Role of Norepinephrine in Depression

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This article reviews the role of norepinephrine (NE) and serotonin (5-HT) in depression and the therapeutic effects of antidepressant drugs from the perspective of human neurotransmitter depletion studies. The data reviewed suggest that both noradrenergic and serotonergic systems are involved in antidepressant action, but the specific impairment that underlies depression is unclear and is likely to vary among patients. Results from neurotransmitter depletion studies in depressed patients who have responded to treatment suggest that, while interactions between NE and 5-HT are likely, neither of these 2 neurotransmitter systems is the final common pathway for the therapeutic effect of antidepressant drugs. NE-selective antidepressant drugs appear to be primarily dependent on the availability of NE for their effects. Likewise, 5-HT–selective antidepressants appear to be primarily dependent on the availability of 5-HT for their effects. Antidepressants that cause effects on both noradrenergic and serotonergic systems—such as mirtazapine—may be dependent on the availability of both neurotransmitters for their effects. Neither 5-HT nor NE depletion induced clinical depression in healthy subjects or worsened depression in unmedicated symptomatic patients with major depression. This finding suggests that the cause of depression is more complex than just an alteration in the levels of 5-HT and/or NE. For some patients, depression may be more directly caused by dysfunction in brain areas or neuronal systems modulated by monoamine systems. We propose that antidepressant drugs may enhance neurotransmission in normal noradrenergic or serotonergic neurons and, through a time-dependent but as yet undiscovered process, restore function to brain areas modulated by monoamine neurons. Future research should focus on understanding the adaptive changes that follow enhancement of synaptic levels of monoamines in neuronal circuits of the frontal cortex, amygdala, and hippocampus. Research investigating the neurobiology of depression may be more informed if the focus is shifted to investigating areas of the brain modulated by monoamine systems rather than the monoamine systems themselves.

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Since the discovery in the mid-1980s that selective serotonin reuptake inhibitors (SSRIs) were effective antidepressants, researchers have focused almost exclusively on trying to understand the role of serotonin (5-HT) in the etiology of depression and its mechanism of antidepressant action. This shift in attention away from norepinephrine (NE) was supported by the theory that enhancement of 5-HT neurotransmission might be a final common pathway for all antidepressants, irrespective of their initial neurobiological effects.[1,2]

The reawakening of interest in the role of NE in depression and antidepressant effects was fueled in part by results from neurotransmitter depletion studies.[3–7] These studies showed that the therapeutic effects of SSRIs could be transiently reversed by rapid depletion of 5-HT but not by depletion of NE. Conversely, the therapeutic effects of an NE reuptake inhibitor (i.e., desipramine) could be transiently reversed by depletion of NE but not by depletion of 5-HT.

This article will review the results of human neurotransmitter depletion studies in depression. These studies have led our group at the University of Arizona[8] to suggest that antidepressants may work through multiple mechanisms, of which NE and 5-HT are just 2. An important implication is that a deficiency of monoamines may not be the sole cause of depression. Some have argued that depression may be due to a deficiency of NE or 5-HT because the enhancement of noradrenergic or serotonergic neurotransmission improves the symptoms of depression. However, this is akin to saying that because a rash on one's arm improves with the use of a steroid cream, the rash must be due to a steroid deficiency. The data to be presented do not prove that depression cannot be caused by a deficiency or dysfunction of NE or 5-HT. However, it is hard to reconcile a deficiency model with the results of studies inducing a depletion of NE or 5-HT in humans.[8]

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NEUROTRANSMITTER DEPLETION PARADIGM

The idea for determining the biological role of a chemical substance by depleting it dates back to studies that investigated the importance of various nutrients on human health. These studies led to the concept of minimum daily requirements for many nutrients, including most amino acids.[9] The first published studies to investigate the effects of neurotransmitter depletion on depression were reported in the 1970s.[10–13] These studies used drugs that inhibit synthesis of 5-HT or NE by blocking the rate-limiting enzymes involved in their synthesis. Para-chloro-phenylalanine (PCPA), a tryptophan (TRP) hydroxylase inhibitor, has been used to inhibit 5-HT synthesis.[12,13] For inhibition of NE and dopamine synthesis, alpha-methyl-para-tyrosine (AMPT), a tyrosine hydroxylase inhibitor, has been used.[10,11]

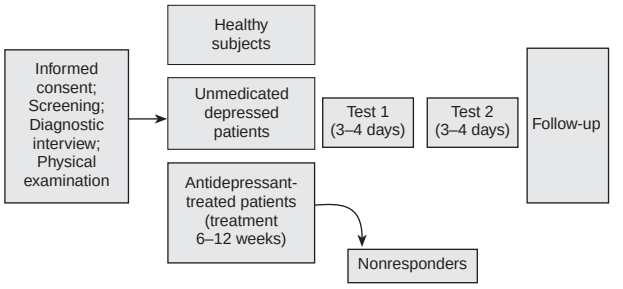
Shopsin and colleagues reported that inhibiting 5-HT synthesis in patients with major depression appeared to rapidly reverse the antidepressant effects of both imipramine[12] and tranylcypromine.[13] Their studies showed that antidepressant-remitted depressed patients experienced a depressive relapse within 24 hours of initiation of PCPA treatment.[12,13] But, within 24 hours of discontinuing PCPA, the patients' depressive symptoms remitted to their pretest state. In contrast, 3 depressed patients who had experienced a therapeutic response to imipramine were challenged with AMPT. The challenge resulted in no significant effect on the antidepressant response.[12] These early depletion studies by Shopsin and colleagues[12,13] were largely ignored because of the lack of a placebo control and the small number of patients tested.

In the mid 1980s, as the first SSRIs (e.g., fluvoxamine) became available for research, the research group at Yale became interested in directly challenging the 5-HT hypothesis of depression and antidepressant action.[14] It was surmised that, if depression were due to a deficiency in 5-HT neurotransmission, one should be able to induce clinically relevant depressive symptoms by depleting 5-HT. Further, if antidepressants work by enhancing 5-HT levels, then one should be able to induce depressive symptoms in patients who have demonstrated a response and continue to take the antidepressant.

Tryptophan Depletion Studies

Because PCPA was not commercially available and had been associated with eosinophilia, it was decided to investigate the use of rapid TRP depletion as an alternative method for depleting 5-HT.[14] TRP is well established as an essential amino acid and, therefore, is not synthesized by humans.[9] It has also been well established that 5-HT levels in the brain are dependent on plasma levels of TRP. Work at McGill University by Simon Young and colleagues[15] had already demonstrated that plasma TRP could be rapidly, safely, and reversibly depleted in young men using an oral

Figure 1. Neurotransmitter Depletion Test Design



administration of a liquid suspension of amino acids lacking in TRP. This mixture induces protein synthesis in the liver, leading to a rapid depletion of plasma TRP as the ingested amino acids are converted into protein.[16] Additionally, the competition for entry across the blood-brain barrier caused by the influx of other large neutral amino acids further reduces the levels of TRP in the brain. A considerable body of work has previously demonstrated that brain 5-HT levels decrease proportionally within 1 to 2 hours of changes in plasma TRP.[3] Further, it has been documented that plasma TRP levels are reduced in healthy subjects within 5 hours of the ingestion of a TRP-free drink with no serious medical or psychological complications.[9,14,15] To monitor the extent of 5-HT depletion following the ingestion of a TRP-free drink, participants in a recent study were evaluated with positron emission tomography, and the results were compared with baseline studies. 5-HT synthesis was reduced in the healthy male and female subjects by 87% and 97%, respectively.[17]

The studies outlined in the following sections of this report involved patients who met criteria for major depression and no other Axis I condition or who were healthy control subjects without a prior diagnosis of mental or physical illness. For those patients on antidepressant therapy who were tested following treatment, testing was provided for a minimum of 6 weeks, frequently for as long as 10 to 12 weeks. The stipulation for testing was that patients had to achieve and maintain at least a 50% decrease in their Hamilton Rating Scale for Depression (HAM-D) scores compared with baseline.[18] Testing typically began within 2 weeks of meeting these criteria.

Patients were scheduled for a depletion or control test a week apart. All tests were conducted in a double-blind, placebo-controlled fashion. The sequence for the testing was assigned randomly in a balanced pattern. Testing was performed in a closely supervised setting. Figure 1 depicts an outline of the testing sequence used in these depletion studies.[3,4,19,20]

A 3-day test period was employed for the TRP depletion studies: baseline, depletion, and follow-up. Subjects were asked to drink the mixture of amino acids (Table 1).

On one test week, the mixture included TRP, and on the alternate week, it did not. Typically, the ingestion of the TRP-free drink led to an 80% to 90% depletion of TRP within 5 hours of consuming the preparation.[3,15] Because cysteine, methionine, and arginine are extremely unpalatable, they were encapsulated. The other amino acids were suspended in approximately 300 cc of water and rapidly gulped down.

Table 1. Composition of the Amino Acid Drink

L-Alanine	5.5 g	L-Serine	6.9 g
Glycine	3.2 g	L-Threonine	6.9 g
L-Histidine	3.2 g	L-Tyrosine	6.9 g
L-Isoleucine	8.0 g	L-Valine	8.9 g
L-Leucine	13.5 g	L-Methionine	3.0 g
L-Lysine HCl	11.0 g	L-Arginine	4.9 g
L-Phenylalanine	5.7 g	L-Cysteine	2.7 g
L-Proline	12.2 g	(± L-Tryptophan	2.3 g)

Catecholamine Depletion Studies

AMPT was used for the evaluation of catecholamine depletion. The production of NE is dependent on a conversion of tyrosine to L-dopa by the enzyme tyrosine hydroxylase. This step can be inhibited by administering AMPT. AMPT is an effective inhibitor of the synthesis of NE (and dopamine), as is evidenced by the decreased excretion of urinary and cerebral spinal fluid (CSF) levels of 3-methoxy-4-hydroxyphenylglycol (MHPG) and homovanillic acid (HVA) following AMPT administration with no concurrent effects on 5-HT metabolism.[10,21,22] A concomitant reduction in brain catecholamine levels has also been demonstrated.[23]

Catecholamine-depletion studies follow a 4-day test cycle: baseline, 2 depletion days, and follow-up. The depletion days involve the ingestion of a total of 3 g of AMPT in divided doses over the middle 2 days. For the "placebo" test, participants were given 25 mg of diphenhydramine 3 times daily instead of AMPT. The rationale for using diphenhydramine was that AMPT is known to cause sedation, and the use of diphenhydramine provided a comparable degree of nonspecific sedation on the control group. Its use, therefore, creates a truly double-blind comparison for the experiment.

Patient Monitoring

For both types of depletion studies, experienced clinicians closely monitored the study participants. Participants were kept in a neutral research environment and questioned at regular intervals. Blood samples for biological measurements were also taken at regular intervals.[3,6,7,19] While both TRP and catecholamine depletion tests and their corresponding placebo tests are stressful, it is noteworthy that there have been no reported depressive responses during placebo testing in antidepressant-treated patients in the published studies.[3,5–7,20]

MECHANISM OF ANTIDEPRESSANT ACTION

The pharmacologic effects of most antidepressant drugs have been relatively well characterized. Despite this, the specific properties that underlie their therapeutic effects are not completely understood. Theories that postulate a central role for the enhancement of 5-HT[2,24,25] or NE neurotransmission[26–29] have the greatest support. The premise of these hypotheses is that antidepressants increase neurotransmission through the noradrenergic and/or serotonergic systems because of a time-dependent process of neuronal adaptation.[30]

The Role of Serotonin: Effects of Tryptophan Depletion in SSRI-Treated Depressed Patients

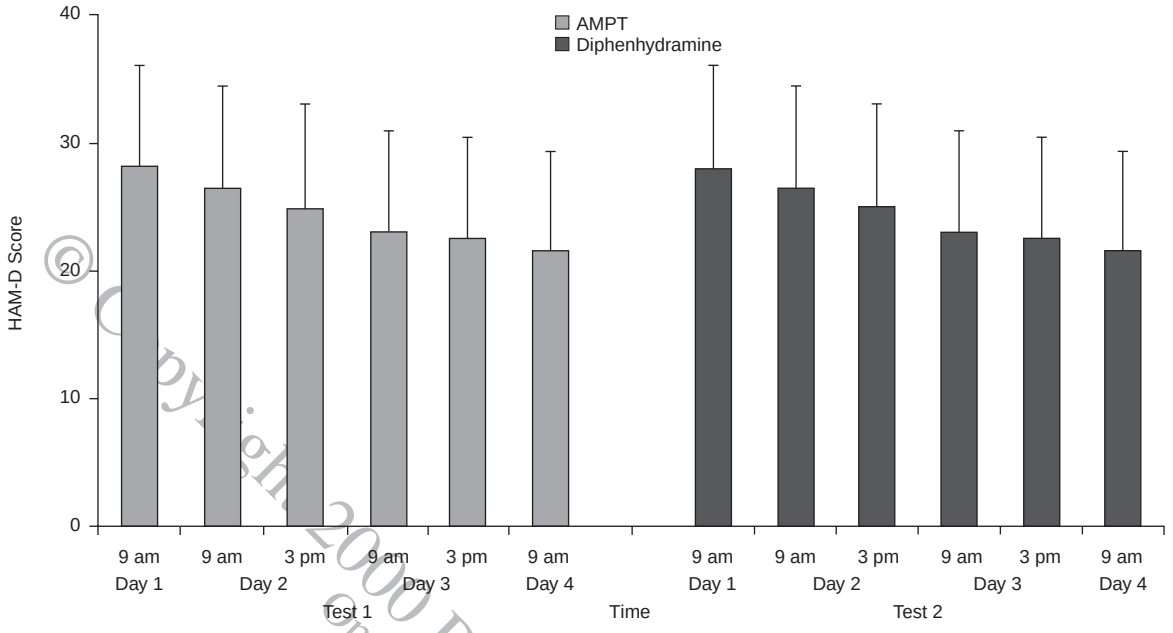
Our first study[3] of the effects of TRP depletion on patients who had been responders showed that 14 (67%) of 21 patients experienced a relapse of depressive symptoms (50% increase in HAM-D score with total score ≥ 17) during TRP depletion but not during control treatment. The symptoms reported by the patients were the same as those they had experienced prior to antidepressant therapy. Patients who had successfully responded to the relatively selective NE reuptake inhibitor desipramine were less likely to relapse (20% relapse rate) than those who had responded to an SSRI or monoamine oxidase inhibitor (90% relapse rate).

These results have been confirmed in a prospective study[20] of patients who were either naive to antidepressant treatment or who had previously responded successfully to treatment. Patients in the confirming study were depleted of TRP in a double-blind manner after at least 6 to 10 weeks of treatment with either desipramine or fluoxetine. Six (46%) of the 13 responders to fluoxetine experienced a relapse, while only 1 (8%) of the 13 desipramine responders relapsed.

Notably in these studies, the depressive symptoms experienced by individual patients during depletion were the same as those experienced by the patient prior to antidepressant therapy and were characteristic of the individual. All of the patients who experienced depressive symptoms recovered within 48 hours of testing, and none of the patients required a change in their treatment due to the effects of testing.

Another notable feature of these studies is the contrast in the rate of relapse in the patients maintained on desipramine therapy compared with those maintained on fluoxetine therapy. Again, no placebo effect was observed, but the rate of relapse with desipramine was much lower on TRP depletion—only 20% to 25%—and the degree of relapse was lower, as judged by the total HAM-D scores. These results led to a renewed interest in the role of NE in antidepressant effects, culminating in the catecholamine depletion studies described below.

Figure 2. Effects of AMPT and Diphenhydramine Challenge on HAM-D Scores in Depressed Patients[a]



[a]Data from Delgado et al.[5] and Miller et al.[7] Abbreviations: AMPT = alpha-methyl-para-tyrosine, HAM-D = Hamilton Rating Scale for Depression.

The Importance of Norepinephrine: Effects of Catecholamine Depletion in Desipramine-Treated Patients With Depression

In order to investigate the relationship of NE to antidepressant responses, we used a design similar to that of the TRP depletion.[5,7] The test periods in this study were conducted on consecutive weeks, and each was conducted over 4 consecutive days, as depicted in Figure 1. Patients who maintained a response to desipramine or mazindol (predominantly noradrenergic antidepressants) or to fluoxetine or sertraline (serotonergic antidepressants) were admitted to the depletion phase of the study. Plasma levels of MHPG and HVA, metabolites of NE and dopamine, respectively, were significantly decreased with AMPT treatment but not with diphenhydramine. Consistent with previous findings, diphenhydramine had no effect on depressive symptoms (Figure 2). Patients who responded to desipramine were at much greater risk of relapse (approximately 80% relapsed) compared with only 20% to 25% of patients maintained on fluoxetine.[5,7] As with TRP depletion, the features of the depressive symptoms were characteristic and unique to each patient.

NEUROBIOLOGY OF DEPRESSION

While our understanding of the clinical aspects of the phenomenology, prevalence, course, and treatment of major depression has advanced considerably, the neurobiological basis of depression remains unknown. For many years, the neurobiological basis of depression has been conceptually linked to the mechanism of antidepressant action. Most models have been largely 2-dimensional, postulating either an actual or a functional deficiency in various monoamine neuronal systems. While strong support exists for the role of monoamine systems in the therapeutic mechanism of antidepressant action, intense investigation has failed to find convincing evidence of a primary dysfunction of a specific monoamine system in the pathophysiology of depression.[31] In part, progress in solving this puzzle has been inhibited by the lack of direct methods for testing these hypotheses in humans. The advent of neurotransmitter depletion paradigms has begun to provide new data relevant to hypotheses regarding the role of monoamine systems in the neurobiological basis of depression and the mechanisms of antidepressant action.

This is not to say that abnormalities in laboratory measures of monoamine function have been lacking, only that the findings frequently have not been replicated and have often involved measures that could be affected nonspecifically by stress, diet, physical activity, or antidepressant drug treatment. A selective review of positive findings in depression includes many provocative reports. Some drug-free patients with depression have been shown to have lower plasma TRP levels.[24] Levels of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) are reported to be lowered in the CSF of patients with depression, although this is not a consistent observation.[32] In addition, reduced levels of 5-HT in the CSF have been found

to correlate with violent suicide.[33] Several observations have been made using platelets as a model for neuronal 5-HT receptors: the level of 5-HT transporter has been found to be reduced in patients with depression,[34] and reduced binding of radiolabeled imipramine has been reported in patients with depression, mainly due to decreased numbers of binding sites on the platelet membranes.[35] Reduced 5-HT uptake by platelets of patients with depression has also been noted.[36] Binding studies in the postmortem brain tissue of suicide victims and patients with depression have not provided consistent findings.[37] A presynaptic dysfunction in patients with depression is suggested by studies showing blunted prolactin responses to TRP,[38] fenfluramine,[39] and clomipramine[40] but normal response to the postsynaptic 5-HT agonist *m*-chlorophenylpiperazine.[41]

Other potential sites of dysfunction in the 5-HT system are the presynaptic and postsynaptic receptors. Altered levels and affinities of 5-HT_1 and 5-HT_2 receptors have also been reported in the brain tissue of suicide victims,[42–44] and increased numbers of 5-HT_{2A} and 5-HT_2 receptors have been observed in the platelets of patients with major depression[45] and suicidal patients,[46] respectively.

With regard to the noradrenergic system, concentrations of the NE metabolite MHPG have been measured in the urine, plasma, and CSF of patients with depression, but no consensus has been reached as to whether there is a correlation between levels of MHPG and depressive symptoms. For example, increased, decreased, and unchanged urinary concentrations of MHPG have been reported in patients with depression.[28,47–50] And patients with low urinary MHPG levels have been reported to be responsive to imipramine treatment.[28] The α_2-adrenoceptor agonist clonidine has been used to investigate any changes in density and sensitivity of the α_2-adrenoceptors in depression, but results of these studies have been inconsistent.[51–53] The effect of antidepressants on the number of receptors is also unclear.[54,55] However, an up-regulation of β-adrenoceptors (autoreceptors) has been consistently observed in patients with depression, and a down-regulation of these adrenoceptors is regarded as a marker of antidepressant efficacy.[56]

Significantly reduced binding of radiolabeled nisoxetine, a ligand for the NE reuptake transporter, has been reported in the postmortem locus ceruleus tissue from suicide victims and patients with depression compared with tissue from control postmortem samples.[57] Animal studies have also shown that chronic administration of desipramine decreases radiolabeled nisoxetine binding in several brain areas, including the hippocampus.[58]

Studies using postmortem tissue indicate that the density and affinity of the α_2-adrenoceptors are increased in the frontal cortex of suicide victims.[59,60] Changes in the sensitivity of the receptor have also been suggested to occur.[61] There is some evidence that the autoreceptors may be supersensitive in depression and that the administration of desipramine reduces the sensitivity.[62]

Table 2. Summary of the Depletion Studies[a]

Subjects	Serotonin Depletion	Catecholamine Depletion
Healthy	–	+/–
History of depression, in remission, off medication	+++	+++
Depressed, no medication	–	–
Recovered on SSRI therapy	++++	+
Recovered on NRI therapy	+	++++
Recovered on NaSSA therapy	++++	++++

[a]Modified from Delgado et al.[8] Abbreviations: SSRI = selective serotonin reuptake inhibitor, NRI = selective norepinephrine reuptake inhibitor, NaSSA = noradrenergic and specific serotonergic antidepressant.

To the extent that the symptoms of major depression are directly linked to abnormal function (either increased or decreased function) of monoamine neurotransmission, it should be possible to acutely change these symptoms by acutely changing monoamine availability. We hypothesized that the depletion of NE or 5-HT should, therefore, lead to an increase in depressive symptoms in unmedicated patients with depression as well as in healthy control subjects.

Neurotransmitter Depletion in Drug-Free Patients With Depression

Unmedicated, currently depressed patients were tested with the same type of depletion studies as have been described for antidepressant-treated depressed patients. Following screening but prior to treatment, patients were administered control and TRP[19] or catecholamine[6] depletion tests. Surprisingly, the type and severity of depressive symptoms did not change in these patients during the time that the depletion was conducted. This was true for both TRP and catecholamine depletion.[6,19] Severity of depression did not alter the behavioral response to either type of depletion: patients did not become more depressed, whether mildly or severely ill. These findings appear to imply that more than a simple disruption of monoamine synthesis is responsible for depression.[8]

Neurotransmitter Depletion in Healthy Controls

Studies in healthy volunteers with no personal or family history of depression showed that depletion alone could not bring about depressive symptoms.[8,63,64] However, of the patients who had recovered from recurrent episodes of major depression and were drug-free, most did experience a transient return of depressive symptoms in response to TRP depletion.[63,65] Similar findings have recently been reported with catecholamine depletion.[66]

SUMMARY AND CONCLUSIONS

The neurotransmitter depletion study findings discussed in this review are summarized in Table 2. There are

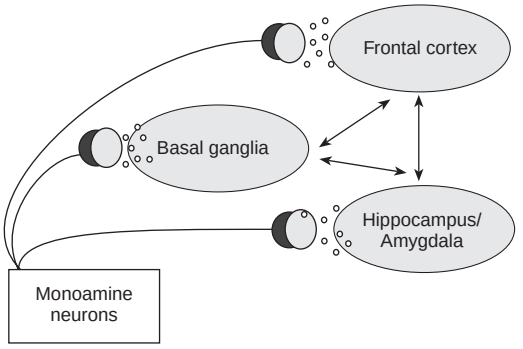
several possible interpretations of these results. The most clear-cut conclusions relate the importance of NE and 5-HT to antidepressant action. The availability of NE appears to be essential for maintaining an antidepressant response to drugs that enhance the release of NE, and 5-HT appears to be vital for maintaining an antidepressant response to drugs that enhance 5-HT. These depletion study data tend to support the hypothesis that there is no single mechanism of antidepressant drug action: both the noradrenergic and serotonergic systems are important. However, it would appear that more than a simple change in the level of monoamine is required to alleviate depression. If the impairment that causes depression lies in the ability of some neurons to appropriately respond to NE or 5-HT, then an acute increase in either of these neurotransmitters would not immediately alleviate depression. Likewise, the acute lowering of NE or 5-HT levels would not worsen depression, as was observed in these studies.

We know that there is a temporary delay in the response to any of the antidepressants (i.e., mood changes are not seen until several days or weeks after administration). Therefore, it may be that antidepressants act by causing an alteration in the ability of postsynaptic neurons to respond to monoamines, an effect that would account for the delay.[67] Only after this had occurred would changes in the monoamine levels have any effect. It is possible that abnormalities in the functioning of the postsynaptic receptors may be subcellular, for example in the G protein–coupling, in second or third messenger systems, or at the level of gene transcription. Some evidence already exists to suggest that the monoamines share common pathways at this subcellular level.[67–69]

While it is well established that there are considerable interactions between NE and 5-HT neurons in the brain, it is also clear that there can be independent effects from changing neurotransmission in either system.[67] This would explain why the pharmacologic profile of the particular antidepressant taken by the study participant is so important to the effects of monoamine depletion. While both neurotransmitter systems generally innervate most brain regions, the pattern of innervation sometimes involves different layers of the cortex and can be selective for some brain regions. In postsynaptic neurons, the intracellular responses can be both common and dissimilar.[67,70,71]

Our studies suggest that a simple deficiency of either NE or 5-HT fails to be the sole cause of depression. If such a deficiency were responsible, then healthy people should become depressed during depletion. Likewise, people who are in an episode of depression (at least those with mild symptoms) would be expected to get worse. We failed to see either of these results in our studies. Our results are consistent with a model in which depression results from dysfunction in areas of the brain that are modulated by monoamine systems, i.e., the frontal cortex, hippocampus/amygdala, and basal ganglia. A cartoon of this model is depicted in Figure 3. It is likely that many different factors could lead to a selective or generalized dysfunction in these brain areas, accounting for the probable heterogeneity in etiology of depression. It is also well known that these areas of the brain are highly sensitive to the effects of stress, possibly accounting for the adverse impact of life stress on depression.

Figure 3. Action of Monoamine Neurons in the Brain



Given what we have learned from the depletion studies, one might expect that there would be differences between selective NE reuptake inhibitors and SSRIs with respect to their efficacy as antidepressants or to the profile of symptoms alleviated. One such report of possible differences in the profile of therapeutic effects appeared in a study of the selective NE reuptake inhibitor reboxetine compared with fluoxetine.[72] The reboxetine-treated patients had greater improvements in social functioning, and those with severe depression had a greater response to treatment, than the fluoxetine-treated cohort. Such differences could simply be due to differences in the ability of NE or 5-HT to modulate the particular kind of dysfunction that is leading to depression in a particular individual's response (e.g., selective response to one type of drug versus another). On the other hand, if the area of dysfunction in a particular patient can be similarly modulated by either NE or 5-HT, then either drug could lead to a similar (but perhaps not identical) therapeutic effect.

While the results from neurotransmitter depletion studies are not conclusive, they do provide important data on which future studies can be based. Many unanswered questions remain. One important question is why some patients who have responded to either a selective NE reuptake inhibitor or an SSRI do not have depressive symptoms during depletion. Can these patients tolerate medication discontinuation without relapse or recurrence? Ongoing studies are exploring this question, as well as many others, about the role of NE and 5-HT in depression and antidepressant action. Neurotransmitter depletion studies have provided important insights and may eventually prove to be clinically useful. It might be that patients at risk of

relapse could be recognized before relapse occurred or that distinct biological subtypes of depression could be identified.

Drug names: clomipramine (Anafranil and others), clonidine (Catapres and others), desipramine (Norpramin and others), diphenhydramine (Benadryl and others), fluoxetine (Prozac), fluvoxamine (Luvox), mirtazapine (Remeron), reboxetine (Vestra), sertraline (Zoloft), tranylcypromine (Parnate).

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