The Biology of Tryptophan Depletion and Mood Disorders

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ABSTRACT

The involvement of the serotonergic system in the pathophysiology and treatment of affective disorders has been strongly implicated. The tryptophan depletion paradigm is widely used to study the effect of lowering serotonin levels. However, the effects observed in such studies are inconsistent and sometimes contradictory. The present review summarizes and discusses these discrepancies, emphasizing the importance of methodological details such as acute vs. chronic tryptophan depletion, patient's diagnosis and disease state (euthymic vs. acute phase) and previous drug treatment. Acute tryptophan depletion as a predictive test for personalized antidepressant treatment is suggested.

INTRODUCTION

Tryptophan is an essential amino acid. Plasma tryptophan levels are determined by the balance between the dietary intake and its removal from plasma by protein synthesis. Protein molar rate of tryptophan is 1.1% compared to ~ 5% of other amino acids, making it the rarest amino acid found in proteins. Tryptophan is transported across the blood-brain barrier (BBB) by a specific carrier for which tryptophan and all other large neutral amino acids (LNAAs) (valine, leucine, isoleucine, phenylalanine and tyrosine) also compete. The differential affinity ratio of 8:1 in favor of tryptophan secures its transport through the BBB into the brain (1). Some studies have shown that the level of brain tryptophan depends on the ratio between plasma concentrations of free tryptophan and other LNAAs (2) while others (3) showed that the ratio of total (free + albumin-bound) tryptophan and other LNAAs in serum is a better predicting parameter for brain tryptophan levels.

Tryptophan is the only precursor of serotonin (5-hydroxytryptamine, 5-HT). The serotonergic cells of the raphe possess a pacemaker-like activity that is modified by 5-HT autoreceptors and noradrenergic receptors. Production of serotonin takes place both in the periphery and in the CNS. After entering the brain tryptophan is converted into serotonin in a two-step synthesis process. L-tryptophan is first converted into 5-hydroxytryptophan by the enzyme tryptophan hydroxylase (Tph). 5-hydroxytryptophan is then decarboxylated by another enzyme, aromatic amino acid decarboxylase (5-hydroxy-l-tryptophan decarboxylase) forming serotonin. There are two isoforms of tryptophan hydroxylase: Tph1 and Tph2. Only the Tph2 isoform is expressed in the raphe neurons (4). Tph is the rate-limiting enzyme in the synthesis of serotonin (Fig. 1) since the Km of Tph for tryptophan is an order of magnitude higher than the Km of 5-HTP decarboxylase and 5-HTP is almost immediately decarboxylated to serotonin (5).

Serotonin modulates a number of developmental events such as neuronal migration, cell differentiation, cell division and synaptogenesis. 5-HT is involved in a wide array of CNS functions including the control of appetite, sleep, memory and learning, temperature

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regulation, mood, behavior (including sexual), perception, cardiovascular function, muscle contraction and endocrine regulation.

Multiple serotonin receptors have been cloned, designated 5HT1 through 5HT7. The 5HT1 group includes the subtypes 5HT1A, 5HT1B, 5HT1C, 5HT1D, 5HT1E, and 5HT1F. There are three 5HT2 subtypes, 5HT2A, 5HT2B, and 5HT2C as well as two 5HT5 subtypes, 5HT5A and 5HT5B. Most of these receptors are coupled to G-proteins that affect the activities of either adenylate cyclase or phospholipases. The 5HT3 class of receptors are ion channels (6).

The major pathway for serotonin degradation is reuptake into the neuron by a specific transporter, followed by degradation by monoamine oxidase (MAO) to yield 5-hydroxyindole acetaldehyde. This intermediate is metabolized by an aldehyde reductase or dehydrogenase to yield 5-hydroxytryptophol or 5-hydroxyindole acetic acid (5-HIAA) (Fig. 2).

Fig. 1: Serotonin degradation

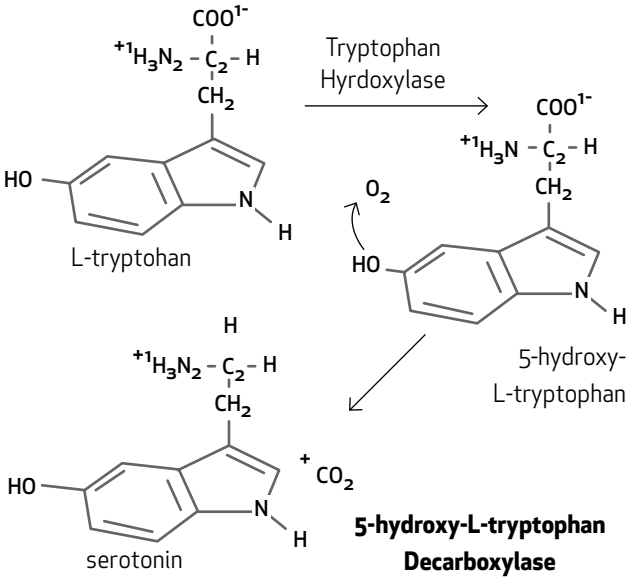
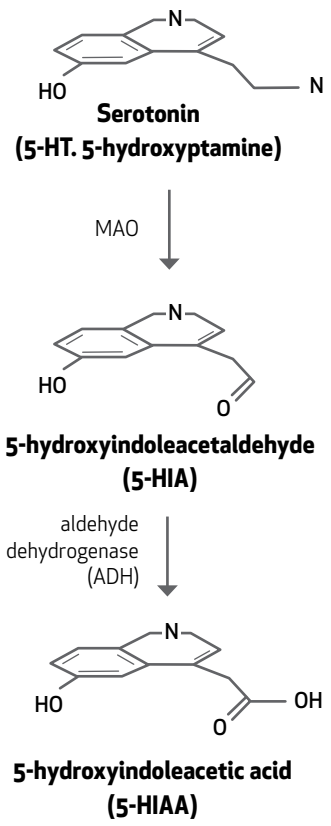


Fig. 2: *Serotonin synthesis*



TRYPTOPHAN DEPLETION AS A RESEARCH PARADIGM FOR THE STUDY OF MOOD REGULATION AND THE NEUROBIOLOGY OF DEPRESSION

Since the rate of 5-HT synthesis is dependent on plasma tryptophan, on how much of it crosses the BBB, and on the activity of brain tryptophan hydroxylase, depletion of plasma tryptophan resulting from an appropriate low-tryptophan diet, or, alternatively, inhibition of tryptophan hydroxylase, may modulate brain activity and mood. The common paradigm to study tryptophan depletion involves an intervention day and a control day one week apart. During the 24 hours preceding each of the days subjects follow a low tryptophan diet (about 160 mg/day) and then remain in fasting overnight and throughout the next day. On the intervention day the subjects consume a tryptophan-free drink containing a 100 gm load of 15 amino acid mixture, including the LNAAs. On the control day subjects consume a nutritionally balanced drink that does contain tryptophan. The intervention has two effects: it stimulates protein synthesis in the liver, which uses up free plasma tryptophan, and the LNAA amino acid family competes with tryptophan for the transport through the active protein shuttle across the BBB. This approach has been shown to produce maximal brain tryptophan depletion with maximal effects seen within 5-7 hrs after the depleting drink (7). The validity of acute tryptophan depletion by dietary amino acid loading as a method to diminish brain serotonin is supported by reports that ingestion

of tryptophan-free amino acid mixture in laboratory animals leads to depletion in plasma tryptophan levels and brain serotonin content (8). In humans ingestion of tryptophan-free amino acid mixture has been shown to produce a 70-90% reduction in plasma tryptophan 5-7 hours following administration (9-12). In healthy volunteers the tryptophan depletion paradigm resulted in 80 - 90% reduced CSF tryptophan levels after 7-10 hrs and 24-40% reduced 5-HIAA after 12-14 hrs (13). Tryptophan depletion was also found to reduce the rate of serotonin synthesis by 90% of baseline values (14).

There are alternative ways to obtain serotonin depletion. One is the administration of serotonin synthesis inhibitors, such as the potent tryptophan hydroxylase inhibitor para-chlorophenylalanine (pCPA) (15, 16). Another way is the administration of high doses of amphetamine analogs, e.g., para-chloroamphetamine (pCA) (17) and d-fenfluramine (d-FEN) (18) which elicit long-lasting decrease in serotonin levels by indirectly inhibiting tryptophan hydroxylase activity (17, 19). More invasive ways include electrolytic (20) or neurotoxic (20, 21) lesions of the median and dorsal raphe nuclei. The obvious advantages of the tryptophan depletion paradigm are that it is highly reliable and easily accomplished and that there is a clear cause and effect relationship. However, it also exhibits several disadvantages. Compounds other than serotonin may also be affected by alteration in tryptophan levels; e.g., plasma melatonin levels are altered by tryptophan depletion (22) and the levels of the tryptophan metabolites tryptamine, kynurenine and quinolinic acid also change. The last may be potentially psychoactive (23). Tryptophan-deficient amino acid mixture induces decline in food intake in rats (24) but not in human subjects (12, 25), suggesting species difference in the response to the imbalanced amino acid mixture. In human subjects it is difficult and unfavorable to repeat the tryptophan depletion paradigm too often. From the practical point of view, the taste of the drink is aversive, and might induce involuntary vomiting. But there is also a health concern. Both forced feeding and *ad-libitum* tryptophan deficient diet compared to control diet resulted in weight loss in rats (26). Early animal studies have shown that prolonged treatment with tryptophan depleted diet caused pathological changes in eyes, liver (27), blood (28), pancreas and spine (29). It is however notable that: 1) The extreme behavioral pattern in tryptophan depleted animals reported in these early studies (27) is absent from more recent studies. 2) The harmful effect of tryptophan depletion could be reversed by seven-day treatment with control diet (27). 3) Some of the pathological findings such as blood changes (28) and eye pathologies (30) were seen after more than 40 days of tryptophan depletion, a period length never applied to human subjects. 4) The experimental animals used in these studies were at their developmental stage which may render higher susceptibility to the depletion of an essential amino acid. In humans, the tryptophan depletion paradigm may obviously only be employed for a relatively short duration, inducing temporary biochemical changes.

The effects of chronic lowered brain serotonin on mood and cognition may differ both quantitatively and qualitatively from those produced by acute tryptophan depletion. Bortolato et al. (31) have shown different effects of acute and chronic tryptophan depletion on prepulse inhibition of the acoustic startle response in rats. It is possible that 5-HT1A and 5-HT2A regulation by acute vs. chronic tryptophan depletion (32) is responsible for the different response. Similar differences are also known to exist between acute vs. chronic administration of serotonin selective reuptake inhibitors (SSRI) (33-36).

In summary, tryptophan depletion provides a useful and interesting tool to investigate the role of serotonin in healthy volunteers, in the pathophysiology of psychiatric disorders, primarily affective disorders, and in understanding the involvement of serotonin in the mechanism of action of drugs such as SSRIs, MAO inhibitors (MAOI), lithium and non-pharmacological treatments such as ECT and sleep deprivation.

EFFECT OF TRYPTOPHAN DEPLETION ON MOOD IN HEALTHY SUBJECTS

Numerous studies but not all (37) have shown that tryptophan depletion does not significantly affect mood in healthy male subjects (7, 10, 13, 38, 39). Healthy women, however, were more susceptible than healthy men to mood lowering following tryptophan depletion (40). This might be related to the fact that tryptophan depletion causes a more significant decrease in the rate of serotonin synthesis in women (41). The gender-related differences in serotonin synthesis could be related to early serotonergic events in brain organization or to effects of circulating gonadal hormones (42-44). People at an elevated genetic risk for mood disorders, such as those with a multigenerational family history of affective disorders, showed an increased vulnerability to

mood alteration during tryptophan depletion (10, 45, 46). However, a subsequent study did not replicate these findings (47).

TRYPTOPHAN DEPLETION IN MAJOR DEPRESSION

Serotonin has strongly been implicated in the pathophysiology of depressive syndromes and in the mechanism of antidepressant drug action. Studies of untreated depressed patients suggest that serotonin function is reduced in depression, and studies of treated patients indicate that the mechanism of action of antidepressants is mediated by the enhancement of serotonin and/or noradrenaline neurotransmission.

TRYPTOPHAN DEPLETION IN UNTREATED DEPRESSED PATIENTS

A study of the effect of tryptophan depletion on untreated depressed patients reported no mood change during the depletion day; however, on the next day there was a bimodal response, with 23% of the patients describing a clinically significant worsening of depression, 37% reporting an improvement in symptoms and the remaining 40% reporting no change in symptoms, all compared to a placebo group (48). The lack of effect on the day of the depletion may have resulted from a floor effect due to maximally reduced serotonin function in these patients. The possibility that the lack of response to tryptophan depletion on the depletion day was caused by a floor effect is supported by the following: out of the initially-studied patients 15 underwent an additional tryptophan depletion session while being euthymic following successful antidepressant treatment. Nine of them did experience relapse of depressive symptoms on the depletion day (9). An alternative suggestion for the lack of response to tryptophan depletion on the depletion day is that depression is not caused by direct aberrant serotonin function but, rather, by dysfunctional serotonin-regulated brain circuits. If so, inducing further decrease in brain 5-HT would not necessarily result in an immediate effect.

In Delgado et al.'s study (48), participants showed strong correlation between the effect obtained on the day after tryptophan depletion and further response to antidepressants. Antidepressant-responders were more likely to improve in depression score following tryptophan depletion, while nonresponders were those whose depressive symptoms worsened following tryptophan depletion. Such bimodal mood changes on the day after tryptophan depletion and their relationship to treatment response should be interpreted with caution. Exclusion criteria for participants did not include personality disorder and 11 out of the 43 depressed subjects studied were concomitantly diagnosed as having a DSM-III-R personality disorder. These subjects were both more likely not to respond to antidepressant treatment and to exhibit increased Hamilton-depression (Ham-D) score. In addition, the authors do not report the exclusion of bipolar depressed subjects. Such a diversity in diagnosis may have led to the different response to tryptophan depletion. Tryptophan depletion directly affects serotonin levels while antidepressants do not achieve their effect just by increasing serotonin levels. If that would have been the case the beneficial effect of the drugs should have been observed earlier than after 2-3 weeks of treatment.

Improvement in depressive symptoms observed in some of the patients on the day after the administration of tryptophan depletion (48), when 5-HT levels are already restored, could be due to regulation of selected serotonin receptors. Binding potential of 5-HT1A autoreceptors but not 5-HT1A postsynaptic receptors was shown to be reduced in rats three hours after acute tryptophan depletion (32). 5-HT2C receptors, shown to exhibit inhibitory effect on dopamine transmission (49, 50), show paradoxical regulation such as down-regulation in response to their blockade (51-53). Receptor modulation could also occur through pre-mRNA editing. Conversion of adenosine to inosine at specific sites of 5-HT2C pre-mRNA can result in protein isoforms with different efficiency to activate G-protein in response to agonist stimulation (54). The editing enzymes recognize five closely spaced adenosine residues (A-E) located within sequences encoding the intracellular domain of the receptor and convert the adenosine residues to inosines. The result of the editing in the C and C' site is expression of 5-HT2c isoforms that are less efficient in activating G-proteins (55). The C' site editing is significantly increased, D site editing is significantly decreased and the C site shows a trend toward increased editing in the dorsal prefrontal cortex of suicide victims with a history of major depression. In contrast, mice treated chronically with fluoxetine showed opposite alterations in 5-HT2C pre-mRNA editing from those detected in suicide victims. The changes in editing-site in response to chronic fluoxetine treatment in mice are paradoxically similar to those detected in serotonin-depleted mice (56). Possible explanation for this phenomenon is that fluoxetine-induced alteration in 5-HT2c pre-mRNA editing is not a result of

simply increasing synaptic serotonin, but rather of receptor blockade by the drug (57). This is further supported by the fact that treatment with a 5-HT2c agonist increased the pre-mRNA editing at the C' site, resulting in expression of less active isoforms of 5-HT2c. It may be suggested that in subjects in whom the regulation of the serotonergic system functions normally, tryptophan depletion would result in increased sensitivity of the system. Namely, serotonin repletion would result in transient improvement of the symptoms. Similarly, depressive patients with functioning regulation of the system would also be expected to respond to serotonergic antidepressants. On the other hand, it is possible that patients whose condition worsened the day after tryptophan depletion (48) may be the ones with primary dysfunction of the regulation of the serotonergic system. In such subjects response to serotonergic antidepressants may not occur. Tryptophan depletion and repletion non-responders possibly exhibit dysfunction of a non-serotonergic system.

Animal studies of chronic serotonin depletion, as well as animal and human studies of acute and chronic serotonin reuptake blockade, and of direct serotonin antagonists, can complement the tryptophan depletion paradigm and support its implications or suggest an alternative mechanism of action of antidepressant drugs.

TRYPTOPHAN DEPLETION IN REMITTED UNTREATED PATIENTS WITH MAJOR DEPRESSION

In remitted untreated subjects with a past history of depression transient reappearance of depressive symptoms following tryptophan depletion was reported in some studies (58-61) but not in others (62, 63). A positron emission tomography (PET) study found that relapse of depressive symptoms in remitted patients following tryptophan depletion is associated with decreased metabolic activity in brain areas implicated in the pathogenesis of depression (64, 65).

TRYPTOPHAN DEPLETION AND DEPRESSIVE SYMPTOMS IN SSRI-TREATED REMITTED PATIENTS

Acute tryptophan depletion resulted in reduced binding potential of 5-HT1A autoreceptors in rats (32), but not in SSRI-treated remitted patients (66). It is well established that chronic SSRI treatment reduces 5-HT1A autoreceptor function (67-69). It is possible that the lack of effect of tryptophan depletion on 5-HT1A autoreceptors under chronic SSRI treatment reflects a floor effect.

Tryptophan depletion was also used to examine the role of serotonin in the mechanism of action of antidepressants. Tryptophan depletion produced relapse in patients responding to SSRIs, MAOIs and phototherapy (61, 64, 65, 70-73), but did not reverse the beneficial effect of tricyclics (9, 70, 71), ECT (74), sleep deprivation (75) or lithium (9, 76, 77). Since SSRIs and MAOIs intervene specifically with the serotonergic system while the tricyclics, sleep deprivation and lithium affect multiple neurotransmitter systems, e.g., adrenergic and dopaminergic, it is conceivable that tryptophan depletion would counteract the effect of SSRIs and MAOIs but not the other treatment modalities. Higher rates of relapse were observed in patients on serotonergic than on adrenergic drugs (71). The rate of transient exacerbation of depressive symptoms in patients on SSRIs or MAOIs was higher (80%) in patients recently remitted than those remitted for an average of 45 weeks (30%) (64). Similarly, recently remitted seasonal affective disorder (SAD) patients after light therapy relapsed following tryptophan depletion (72, 75), while for long-term remitted patients, one study resulted in a relapse rate of 73% (61), but another showed no effect of tryptophan depletion on symptoms (63). It may be that tryptophan depletion induced relapse in the more recently remitted patients because increasing synaptic serotonin is a primary step in a cascade of events that results in adaptive changes in serotonergic neurons, while at the adaptive stage an interruption to serotonin function is less adversive.

TRYPTOPHAN DEPLETION IN BIPOLAR DISORDER

Tryptophan depletion in recovered bipolar patients (77), lithium-maintained patients (74, 78) and recently remitted bipolar patients (79) did not result in relapse of bipolar symptoms. In addition, tryptophan depletion did not produce noticeable effects on the neuropsychological performance of euthymic bipolar patients (80). Shopsin et al. (81) studied five depressed patients out of whom two had bipolar depression. All patients responded to the MAO inhibitor antidepressant, tranylcypromine, and relapsed when the tryptophan hydroxylase inhibitor pCPA was added to the treatment. One of the bipolar patients became manic after four weeks of tranylcypromine treatment while a week of combined treatment with pCPA brought the patient to a euthymic state. This kind of cycling was repeated in this subject but the pattern was not observed in the other bipolar patient.

Recently, a new therapeutic use for tryptophan depletion has been evaluated in acutely manic patients (82).

The results from the double blind placebo controlled study of 17 manic patients showed a clinically and statistically significant difference in the Young Mania Scale between nine patients who received the tryptophan free amino acid drink and the eight patients who received the placebo drink. In the latter study the composition of the amino acid mixture used to cause tryptophan depletion was the same as previously reported (77, 80) but the regime of seven-day administration of the tryptophan depleted mixture did not include pretreatment with low tryptophan diet. Since plasma tryptophan levels were not assayed, Applebaum et al.'s (82) study lacks an objective proof of the degree of tryptophan depletion obtained. The recruited patients were in a manic state and received valproic acid during the trial while euthymic bipolar subjects were studied previously (74, 77, 78). It is possible that reduction in brain serotonin affects differently remitted vs. manic patients, as seems to be the case in depressed vs. remitted unipolar patients.

TRYPTOPHAN DEPLETION AND GENETICS

The response to tryptophan depletion could depend on genetic factors. Polymorphisms in the promoter region of the serotonergic transporter (5HTTLRP) have been found to be associated with unipolar depression (83-87). Moreno et al. reported an increased risk of drug-treated remitted major depressive patients with the l/l genotype of 5-HTTLRP for depressive mood response to tryptophan depletion (88). In healthy subjects, however, Neumeister et al. (89, 90) found an inverse association.

SUMMARY

Despite several decades of studies of the effect of tryptophan depletion using different paradigms, there are yet multiple open issues. Booij et al. (91) reported that high but not moderate tryptophan depletion induced depressive symptoms in patients remitted from depression. Information regarding different regulation pattern of serotonin receptors by acute vs. chronic tryptophan depletion, and discrepancies among studies suggest that methodology details might be critical for the treatment outcome (92). However, given the potential risk in applying chronic tryptophan depletion to human beings (93, 94) further studies of this paradigm should be used for basic research, as a tool towards the understanding of the relationship between serotonin and mood disorders. Potentially, if the finding that depressive patients who responded to acute tryptophan depletion and repletion were more likely to be antidepressant responders would be substantiated, response to acute tryptophan depletion may be found of predictive value to personalized antidepressant treatment.

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