) (Davis, Whittington, Ruth, and Bryson,1997; de Boer and Ruigt, 1995)

2.4.2 Mianserin

Mianserin is a tetracyclic piperazino-azepine compound and structurally different from tetracyclic drugs of the maprotiline type. It has only weak effects in blocking monoamine reuptake but it blocks presynaptic α2-adrenoceptors, thereby increasing NA turnover. It has antagonizing effect on 5-HT1, 5-HT2, and 5-HT3 receptor.

2.5 Selective NE/5-HT reuptake inhibitors

2.5.1 Venlafaxine

Venlafaxine(Effexor) is chemically unrelated to tricyclic, tetracyclic, or other available antidepressant agents. It is a serotonin and norepinephrine reuptake inhibitor and weak inhibitors of dopamine reuptake. Venlafaxine and its active metabolite, o-desmethylvenlafaxine (ODV) have no significant affinity for muscarinic,

histaminergic, or α-1 adrenergic receptors. Pharmacologic activity at these receptors is hypothesized to be associated with the various anticholinergic, sedative and cardiovascular effects seen with other psychotropic drugs. They do not posses monoamine oxidase inhibitory activity.

2.6 Mixed Norepinephrine/Dopamine reuptake inhibitors

2.6.1 Nomifensine

Nomifensine (Merital) is an antidepressant with a novel tricyclic structure, and it is a potent reuptake inhibitor of dopamine at central synapes. It also inhibit norepinephrine reuptake but is only a weak inhibitor of 5- hydroxytryptamine (Fields, 1982). The usual effective dose of nomifensine is 100-200 mg daily in divided dose. Nomifensine has caused few side effect, minimal anticholinergic and sedative effect (Kinney, 1985)

2.6.2 Bupropion

Bupropion HCl(Wellbutrin) is a potent inhibitor of the dopamine neuronal reuptake system and down-regulates β-adrenoceptor function in about 40 percent of the animals tested following chronic dosing.

Because of a low incidence of side effects, bupropion is well tolerated by patients of all ages, including the elderly including nervousness and insomnia; higher dose may cause seizures (4/1000 patients) Thus, bupropion is contraindicated in aptients with a history of seizure or head trauma, or those who are taking medications that lower the seizure threshold (antipsychotics, TCA drugs).

2.7 Noradrenergic Drugs

2.7.1 Maprotiline

Maprotiline(Ludiomil), a tetracyclic antidepressant, strongly inhibits NA reuptake but does not block 5-HT. It has a strong antihistaminic and a weak anticholinergic action (Khanna, 1967)

2.8 Dopaminergic Drugs

2.8.1 Amineptine

Amineptine is an atypical tricyclic, differing from the typical compounds in its 7-aminohepatanoic acid side-chain. It has a distinct pharmacological action in its ability to both inhibit the uptake of dopamine and increase its release (Philip et al, 1995). Chronic treatment with amineptine induces downregulation of dopamine D2, beta- and alpha 2-adrenergic receptors (Garattini, 1997). Major problem with amineptine are a significant potential for abuse and hepatotoxicity.

2.8.2 Minaprine

Minaprine is a nontricyclic compound that enhance dopaminergic and serotonergic transmission. It has no effect on DA release or uptake and also has no affinity for DA D1 or D2 receptors. Behaviour consistent with enhancement of DA activity such as low dose of minaprine antagonize haloperidol-induced catalepsy; it induces stereotypic behavior in rats and it induces contralateral rotations in mice with unilateral striatal lesions. (Kan, Mouget-Goniot, Worms, and Biziere, 1986) Minaprine action on serotonin to increase 5-HT and decrease 5-HIAA level in many brain areas, the mechanism of this effect is unclear. Minaprine affect neither the release nor thr uptake of 5-HT. Similarly, it has no affinity for either 5-HT1 or 5-HT2 receptors. (Mitchell,1995)

ANIMAL BEHAVIORAL MODELS FOR THE EVALUATION OF NEW ANTIDEPRESSANT DRUGS

The purpose of studying the effect of drugs in animals is to predict the effect of the same drugs in man. Animal test systems are required in studies designed to clarify not only the safety and efficacy of drugs in humans but also the mechanism of drug action.

A model is defined as any experimental preparation developed for the purpose of studying the same condition in different species. Typically, models are animal preparations that attempt to mimic a human condition, including human psychopathology (Geuer And Markou , 1995)

Many animal experimental procedures are used to screen, predict, and evaluate the potential therapeutic activity of psychotropic drugs such as antidepressants. In addition to biochemical and electrophysiological methods, behavioral models play a major role in these studies. Behavioral procedures for studying antidepressants use drug-, lesion-, or environment-induced behavior changes. However, human psychiatric disorders cannot be induced in animals. Therefore the models cannot be claimed to reproduce human psychopathology, but they are intended to induce changes that are sensitive to the therapeutic agents, in a manner predictive of their effects in humans (Thiebot, Martin, and Puech, 1992).

1. Reserpine reversal

At one time, reserpine was an agent commonly used in the treatment of agitated psychotics but the nature and high incidence of its side effects largely precluded continued usage. The most serious side effect was the occurrence of mental depression. Reserpine-induced depression also provided evidence in favor of the catecholamine theory of depression. Reserpine depletes the brain, as well as the

periphery, of both catecholamines and serotonin. This probably occurs as a result of inhibition of uptake of biogenic amines into storage granules and their subsequent intraneuronal metabolism by monoamine oxidases. Dibenzazepines are thought to antagonize reserpine by inhibiting presynaptic reuptake of NE after its release; that is, the effects of intraneuronal NE depletion are supposedly countered by an increase in synaptic NE (Glick, 1976)

The syndrome induced by reserpine and related agents, such as tetra-benazine, is characterized by ptosis (eye closure), hypothermia and akinesia. These effects can be blocked or reversed by a variety of antidepressant drugs (Sigg, 1967)

1.1 Reserpine-induced ptosis

Reserpine induced ptosis in rodents. The dose of reserpine in mice is 2.5 mg/kg by intraperitoneal route. The test drugs were administered 4 h after reserpine and palpebral ptosis was scored every 30 min for 2 h. Reserpine-induced ptosis is rated 0-4. In each animal the optimal score (the most effective) is 8 (both eyes).

The score system for eyelid opening is that of Rubin, Malone, Waugh, and Burke (1957).

0: eyelids completely closed; 1: eyelids ¼ opened; 2: eyelids ½ opened; 3: eyelids ¾ opened; 4: eyelids completely opened

1.2 Reserpine-induced hypothermia

Reserpine induces a marked and prolonged hypothermia in rodent. Antidepressants, such as dibenzazepine, administered after reserpine readily produce a prolonged reversal of the hypothermia. In each experiment, the rectal temperature was recorded twice with a probe thermometer at a constant depth. At first the temperature was measured at 3.5 h after reserpine administration in mice at dose of 2.5 mg/kg i.p.. The mice were divided into groups in such a way that the mean rectal temperature was

the same in each group. Drug administration was given 4 h after reserpine and temperature was measured 90 min after drug administration (Simiand *et al.*,1992)

1.3 Reserpine-induced immobility (akinesia)

Reserpine depresses locomotor activity and, at higher doses, induces immobility and catalepsy in rodents. Reduction of motor activity appears to be related to a decrease in dopaminergic activity Antidepressants such as tricyclic or MAO Inhibitor antagonise this effect (Hollinger *et al*, 1969).

This test is usually performed at the same time as the 2 preceding tests. Mice are housed in individual cages (20×10×10 cm) and reserpine is injected. Akinesia is assessed 30 min after test drug administration. The reserpine induced akinesia is only considered to be antagonised if a mouse changes position is such a way that its body was not prostrate and the mouse had to walk a distance equal to at least the whole length of its body. Antagonism of akinesia is used as a criterion for a positive effect in at least 50 % of the mice tested even if the effect is not dose-dependent (Bourin, 1990).

The reserpine-induced akinesia is only considered to be antagonized if a mouse changed position is such a way that its body was not prostrate and the mouse had to walk a distance equal to at least the whole length of its body. Antagonism of akinesia is used as a criterion for a positive effect in at least 50% of the mice tested.

2. Amphetamine potentiation

Many antidepressants such as dibenzazepines potentiate behavior effects of amphetamine in animals. This occurs because amphetamines are thought to release catecholamines from presynaptic terminals and also increase synaptic concentrations of NE and inhibit NE reuptake. Dibenzazepines inhibit presynaptic reuptake of NE resulting in presumably higher synaptic concentrations of NE. Because both kinds of drug have similar action that would be expected to synergize, the

interaction of antidepressants with amphetamine has been predictably useful screening procedures. Some of these procedures include the following

2.1 Amphetamine-induced hyperthermia

2.2 Amphetamine-induced stereotypy

Arntred and Randrup (1968) have suggested the existence in the brain of a dopaminergic-cholinergic balance in the nigrostriatal system influencing stereotypy. These amphetamine-induced effects are also potentiated by dibenzazepine and other antidepressant drugs. Amphetamine 5 mg/kg i.p was selected as the dose which would reliably induce a stereotyped behavior in rats that were placed in individual cages measuring 30cm×20 cm and 15 cm high (Costall, Naylor, and Olley, 1972). Intensity was assessed at 15-min intervals according to the scoring system. The maximal effect was attained 60 min after amphetamine administration.

Scoring system used for estimation of the intensity of stereotypy

Score	*Description of stereotyped behavior*
0	The appearance of the animals is the same as saline treated rats
1	Discontinuous sniffing, constant exploratory activity
2	Continuous sniffing, periodic exploratory activity.
3	Continuous sniffing, discontinuous biting, gnawing or licking. Very brief periods of locomotor activity.
4	Continuous biting, gnawing or licking; no exploratory activity.

3. Behavioural despair test

In this procedure, mice or rats are forced to swim in a confined space. The animals initially make vigorous attempts to escape, but then assume increasingly

long periods of immobility, during which the only movements made are those that are necessary to keep the nose above the water level. This state has been named 'behavioral despair'. It is assumed that the animals have 'given up hope of escaping' (Persolt, Bertin, Blavet, Deniel, and Jalfre,1977, Porsolt, Anton, Blavet and Jalfre, 1979). The total immobility time is reduced by the majority of antidepressants, including tricyclics, MAOIs, atypical antidepressant, newer antidepressants. (Thiebot, Martin, and Puech, 1992) Nevertheless, many false positives have been reported for stimulants, convulsants, anticholinergics, antihistamines, pentobarbitals, opiates and other brain peptides, and a number of other drugs. (Willner, 1984; Borsini and Meli, 1988) The immobile behaviour cannot be explained by physical exhaustion as, in other situations, animal are able to swim vigorously for much longer than the usual duration of the forced swimming test. Also, antidepressant-induced reduction of immobility cannot be explained by non-specific behavioural stimulation. Indeed, most antidepressants tend to decrease motor activity

4. Learned helplessness test

This test is based on the observation that exposure to uncontrollable stress produces performance deficit in subsequent learning tasks that are not seen in subjects exposed to identical stressors but able to control it. (Geyer and Markon, 1995; Willer, 1984) Learned helplessness could be reversed by subchronic treatment (4-7 days) with a variety of antidepressants.

The test method described by Mannisto *et al.* (1995): Preconditioning with inescapable electric foot shock was performed on day 1. A constant current shocker delivered 60 scramble, inescapable shocks (15 sec duration, 0.8 mA, every 1 min) to the grid floor. Conditioned avoidance sessions were performed on day 5. Animals were placed individually into the shuttle-box that divided into two equal chambers and had an open door between them. The animals were subjected to 30

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trials. The rats could avoid shocks by moving into the other compartment of the box. Failing to escape during the shock period was recorded.

5. Dopa-potentiation

Dopa, the precursor of the catecholamines dopamine (DA) and noradrenaline (NA) is administered following pretreatment with an MAOI to protect newly synthesized amines. Signs of adrenergic stimulation are seen, including piloerection, locomotor activity, irritability and aggression. These effects are potentiated by pretreatment with tricyclic antidepressants

6. Yohimbine potentiation

Yohimbine is an alpha$_2$-receptor antagonist. The dose of yohimbine 25 mg/kg, subcutaneously, induced death on average by 1 mouse in each of 10 (Quinton,1963). The lethality of yohimbine was increased in mice by tricyclic antidepressants and, MAOIs, but also by a variety of other drug classes. However some drugs such as anticholinergics, antihistamines, appear as false positive response. (Sangvi and Gershon, 1969)

Drugs were administered i.p. to mice, 30 min before yohimbine hydrochloride injection (25 mg/kg s.c) and mortality was observed at 1,2 and 3 h and recorded at 24 hours in comparison with controls.

7. L-5-HTP potentiation

5-HTP is the precursor of 5-HT. It induced behavioral effects in mice for example tremor, head-twitch and hind-limb abduction. The most antidepressants potentiated L-5-HTP-induced behavioral effect in mice (Hiromi *et al.* 1997).

Mice were dosed orally at 48 min or 30 min before administration of L-5-HTP (90 mg/kg i.v) according to the method of Bogdanski *et al* (1958). The mice were rated for the presence of each symptom at 5-10 min after L-5-HTP treatment. In fact, the model was explicitly developed as a behavioral system within which to test the effects of drugs on 5-HT neurotransmission.

8. Separation models

This model is present to some extent in many species, including cats, dogs, rodents and precocial birds. The only worthwhile animal models of depression are those involving separation phenomena in nonhuman primates (Willner, 1984).

Infant monkeys respond to maternal separation by an initial stage of 'protest' characterized by agitation, sleeplessness, distress calls and screaming, followed after 1 or 2 days by 'despair', characterized by a decrease in activity, appetite, play and social interactions, and the assumption of a hundred posture and sad expression (Mckinney and Bunney, 1969; Willner, 1984).

9. Muricide antagonism

Male rats are isolated and deprived of food for a day before being placed together with a mouse. The mouse-killing behavior (muricide) of rats (by biting the cervical vertebrae) is antagonized by any antidepressant such as imipramine and other tricyclics. Although the behavior employed in the test is rather extraordinary, there is a rational explanation for its use. The pertinent theory is that antidepressants act by inhibiting the amygada. The theory (Horovitz, 1966) is supported by observations that bilateral amygdaloid lesions abolish muricide behavior that antidepressants inhibit amygdaloid after discharges (Glick, 1976)

10. Olfactory bulbectomy

Rats subjected to bilateral lesions of the olfactory bulbs show a variety of behavioral changes that can be reversed by antidepressant drugs. These changes, including irritability, hyperactivity and an elevation of circulating levels of plasma corticosteroids, as a result of their hyperactivity the animals are also deficient in passive avoidance leaning (Willner, 1984).

The lesion procedures make use of the behavioral and biochemical changes caused by the destruction of a limited area of the brain: for example, rats subjected to bilateral lesions of the olfactory bulbs show a variety of changes. These behavioral changes can be reversed by antidepressants.

11. Intracranial self-stimulation model

The intracranial self-stimulation (ICSS) paradigm requires that animals be prepared with intracranial electrodes aimed at specific areas (for example, the medial forebrain bundle) of the brain in rewarding. Animal will readily learn to press levers in order to receive such stimulation. Both the rate of response for ICSS and the psychophysically defined threshold for ICSS have been used as measures of the reward value of the stimulation.

12. Isolation-induced hyperactivity

If animals such as rats are reared in social isolation from an early age (2-3 weeks) they will show a marked hyperactivity when compared to group-reared control. The activity difference between isolated and group reared animals was abolished by acute treatment of a variety of antidepressants (Garzon, 1979; 1981).

13. Restraint-stress

In this procedure, rats are subjected to an acute two-hour restraint stress. Twenty-four hours later, they show behavioral changes including a reduction of locomotor activity and food intake. After repeated restraint (2 h/day for 7 days), the behavioral deficits disappear (Kennett, 1987). The locomotor deficit was prevented by chronic pretreatment with antidepressants. Similar behavioral effects which are sensitive to antidepressants administered chronically have also been described with chronic, unpredictable stresses (Thiebot, Martin, and Puech, 1992)

14. Chronic unpredictable stress

During a 3-week period, rats were subjected to a variety of different stressors, including, among others, electric shocks, immersion in cold water and reversal of the light/dark cycle. At the end of this period, they received a session of exposure to loud noises and bright lights, followed immediately by an open field test. In unstressed animals, the noise/light session caused an increase in open field activity, but this effect was not seen in chronically stressed animals. The effect was, however, restored by daily antidepressant treatment during the chronic stress period (Willner, 1984; Katz, 1982).

SYNTHETIC CHEMICALS FOR INVESTIGATION OF ANTIDEPRESSANT ACTIVITY

CU 763-14-07 and CU 763-14-10

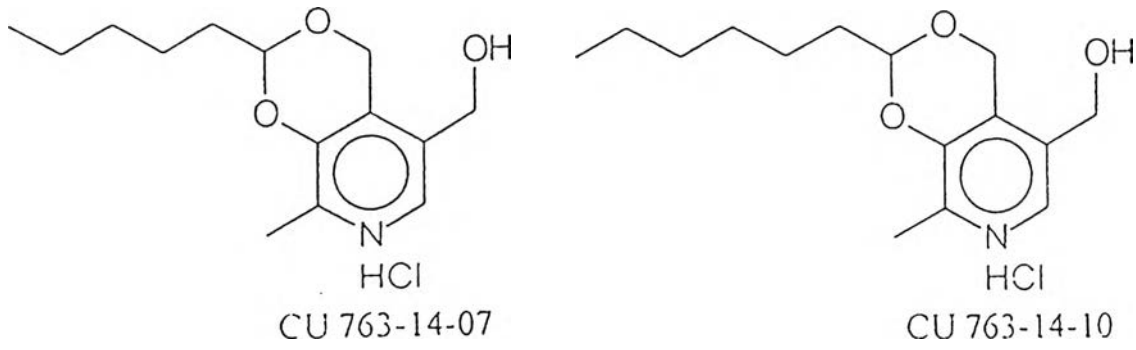

Figure 9. Structural formulae of CU 763-14-07 and CU 763-14-10

CU 763-14-07 and CU 763-14-10 are cyclic acetal derivatives of pyridoxine (Sutthatip, 1997). They were synthesized by Dr. Chamnarn Patarapanich's group in Department of Pharmaceutical Chemistry, Chulalongkorn University.

Previous studies *in vitro* by Chanika Ratanachol with CU 763-14-07 and Nopawan Wongsomnuk with CU 763-14-10 on isolated rat liver mitochondrial monoamine oxidase activities were made using tyramine (nonspecific substrate), 5-hydroxytryptamin (prefered substrate for MAO-A), and benzylamine (prefered substrate for MAO-B) as substrate. The resulted revealed that CU 763-14-07 and CU 763-14-10 can inhibit monoamine oxidase enzyme both MAO-A and MAO-B. They behave as the nonspecific inhibitor. Moreover it appears that MAO-A and MAO-B was nearly inhibited by CU 763-14-07. The IC50 (concentrations producing 50 % inhibition) were 11 and 10 μM respectively (Ratanachol, 1997). Whereas CU 763-14-10 is being more potent inhibitors of the type B enzyme species than type A. The IC50 was 1.9 μM when used benzylamine as substrate but the IC50 was 18.82 μM by using 5-hydroxytryptamine as substrate. The value was higher than 10 fold indicated that CU 763-14-10 can inhibit MAO-B higher than MAO-A (Wongsomnuk, 1998)