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FEATURE REVIEW

Monoamine depletion in psychiatric and healthy populations: review

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A number of techniques temporarily lower the functioning of monoamines: acute tryptophan depletion (ATD), alpha-methyl-*para*-tyrosine (AMPT) and acute phenylalanine/tyrosine depletion (APTD). This paper reviews the results of monoamine depletion studies in humans for the period 1966 until December 2002. The evidence suggests that all three interventions are specific, in terms of their short-term effects on one or two neurotransmitter systems, rather than on brain protein metabolism in general. The AMPT procedure is somewhat less specific, affecting both the dopamine and norepinephrine systems. The behavioral effects of ATD and AMPT are remarkably similar. Neither procedure has an immediate effect on the symptoms of depressed patients; however, both induce transient depressive symptoms in some remitted depressed patients. The magnitude of the effects, response rate and quality of response are also comparable. APTD has not been studied in recovered major depressive patients. Despite the similarities, the effects are distinctive in that ATD affects a subgroup of recently remitted patients treated with serotonergic medications, whereas AMPT affects recently remitted patients treated with noradrenergic medications. The evidence also suggests that ATD and APTD affect different cognitive functions, in particular different memory systems. Few studies investigated cognitive effects of the procedures in patients. Patients who are in remission for longer may also be vulnerable to ATD and AMPT, but the relationship with prior treatment is much weaker. For these patients, individual vulnerability markers are the more important determinants of depressive response, making these techniques potentially useful models of vulnerability to depression.
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Introduction

Monoamines play a role in the regulation of mood and cognitive functions, and low monoamine function is associated with a range of psychopathological conditions, particularly with mood and anxiety disorders. Consistent with these notions, medications that augment the activity of the monoamine systems (tricyclic antidepressants (TCAs), selective serotonin re-uptake inhibitors (SSRIs) and monoamine oxidase inhibitors (MAOIs)) are effective medications for anxiety disorders and for depression.[1,2]

A number of experimental procedures can be employed to investigate the role of monoamine neurotransmission in psychopathology. These include administration of drugs (eg receptor-specific agonists or antagonists) and methods that lower monoamine functioning by restricting the intake of precursor amino acids (AAs) and/or blocking their synthesis (depletion methods). Lowered catecholamine (CA) brain function can be investigated experimentally in two ways: blocking its synthesis by administration of alpha-methyl-*para*-tyrosine (AMPT), or by dietary restriction of its immediate precursors phenylalanine and tyrosine: acute phenylalanine/tyrosine depletion (APTD). Lowered serotonin (5-HT) neurotransmission can be induced experimentally by the acute tryptophan depletion (ATD) method. ATD involves dietary restriction of the 5-HT precursor l-tryptophan (Trp), in combination with the consumption of a large quantity of other AAs that compete with Trp at the blood–brain barrier.[3] Central 5-HT function can also be lowered by administration of *para*-chlorophenylalanine (PCPA), a selective inhibitor of Trp hydroxylase. However, the use of this procedure is limited by its toxicity.[4]

Monoamine depletion research has had important implications for biological theories of depression. For instance, the earlier idea of an absolute deficit in 5-HT neurotransmission as a primary cause of mood disorders, or as a predictor of response to 5-HT-enhancing medications, has proven to be untenable.

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Incompatible with a simple, low 5-HT model of depression are the findings that ATD does not worsen the symptoms of currently depressed patients[5] and that ATD induces no symptoms or only mild dysphoria in healthy volunteers.[3] The concept of 'serotonergic vulnerability' more adequately describes the association between serotonin and depression. The categorical view (ie low 5-HT is characteristic of specific diseases) has been replaced by the dimensional view that impaired 5-HT function (eg as reflected in a mood response to ATD) is a manifestation of a vulnerability to mood lability and/or cognitive dysfunction, across different diseases.[6,7] According to this view, impaired 5-HT function signifies the presence of a biological risk factor, resulting from either genetic loading and/or early environmental insult. Neither of these may be sufficient; however, in combination with other genetic factors and/or environmental stressors, this high-risk model predicts the triggering and maintenance of low mood and cognitive dysfunction. A similar vulnerability model may be postulated for noradrenergic (NE) and dopaminergic (DA) neurotransmission.

Recently, many studies have investigated mood and cognitive functioning before and after manipulations of 5-HT or CA levels. The complex pattern of 5-HT and DA effects on behavior is readily apparent when considering the major pathways of these neurotransmitters in the central nervous system (CNS). However, by focusing on differences, similarities and interactions, hypotheses may be generated about the specificity of effects of manipulating single neurotransmitter dimensions. The lowering of both 5-HT and DA may be expected to induce negative affect, since negative affect may be the result of many different projections. However, differential effects—on the level of symptoms, neuropsychological functions or hormonal responses—may also be expected. Typical findings associated with low 5-HT function are: increased anxiety and depression, increased appetite, increased aggression due to impaired impulse control, increased cortisol levels and a decreased cortisol response to neuroendocrine and stress challenges and impaired cognitive functioning, in particular memory consolidation.[8–10] Low CA function (in particular, low DA) is typically associated with proneness to addiction, decreased vigor, impaired emotional memory, increased prolactin and impaired working memory.[11–14]

The purpose of this paper is to review monoamine depletion studies in humans: ATD, AMPT and APTD. The specificity of these procedures as manipulations of 5-HT and CA function (with an emphasis on DA) will be assessed. For this purpose, the effects of these challenges in psychiatric patients as well as in healthy individuals will be reviewed. The following hypotheses will be tested: (1) there are distinct and dissociable influences of manipulations of 5-HT *vs* CA neurotransmitters on behavioral parameters (mood and cognition) and (2) these challenges are meaningful models of vulnerability to mood disorders, particularly major depression. A review of the ATD method has recently been published.[15] Since this procedure continues to be used intensively, the present paper will present an update of findings published between July 1999 and December 2002.

Materials and methods

Electronic libraries (Medline and Web of Science) were used to identify relevant literature on monoamine depletion studies (period between 1966 and December 2002). The keywords used were 'AMPT', 'tryptophan depletion', 'catecholamine depletion', 'APTD' and 'tyrosine depletion'. In addition, reference lists of the retrieved papers were checked.

Results

Acute tryptophan depletion

Mechanism, procedure ATD commonly involves the restriction of Trp intake during 1 day, followed by the consumption of a large quantity of AAs, but no Trp, the next day morning. The usual amount is 100 g, mixed with 300 ml water and artificial flavor.[3] The AAs stimulate protein synthesis, which requires Trp, while Trp competes at a relative disadvantage for the same transport system into the brain. When using a 100 g AA mixture, plasma levels of Trp drop by 70–90% within 4–7 h after consumption of the drink. Some studies do not employ the 24-h dietary restriction of Trp prior to ingestion of the AA drink. Other ATD studies have used only essential AAs. Another variation is the use of lower dosages (75 g or even 50 g or 31.5 g AAs), which usually (but not always) results in plasma Trp reductions of 60–80%.

The physiological effects of ATD appear to be specific for 5-HT: its metabolite 5-HIAA in CSF is reduced after ATD, whereas homovanillic acid or other metabolites are not reduced.[16] A more detailed explanation of the procedure can be found elsewhere.[3,4]

Two control procedures are commonly used: one in which a small amount of Trp is added to the AA mixture, and one in which 25 g instead of 100 g of AAs is consumed.[17] The latter procedure was used in the United States during the 1990s, when Trp was banned from the US market. The results are not the same: the first procedure leads to a rise in plasma Trp that can vary in size, whereas the latter procedure usually leads to modest reductions of plasma Trp.

As central 5-HT function depends on Trp as well as on large neutral amino acids (LNAAs), the Trp/LNAA ratio in plasma is a better index of central 5-HT function than plasma Trp. Some studies still fail to report Trp/LNAA ratios, although it was demonstrated many years ago that Trp/LNAA ratios may decrease while plasma Trp levels increase.[18] Both the ATD and control procedures have the drawback that the AA mixtures taste unpleasant and have side effects, in particular nausea and vomiting. Some

researchers have administered some or all of the AAs in capsules. The amount of AAs is then reduced to 31.5 g, still requiring 50 capsules. This procedure has been shown to induce sufficient depletion,[19] and may also cause fewer side effects, although there are insufficient data to determine whether side effects are prevented. Up to 25% dropout rates have been reported due to side effects, although in most studies, dropout rates have been low.[20] Nevertheless, the introduction of a new, protein-based Trp depletion method may be an important development in terms of tolerability.[21,22] This procedure uses a gelatin-based protein comprising the entire range of AAs (except Trp) in the form of peptides.[21,22] A second potential advantage of this new method is that much higher reductions of Trp were found in rats than are usually obtained with the AA method.[23,24] However, no studies on this new ATD method in humans have yet been published.

Prior review A previous review of the literature up to July 1999[15] showed that ATD has a mood-lowering effect in subgroups of recovered depressed patients, patients with seasonal affective disorder (SAD) and in genetically vulnerable, healthy subjects. The mood effect in former patients is much larger, and also seems to be of a different quality than the effect in vulnerable healthy subjects. The response to ATD was clearly associated with prior treatment with medication affecting the 5-HT system. In SSRI-treated patients, the 'relapse' rates during ATD were 50–60%, and several recent studies failed to produce the effect. Preliminary evidence existed for an effect of ATD on bulimia nervosa, autism, aggression and substance dependence.

Behavioral effects in patients with mood disorders and in genetically vulnerable individuals Although the mood-lowering effect of ATD in a subgroup of recovered depressed patients, particularly those who had been treated or were still being treated with an SSRI, was already well established at the time of our previous review, a number of studies had not been able to replicate the effect. Further research support, however, has since become available.[25–28] Although each of these studies comprised a relatively small number of patients, the replications are important because of the negative findings mentioned above.

The serotonergic treatment specificity of response to ATD also received further support. In a study with 20 bipolar and 10 unipolar depressed patients who were all stabilized on lithium treatment, no clinically relevant mood changes were induced by ATD.[29] The same result was found in a study with 19 lithium-treated patients with bipolar-I disorder.[30] This corroborates two earlier studies that had shown that euthymic patients who were being treated with lithium were unaffected by ATD.[31,32] Bipolar patients treated with an SSRI, however, are probably as vulnerable to ATD as unipolar patients.[15]

More is known now about what other factors determine response to ATD in recovered depressed patients. This question had arisen not only because of the occasional unsuccessful attempt to replicate, but also because of the common finding that even in SSRI-treated patients, no more than 50–60% respond to ATD. Previous attempts to find predictors of response[28,33,34] had been inconclusive because of the substantial number of cases that is needed for prediction studies. In a recent study, the data from six prior ATD studies were pooled and reanalyzed, in order to increase statistical power. Results showed that chronicity (more than one prior episode), SSRI treatment, gender (female), and previous serious suicidal thoughts or behaviors are independent predictors of depressive response to ATD.[20] Chronicity of illness was the most powerful predictor, whereas residual depressive symptoms were not found to predict response to ATD. Comparison of these findings with those of Delgado *et al*,[35–37] which were not included in the reanalysis, suggests that depressive response to ATD may reflect: (1) the mechanism of action of SSRIs (if ATD is applied soon after onset of remission); or (2) individual differences in biological vulnerability to depression (if ATD is applied during a later phase of remission). This concept of ATD as a model of vulnerability to depression may be independently validated by studying the response to ATD of healthy individuals who may be genetically vulnerable to depression (this will be further discussed below) and by brain imaging studies.

A recent PET 5-HT_2 receptor ligand-binding study, mapping 5-HT_2 binding potential using ^{18}F-setoperone, showed a significant decrease in brain 5-HT_2 receptor density following ATD in healthy women.[38] No mood effects were observed in this study, and the authors suggest that downregulation of 5-HT_2 receptors may have an adaptive function in the prevention and relief of depressive symptoms. This hypothesis could explain why patients treated with noradrenergic drugs typically do not respond to ATD, because animal studies have shown that these drugs (in contrast with SSRIs) consistently downregulate 5-HT_2 receptors.[38] A study of ATD followed by Trp infusion indicated that cortisol response to Trp is enhanced by ATD.[39] This phenomenon probably reflects acute dysregulation of postsynaptic 5-HT_2 receptors, which also occurs as a compensatory response to stress and low 5-HT, in an attempt to maintain normal 5-HT turnover.[40] Theoretically, prolonged duration of stress and low 5-HT, the latter putatively induced by the former, ultimately leads to dysregulation of the 5-HT system.[41]

An important recent development concerns the relationship between genetic variability and mood response to ATD. Two studies, however, show contradictory findings, albeit in different populations. Both studies investigated the relationship between mood response to ATD and a functional polymorphism of the promoter region of the serotonin transporter gene

(SLC6A4). In one of these studies, remitted depressed patients who were homozygous for the long allele of the promoter region had a mean change in Hamilton ratings that was much larger than patients who were heterozygous or homozygous for the short allele (11.0 *vs* 5.5 *vs* 3.4 points change, respectively).[42] Patients in this study were on diverse treatment regimes: 12 were treatment-free, 19 were on SSRIs, three on other medications and nine underwent cognitive therapy. The sample size did not allow independent testing of treatment history and other clinical variables associated with response to ATD. Inconsistent with these results, a larger response to ATD was associated with the homozygous *short* genotype in 45 healthy women, irrespective of a family history of depression.[43] The women with l/l genotype did not show a mood response. Women with the s/l genotype and a family history (FH+) had similar mood responses as the s/s genotypes, whereas the s/l genotypes without family history (FH−) showed an intermediate mood response. How to explain these contradictory findings? Since both *in vitro* and *in vivo* studies have indicated that the long allele is associated with higher basal and induced transcriptional activity of the serotonin transporter, the s/s allele pair could be expected to be the most vulnerable genotype, and the heterozygous genotypes intermediate.[43] It could also be argued that when the decreased availability of 5-HT during ATD is combined with a greater uptake of 5-HT into the presynaptic cell (in l/l genotypes), a greater decrease in 5-HT neurotransmission may occur.[42] Insufficient depletion has previously been proposed as a potential explanation as to why the response to ATD is so varied.[44] This genetic variability may cause different extents of brain 5-HT depletion, while peripheral markers of 5-HT function are comparable. Although speculative, the fact that the l/l genotype was observed to be the most responsive to ATD in remitted patients[42] could also be due to the use of SSRIs. There is some evidence that use of SSRIs alters or masks the effect of vulnerability by scarring the neurotransmitter system, at least shortly after the cessation of experimental SSRI treatment of only 4 weeks. Healthy individuals treated with fluoxetine had blunted hormonal responses to 5-HT_{1A} challenge, indicative of down-regulation of both pre- and postsynaptic 5-HT_{1A} receptors.[45] Furthermore, 3-week treatment of healthy volunteers with paroxetine led to desensitized 5-HT_2 receptors as evidenced by a blunted prolactin response.[46] Since many participants in the Moreno *et al* study were on SSRIs (and some may have been on SSRIs in the past), one could speculate that SSRIs may affect the function of 5-HT neurotransmission particularly in l/l genotype individuals. It should also be noted that the mood response to ATD in the study with healthy women was remarkable: the average change of Hamilton (21 item version) scores was approximately 5.5 points in FH+ women and 3.1 in FH− women (averaged over genotypes). In prior ATD studies using genetically vulnerable but healthy samples, only small changes in mood, if any, were detected. The effect of ATD on depression ratings in this population is in fact so small that it was inconclusive at the time of our earlier review.[6,47] The evidence now indicates that healthy relatives of patients with depression or bipolar disorder are more vulnerable to the effects of ATD than individuals without a family history of mood disorders,[48–50] but the mood effect is quite small. Furthermore, the large change in the s/s genotype healthy sample occurred despite the fact that a much smaller amount of AAs (31.5 g) was used than that usually used in ATD studies (100 g). The whole dosage was encapsulated, however, which may have ensured sufficient depletion (about 80% reduction of free and total plasma Trp), and since this procedure is uncommon, it cannot be ruled out that this has been responsible for the large effect. Finally, perhaps clinical depression rating scales are more sensitive to the effects of ATD than visual analogue ratings of mood states. Although this may sound counterintuitive, in one study with remitted patients a greater effect of ATD was found on the Hamilton scale than on self-ratings[28] (this particular finding was not published). In an ongoing study, different sensitivities of different depression scales are being observed. Surprisingly, the Hamilton scale has not been used previously in ATD studies of healthy samples, so this may be investigated further. To summarize, ATD was associated with a larger mood response in l/l genotype remitted patients, and in s/s genotype healthy women. One possible explanation might be that pharmacotherapy has different effects on 5-HT neurotransmission in l/l and s/s individuals. However, there are many differences between both studies. Combined with the facts that the patient sample was rather heterogeneous, and that the large mood response in the healthy women is anomalous, a replication of both studies seems warranted.

Family history seems to be a better predictor of response to ATD than is neuroticism.[51] Opposite effects were found in subjects who had relatives with either bipolar I or bipolar II disorder: the type II relatives showed an elevation of mood, whereas the type I relatives had a lower mood after ATD.[50] In both groups, but not in controls, cortisol levels decreased after ATD. Although the cortisol response to stress was attenuated after ATD, this was not statistically significant.

At the time of our previous review, several studies had already shown that remitted (light treatment) SAD patients respond to ATD, whereas symptomatic SAD patients do not. It has since been shown that eight of 11 SAD patients who were in natural remission during summer responded to ATD.[52] This study also showed that seven of eight SAD patients who reacted to ATD in summer developed a depressive episode during the next winter, whereas two of three patients who did not respond to ATD remained well.[52] In another study on SAD during natural summer remission,[53] six of 12 medication-free

patients had a clinically significant increase of symptoms after ATD, but three patients also reacted to sham depletion. As the difference was nonsignificant, it was concluded that summer remission is not dependent on plasma Trp levels in the same manner as remission after light therapy. This study has since been cited as a failure to replicate,[54,55] but this conclusion is unwarranted: the 50% response rate to ATD is well within the normal range.[56] The response to sham depletion was unusually high in this study, which may have been due to unintended reductions of the Trp/LNAA ratio during control sessions. Thus, the evidence for the importance of 5-HT mechanisms during summer remission is as strong as it is for remission after light therapy.

Tryptophan depletion in other psychiatric conditions Significant effects of ATD on patients with *bulimia nervosa* (BN) have been confirmed. ATD had a small effect on mood and desire to binge, but no effect on food intake in 22 women with BN as compared to 16 healthy women.[57]

The evidence in *panic disorder* had been mixed; however, three recent studies have shown that ATD increases the vulnerability of PD patients to provocation with flumazenil,[58] 5% CO_2[59] and 35% CO_2.[60] The latter study found a possible protective effect of the control condition (which often raises plasma Trp levels).

In *alcoholism*, ATD does not seem to have an effect, neither on the amount of alcohol consumed in an experimental setting,[61] nor on craving[62] or mood.[63] However, a cognitive effect was found in one of these studies (see below).

One study on *schizophrenia* found no effect of ATD on mood, psychotic symptoms or movement disorders.[64] Added to two earlier studies with very small and opposite effects, it seems safe to conclude that acute serotonin deficiency induced by ATD does not affect schizophrenia symptoms. However, it may affect executive function, as assessed by a neuropsychological planning test.[64]

Tryptophan depletion and aggression The relationship between serotonin and aggression continues to be an important and fruitful area of research. At the time of our previous review, a number of studies had shown that ATD increased aggressive responses, particularly in individuals with high trait aggression scores. However, negative findings had also been published. It has again been shown that ATD increased aggression in men with high baseline levels of aggression, and also that ATD does not affect aggressive behavior in men with low baseline levels of aggression.[65] Women who took ATD during the premenstrual phase showed increased aggression in response to provocation.[66] In another small study, mood effects of ATD were only observed in high trait-aggressive women, and not in low trait-aggressive women and men.[67] The hightrait-aggressive men in this study had relatively low aggression scores, so these results are not necessarily incompatible with Bjork *et al*. Another study studied aggressive behavior in 12 women under ATD conditions, Trp augmentation and fasting.[68] Trp augmentation decreased aggressive behavior and ATD increased aggressive behavior. These effects were restricted to women with higher plasma Trp during the fasting control condition. In earlier studies with men, Trp augmentation did not affect aggression.[69] Furthermore, although self-reported (trait) aggression and impulsiveness were unrelated to aggression following ATD or Trp augmentation, these self-reports were related to the women's sensitivity (change of plasma Trp levels) to ATD and Trp augmentation.

In summary, these studies provide further support for the relationship between 5-HT and aggression, and also provide explanations for apparently conflicting data by drawing attention to differences between men and women, and between certain subgroups (in particular, individuals with high trait aggression scores or high plasma levels of Trp).

Cognitive effects of ATD in patients and healthy samples Some general trends are emerging from studies into the cognitive effects of ATD in normal samples, despite the fact that researchers use many different tests or test versions to measure cognitive functions. ATD has a specific negative effect on long-term memory (memory consolidation),[70] irrespective of family history for depression,[49,71,72] and particularly for words with positive or neutral connotations.[49] Furthermore, it has a *positive* effect on focused attention as measured by Stroop word–color interference.[70] Performance on a planning task (Tower of London) was impaired after ATD in first-degree relatives of bipolar disorder patients,[72] but not in healthy volunteers.[72,73] In euthymic bipolar patients, the effect of ATD on planning was small (statistically a trend) and there was no effect on attention, vigilance and learning.[74]

Another study also points to the importance of individual differences, in this case family history of alcoholism. ATD had opposite effects on performance on a behavioral inhibition task in 20 men with and 20 men without an alcoholic father (impaired and improved performance, respectively).[63] ATD had no effect on mood in this study, nor did it affect performance on a delay discounting task. Finally, ATD caused a small but significant reduction of scores on the Mini Mental State Examination in 16 patients with dementia of the Alzheimer type,[75] while it had no effect on the cortisol levels of Alzheimer patients.[76]

Alpha-methyl-para-tyrosine

Mechanism, history and procedure The AA tyrosine is the precursor of CA, DA and NE. After crossing the blood–brain barrier, tyrosine is converted into 3,4-dihydroxyphenylalanine (L-dopa) by the rate-limiting

enzyme tyrosine hydroxylase. Next, L-dopa is converted via aromatic L-amino acid decarboxylase to DA and via DA-β-hydroxylase to NE.[77] CA activity in the CNS can be reduced by oral administration of AMPT. AMPT temporarily reduces L-dopa availability by blocking the enzyme tyrosine hydroxylase, which consequently leads to reduced CA synthesis.[77]

Daily administration of AMPT (1–4 g/day) generally reduces urinary and CSF levels of MHPG and HVA (CA metabolites) by more than 50%. CSF levels of 5-HIAA (metabolite of 5-HT) remain unchanged,[78] indicating that the effects are specific. CA metabolite levels are assumed to reach their minimum within 2–3 days after starting AMPT administration and are normalized within 3–4 days after withdrawal of the drug.[77,79,80]

Early treatment studies have shown that daily, long-term treatment with AMPT could relieve symptoms in those medical conditions in which an excess of CAs may play a role, including phaeochromocytoma,[81] hypertension, glaucoma or tardive dyskinesia, whether or not in combination with conventional medicines.[79,81] The clinical use of AMPT in patients with mood disorders has been investigated in the 1960s. In a sample of seven manic and three psychotically depressed patients, administration of 2–4 g AMPT daily (5–48 days) decreased manic symptoms in five patients, whereas the depressed patients all became more depressed. Mood state returned to baseline levels after discontinuation of the drug, except for three manic patients who retained the reduction in symptoms during the 5-day post-AMPT observational period.[78,80] In a treatment study with 20 patients with essential hypertension, six had a history of depression. Three of these depression-prone patients experienced increased agitation after receiving AMPT, requiring withdrawal of AMPT treatment.[79] To conclude, chronic AMPT treatment generally does not affect mood in medically ill, nonpsychiatric patients, but it may affect mood in depressed patients or in individuals vulnerable to mood disorders.

The first 'challenge' study in which AMPT was used dates back to the mid-1970s. Three depressed patients were concomitantly treated with AMPT after they had responded well to 300 mg imipramine (a tricyclic antidepressant). AMPT did not reverse the imipramine response in any of these patients (two unipolar, one bipolar), not even in a patient who received AMPT daily prior to imipramine treatment and continued to use it daily concomitant with imipramine.[82] In contrast, two imipramine responders (one unipolar, one bipolar patient) in the same study temporarily relapsed within 2–3 days after starting PCPA administration (a Trp hydroxylase inhibitor). The authors concluded that 5-HT rather than CAs may be involved in the mechanism of action of imipramine.

The Yale group was the first to use the AMPT challenge procedure for relatively short periods in a placebo-controlled design. Since the early 1990s, many experimental studies that used AMPT as a challenge were published (Table 1). Nowadays the AMPT depletion procedure usually involves a 48 h study period in which participants receive a total amount of 4–8 g AMPT in divided doses (1.5–4 g daily) and a post-AMPT follow-up day. Placebo testing usually consists of the administration of a total amount of 250–500 mg diphenhydramide hydrochloride or promethazine.[83,84] Both tests are active placebos, since they induce comparable levels of sedation.[84,85] Usually, participants are kept on a low monoamine diet during the study periods in order to standardize food intake.

Although the dosages vary between AMPT challenge studies, the magnitude of reduction of CA metabolites (measured in plasma) is generally equivalent to the change in CA metabolite levels in urine reported in the early treatment studies (Table 1). The nature of the dose–response relationship is not clear, as the few studies that use a higher dosage of AMPT did not report HVA or MHPG levels. Maximum effects occur within 1–2 days after starting AMPT administration and disappear within 2–3 days after discontinuation.[83,86]

Behavioral effects of AMPT in mood disorders In a pilot study with 14 recently remitted depressed patients who had responded well to NE re-uptake inhibitors (NRIs) (n=5) or SSRIs (n=9), five patients had a brief 'full relapse' following AMPT (ie 50% increase on HRSD-25 and score $\geq$18).[83] This depressive response appeared to be treatment specific, as four of the five 'responders' had been treated with an NRI. The pilot sample was enlarged, and the findings were that eight of nine patients treated with an NRI relapsed after AMPT (mean Hamilton scores changed from 8.9 to 23.4), whereas none of the 10 SSRI-treated patients relapsed.[87] Not all the SSRI responders from the pilot study were included in the second report.

AMPT also led to a transient depressed mood in a sample of 11 remitted depressed patients who had responded well to mirtazapine (an antidepressant that affects both the NE and 5-HT systems).[88] Some of these patients also responded to ATD (mean Hamilton scores changed from 6 to 18). The effect sizes were equivalent, but there were some differences when symptoms were compared separately. Regarding depression, both depletions led to loss of energy, concentration problems and retardation. ATD also led to depressed mood and somatic anxiety, whereas AMPT led to decreased interest and agitation. It is of interest that the effects of AMPT in mirtazapine-treated patients (change score: 7) fall between the effects in patients treated with an SSRI and those treated with an NRI in the study by Miller *et al*[87] (change scores 1.1 and 14.5, respectively).

The brief relapse following AMPT has been replicated in a sample of medication-free fully remitted depressed patients who were in remission for at least 4 months (mean 18 months).[89] A total of 10 of 14

Table 1 Summary of the results of AMPT challenge studies in humans

Study reference	*Population, design*	*Treatment*	*Total AMPT dosage (g)*	*% decline in plasma AMPT*		*Main findings*
				HVA	*MHPG*	
Mood disorders						
Shopsin *et al*[82]	3 MDD, 2 BP (2 males). Imipramine + PCPA (1 MDD, 1 BP). Imipramine + AMPT (2 MDD, 1BP). No placebo	Imipramine	3–4 g/day			No 'relapse' after AMPT. 2/2 patients relapsed after PCPA
Delgado *et al*[83]	14 MDD (8 males). Remission. HAMD-25 $\leq$ 15. Within SS design: AMPT/placebo	3 desipramine; 2 mazindol; 5 fluoxetine; 4 sertraline	5	71	44	4/5 NA-treated patients relapsed after AMPT. 1/9 SSRI-treated patients had a relapse
Miller *et al*[87]	19 MDD (13 males). Remission $>$2 weeks. HRSD $\leq$15. Within SS design: AMPT/placebo	9 fluoxetine; 1 sertraline; 7 desipramine; 2 mazindol	5	86	51	8/9 NA-treated patients relapsed after AMPT. 1/9 after placebo. 0/10 of SSRI-treated patients relapsed
Miller *et al*[85]	17 MDE (13 males). HRSD $\geq$20. Within SS design: AMPT/placebo	Medication-free	5	70	50	No mood effect of AMPT. Slight significant improvement after placebo (HRSD: 31–$>$24[a])
Neumeister *et al*[90]	13 SAD (9 MDD, 4 BP; 4 males). Remission $\geq$2 weeks. SIGH-SAD $<$12. Within SS design: AMPT/ATD/ placebo AMPT	Medication-free $\geq$6 months. Last treatment: light therapy	4	61	63	ATD: 10/13 patients relapsed AMPT: 7/13 relapsed
Berman *et al*[89]	15 MDD (5 males). Remission $\geq$4 months. HRSD-25 $<$10. Within SS design: AMPT/ placebo	Prior treatment: SSRI: n=10; SSRI + TCA/MAOI: n=2; TCA: n=1; no medication: n=2	5	86	51	10/14 patients relapsed after AMPT. 1/13 patients after placebo
Anand *et al*[86]	8 BP patients (4 males). Euthymic in remission. Within SS design: AMPT/placebo	Lithium $\geq$3 months	5	73[a]	65[a]	No mood change during AMPT Hypomanic symptoms 24–48 h after last dose
Lam *et al*[91]	9 SAD (1 male). Summer remission $>$11 weeks. SIGH-SAD $\leq$10. Within SS design: AMPT/placebo	No treatment $\geq$8 weeks	5	49	57	9/9 patients relapsed after AMPT. 2/9 patients relapsed after placebo
Berman *et al*[92]	10 MDE (5 males). Within SS design: AMPT + ATD/placebo + ATD	Medication-free $>$2 weeks	5	63	Not reported	Small decrease in HRSD-25 after AMPT + ATD and placebo + ATD

Table 1 Continued

Study reference	*Population, design*	*Treatment*	*Total AMPT dosage (g)*	*% decline in plasma AMPT*		*Main findings*
				HVA	*MHPG*	
Delgado *et al*[88]	11 MDE (4 males). In remission. 10/11 with SSRI-induced sexual dysfunction. Within SS design: AMPT/ATD	Mirtazipine. Prior medication: SSRI (n=10); St John's wort (n=1)	5	65	48	Increase in HRSD-25 after both ATD and AMPT. No difference in effect size between ATD and AMPT
Other mental disorders						
Stine *et al*[96]	10 cocaine users (8 males). Within SS design: AMPT + cocaine/placebo + cocaine/placebo + AMPT/ placebo + placebo	No treatment	3	84	64	AMPT increased prolactin levels. AMPT + cocaine decreased feelings of 'high' (NS). No effect on cocaine levels
Longhurst *et al*[93]	6 OCD patients (4 males); 4 with comorbid depression. Within SS design: AMPT/placebo	Medication-free ≥ 5 weeks. Prior treatment: SSRI	9	Not reported	Not reported	No change in HRSD or Y-BOCS scores
Abi-Dargham *et al*[94]	18 patients with schizophrenia (11 males). 18 controls (11 males). Between SS design: baseline SPECT/ AMPT + SPECT. No placebo drug	No neuroleptics for about 139 days	8	Not reported	Not reported	Effect of AMPT on D_2 receptor availability larger in patients than in controls (9 *vs*19%). AMPT reduced positive symptoms in patients (decrease PANSS positive scores: 3.7 points): inverse correlation with change in D_2 receptor binding. D_2 receptor binding after AMPT predicted response to antipsychotic treatment
Voruganti *et al*[95]	Between SS design. N=2 × 6 patients. With schizopherenia: 'dysphoric' responders to previous neuroleptic drugs *vs* 'nondysphoric'. Baseline SPECT scan *vs* AMPT + SPECT. No placebo drug	Unmedicated. Prior treatment: neuroleptica	8	Not reported	Not reported	Increased dysphoric symptoms after AMPT. Effect larger in 'dysphoric' group. Reduced psychotic symptoms after AMPT. Inverse correlation between change in receptor binding ratio/change in dysphoric symptoms. Increased binding ratio in D_2 receptors after AMPT; effect more pronounced in 'nondysphoric' group than in 'dysphoric' group (19.1 *vs* 5.1%)

Healthy samples

Tychsen and Sitaram[104]	N=3 (2 males) Within SS design: AMPT/placebo	None	9	Not reported	53% (urine)	Increase in saccadic eye movements (amplitude/frequency)
McCann *et al*[100]	N=2 × 12 (males). Between SS design: AMPT/AMPT + 64h SD	None	Max. 8.25	Not reported	Not reported	Dystonia in 5/24 males after AMPT. Symptoms disappeared after treatment with diphenhydramine or massage and posture change
McCann *et al*[103]	N=4 × 10 (males). Between SS design: AMPT/AMPT + 40h SD/lactose/lactose + 64h SD	None	5.25	Not reported	Not reported	Increased subjective sleepiness after AMPT, SD or AMPT + SD. Effect larger after AMPT + SD. Decreased sleep latency after AMPT, SD and AMPT + SD, effect not larger after AMPT + SD. Change in speed and decreased accuracy after AMPT + SD, small cognitive effects after AMPT or SD alone. AMPT and AMPT + SD increased prolactin levels. Difference between AMPT and AMPT + SD not significant
McCann *et al*[99]	N=4 × 10 (males). Between SS design: AMPT/AMPT + 40h SD/lactose/lactose + 64h SD	None	5.25	Not reported	Not reported	AMPT and SD increased sleepiness and fatigue and decreased alertness; effects larger in the AMPT + SD condition. No mood effect after AMPT or SD. AMPT + SD increased negative mood (POMS/VAS). Behavioral effects persist 12–16 h after discontinuation of AMPT
Zimmermann *et al*[106]	N=7 (3 males). Within SS design: AMPT/placebo	None	5	Not reported	Not reported	AMPT decreased nocturnal melatonin secretion. 24-h urinary 6-SM excretion correlated highly with 24-h melatonin secretion during AMPT condition (r=0.93)
McCann *et al*[97]	N=4 × 10 (males). Between SS design: AMPT + placebo/AMPT + L-dopa/placebo + L-dopa/placebo + placebo	None	6	Not reported	Not reported	AMPT increased prolactin levels. Effect reversed by L-dopa. AMPT increased sleepiness and anger and decreased alertness, calmness

Table 1 Continued

Study reference	*Population, design*	*Treatment*	*Total AMPT dosage (g)*	*% decline in plasma AMPT*		*Main findings*
				HVA	*MHPG*	
						and sleep latency. Slight decrease in VAS happiness. Effects on calmness, sleep and alertness reversed by L-dopa
Zimmermann *et al*[105]	*N*=10 (5 males). Within SS design: AMPT/placebo	None	5	Not reported	67% (urine)	AMPT increased prolactin levels; effect larger in females than in males. AMPT decreased 24 h urinary 6-MS secretion. No mood effect
Salomon *et al*[101]	*N*=8 (4 males). Within SS design: AMPT + balanced amino-acid mixture/AMPT + ATD mixture	None	5	67%[a]	54%[a]	No effect of AMPT + ATD on mood
Laruelle *et al*[98]	*N*=11 (males). Baseline SPECT/ AMPT + SPECT. No placebo drug	None	6	70%	66%	AMPT decreased VAS scores on happiness, increased sleepiness, restlessness, parkinsonism. No effects on anxiety or energy. Increased D_2 receptor binding potential (+28%)
Zimmermann *et al*[107]	*N*=10 (5 males). Within SS design: AMPT/placebo	None	5	Not reported	67% (urine)	No effect of AMPT on leptin secretion
Krahn *et al*[84]	*N*=10 (5 males). Within SS design: AMPT/placebo	None	5	Not reported	?	Small increase after AMPT on HRSD-25 (3 points). No effect on POMS or HAM-A. Decreased nocturnal and daytime urinary 6-SM excretion
Fujita *et al*[108]	*N*=8. 2 baseline SPECT/ AMPT + SPECT. No placebo drug	None	5.5/70 kg body weight	64%	51%	No change in PANSS subscales. Increased D_2 receptor ligands binding in temporal cortex (+13%), not in thalamus (+1.8%). Significant correlation between dysphoric mood and potential binding in the temporal cortex (r=0.88)
Verhoeff *et al*[102]	*N*=6 (4 males). Baseline PET/ AMPT + PET. No placebo drug	None	4.5	73%	53%	AMPT increased VAS scores on tiredness, sleepiness and drowsiness and decreased

					happiness. No change on POMS scores. Decreased selective attention and alertness. Increased neostratal dopamine D_2 receptors availability (+18.5%). Increased prolactin levels
Verhoeff *et al*[109]	*N*=6 (1 male). 2 baseline PET sessions. AMPT + PET (2 times). No placebo drug	None	5.25	62%	66%	Increased prolactin levels after AMPT. AMPT increased neostriatal D_2 receptor binding (+11%). No effect on D1 receptor binding

MDD: major depressive disorder; MDE: major depressive episode; BP: bipolar disorder; SAD: seasonal affective disorder; HRSD: Hamilton Rating Scale for Depression; SD: sleep deprivation. [a]Estimated values (based on values expressed in graphs).

patients had a relapse after AMPT, with HRSD-25 scores increasing from a mean of 3.1 to 23.9. A high baseline cortisol level was associated with the depressive response, whereas duration of remission, history of medication use and changes in HVA or MHPG levels were not.[89] In contrast with the findings in recently remitted samples,[83,85,87] the depressive response was unrelated to prior treatment. Of the 15 patients, 12 had been successfully treated with an SSRI during their latest episode, and three had received NRI treatment in the past. Thus, the depressive response to AMPT may not only reflect (prior) antidepressant mechanisms, but also reflect individual vulnerability to depression.

Furthermore, AMPT induced clinically significant depressive symptoms in seven of 13 patients with SAD who had recently responded to light therapy.[90] Six of these patients also responded to ATD, whereas four patients only responded to ATD. In a sample of nine SAD patients in summer remission, all patients relapsed after AMPT.[91]

In a sample of eight recovered bipolar patients who were stable on lithium, there was no mood effect during AMPT administration. However, manic symptoms increased 24–48 h after discontinuation of AMPT.[86]

In an early treatment study on symptomatic depressed patients, all three patients had a depressive response to AMPT.[78] However, this finding seems to be anomalous, as a later study found no mood change in a sample of 17 unmedicated symptomatic depressed patients.[85] Combined AMPT and ATD had no effect on mood in a sample of 13 unmedicated depressed patients.[92]

AMPT in other psychiatric disorders AMPT had no effect on symptoms or mood in a sample of six unmedicated symptomatic patients with *obsessive-compulsive disorder* (OCD).[93] In unmedicated patients with *schizophrenia*, AMPT led to a small but significant improvement of psychotic symptoms.[94,95] In 12 drug-free symptomatic patients with schizophrenia who had previously been treated with neuroleptic medication, AMPT also induced dysphoric symptoms.[95] In a sample of 10 patients with *cocaine abuse,* AMPT tended to reduce the cocaine-induced feelings of 'high' (P=0.10).[96]

AMPT in healthy volunteers AMPT generally does not induce depressive symptoms in healthy volunteers, as measured with the Hamilton Depression Rating Scale. However, one study found an average 3-point increase in Hamilton scores, although there were no changes in self-report mood ratings.[84] Some studies in healthy samples found small changes in the ratings of 'happiness' 'anger' or 'tension' using Visual Analogue Scales,[97,98] but these results are inconsistent. AMPT induced negative mood when it was combined with 40.5 h sleep deprivation.[99] AMPT commonly has sedative effects, including sleepiness, fatigue and decreased

subjective alertness. Not all studies in healthy samples use an active-sedative control drug. This implies that the mood effects after AMPT in these samples might be due to nonspecific side effects. In some individuals, AMPT also induced dystonic reactions,[100] tremors and restlessness,[98] which could persist for 12 h after discontinuation of AMPT. Administration of L-dopa reversed the AMPT-induced effects on sleep, alertness and calmness, but not on anger.[97] ATD did not change the response to AMPT in healthy volunteers.[101]

The effects of AMPT on cognitive functions have also been investigated. In a D_2 ligand-binding PET study using ^{11}C-raclopride in healthy volunteers, AMPT impaired attention, which was associated with increased raclopride binding potential, whereas psychomotor speed remained unchanged.[102] This study did not include a placebo condition, but can nevertheless be taken to indicate that the effects of AMPT on cognitive performance are associated with lowered DA function. Another study reported decreased performance on memory and attention tasks relative to lactose administration when AMPT was followed by 40 h sleep deprivation, but the effects were not significant after AMPT or sleep deprivation alone.[103] A small study (N=3) also found that AMPT led to an increase in saccadic eye movements.[104]

In summary, AMPT may slightly lower mood in some healthy individuals. However, the mood effect found in healthy samples is not comparable to the effect observed in remitted depressed patients or SAD patients. No studies were found that explicitly focused on the mood or cognitive effects in healthy first-degree relatives of depressed patients, or that even mentioned psychiatric family history. Perhaps the small inconsistencies are due to varying numbers of genetically vulnerable participants in the samples. However, methodological differences between the studies may also be responsible. It seems safe to conclude that, in nonvulnerable individuals, AMPT has very small mood effects, if any. The effects on attention and alertness are also quite small, and appear to be modulated by dopaminergic function.

Neuroendocrine effects of AMPT AMPT consistently produced a two- to four-fold increase in prolactin (Prl) levels (see Table 1). This is not surprising given that DA inhibits Prl release.[105] The magnitude of AMPT-induced Prl release is related to gender, with women showing a larger AMPT-induced Prl release than males. Estradiol enhances the synthesis and release of Prl by the pituitary gland, which may explain the gender difference.[106] Administration of AMPT also decreased the nocturnal release of melatonin[105,106] and the 24 h secretion of urinary 6-hydroxymelatonin (6-MS) (melatonin metabolite).[84,106] Leptin secretion was not affected by AMPT.[107] No studies were found that investigated the effects on neuroendocrine measures in patient samples.

Brain imaging studies and AMPT Since the AMPT procedure is too short to affect D_2 receptor upregulation,[98] the AMPT procedure combined with imaging techniques has been used to study occupancy of D_2 receptors in the absence of any pharmacological treatment ('baseline levels'). Assuming complete depletion of DA following AMPT, baseline DA receptor occupancy can be estimated by comparing the binding potential of D2 radiotracers before and after AMPT administration, using single photo emission computed tomography (SPECT)[95,98,108] or positron emission tomography (PET).[102,109] Depending on the investigated sample, brain region, magnitude of depletion in the synapse and possibly on the type of radiotracer, AMPT increased D_2 receptor binding potential in both patients and healthy samples by 9–28% (see Table 1). At least in healthy volunteers, AMPT does not affect D_1 receptor binding.[109] In patients with schizophrenia, the increase in receptor binding after AMPT is larger than in controls.[94] If behavioral changes in healthy volunteers occur, increased receptor binding (indicating higher 'baseline' synaptic DA activity) has been found to correlate positively with these changes in mood and cognition,[98,102,108] although there are also studies that found no relationship with mood.[109] In patients with schizophrenia, the change in receptor binding in the striatum correlated positively with positive symptoms.[94] However, the change in receptor binding was negatively associated with the change in dysphoric symptoms following AMPT in patients with schizophrenia.[95] Interestingly, the AMPT-induced increased D_2 receptor binding potential was associated with a decrease in positive symptoms after 6 weeks of antipsychotic treatment.[94] No imaging studies were found that investigated the effects of AMPT in depressed patients.

Limitations of the AMPT method Although the AMPT procedure facilitates the study of low CA function under standardized conditions within a relatively short time interval, there are some limitations. Firstly, high amounts of AMPT may cause sedation. It should be noted, however, that sedation is an ill-defined concept, originating from the clinical observation of overt drowsiness, caused by an underlying general decrease of (sympathetic) activation in the CNS. Claims of drug-induced 'sedation' are sometimes based on minute prolongations of reaction times.[110] Another pitfall of the concept of sedation is the implicit assumption of unidimensionality. However, when using fine-grained measures, it appears that drugs may have sedative effects in one system and stimulant effects in another system. For instance, SSRIs impair vigilance[111] but enhance motor output and shorten reaction times.[112]

One study found dystonic reactions[100] to AMPT, which were alleviated by diphenhydramine administration or massage and posture change. This probably indicates that AMPT disturbed the cholinergic–adrenergic balance. AMPT can also induce the

Table 2 Summary of the results of APTD challenge studies in humans

References	*Population, design*	*% reduction after APTD*		*Main findings*
		Tyrosine	*Phenylalanine*	
Healthy volunteers				
Moja *et al*[116]	*N*=5 (5 males). Within SS design: 15.75 g APTD/31.5 g APTD/fasting/19.05 g placebo	45.2 (15.75 g) 70.2 (31.5 g)	40.4 (15.75 g) 87.3 (31.5 g)	APTD decreased blood pressure
Sheehan *et al*[120]	*N*=8 (5 males). Within SS design: 45.0 g APTD/57.5 g placebo/water. Essential amino acids only	50	40	No effect on evening melatonin levels
McTavish *et al*[125]	*N*=15 (12 males). Between SS design: 90 g APTD + D-amphetamine (*n*=7)/115 g placebo + D-amphetamine (*n*=8). Essential amino acids only	68[a]	Not reported	APTD alone had no effect on mood APTD + D-amphetamine decreased VAS score on 'mind racing' and increased 'depression'
Leyton *et al*[118,119]	*N*=41 females, screened for psychiatric family history. Between SS design: 86.0 g placebo (*n*=14)/APTD (*n*=12)/ATD (*n*=15)	76.5 TYR/LNAA: 79.3	52.6 PHE/LNAA: 88.6	APTD and ATD both decreased energy and calmness (VAS). APTD and ATD decreased mood after a stress task (POMS). ATD increased anger after stress; APTD increased boredom before and after stress
Leyton *et al*[124]	*N*=39 female social drinkers (>11 consumptions/week), screened for psychiatric family history. Between SS design: 86.0 g placebo (*n*=13)/APTD (*n*=12)/ATD (*n*=14)	76.4	52.6	APTD decreased voluntary alcohol consumption in a 'free choice' test. No effect of APTD on liking of alcohol
Harmer *et al*[122]	*N*=12 (7 males). No personal history of psychiatric illness. Within SS design: APTD (90 g)/placebo (115 g). Essential amino acids only	87 (TYR + PHE)/LNAA: 99	Not reported	APTD increased prolactin levels. APTD impaired spatial working memory and recognition memory and increased feelings of 'less good'. No effect
Coupland *et al*[127]	*N*=5 (5 males). No history of psychiatric illness. Within SS design: APTD + pentagastrin/placebo + placebo/placebo + pentagastrin	Not reported	Not reported	Pentagastrin increased anxiety; APTD did not change this effect
Grevet *et al*[123]	*N*=10 (10 males), 6 with a positive family history of mental disorders. Within SS design: 100 g APTD/placebo	56	11	APTD increased anxiety (POMS) and tended to increase hostility. APTD decreased the delayed word

Table 2 Continued

References	*Population, design*	*% reduction after APTD*		*Main findings*
		Tyrosine	*Phenylalanine*	
				recognition of words. No effect on figures or attention. No effect of family history
McTavish *et al*[117]	*N*=16 (8 males). Within SS design: 90 g APTD + D-amphetamine/115 g placebo + D-amphetamine. Essential amino acids only	65	Not reported	APTD increased prolactin levels
		Ratio (TYR + PHE)/LNAA: 95		No mood effects after APTD alone. APTD reduced the psychostimulant effects of methamphetamine (VAS scales/cognitive tests)
	N=2 × 10 manic patients (8 males). Antipsychotic medication (*n*=20). Mood stabilizers (*n*=3).Between SS design: 90 g APTD/115.0 g placebo	Not reported	Not reported	Decrease in manic symptoms after APTD (35%)
Gijsman *et al*[14]	*N*=12 (7 males). Within SS design: 10/30/60 g amino acids (valine, isoleucine and leucine)	68 (10 g) 80 (30 g) 85 (60 g)	47 (10 g) 73 (30 g) 75 (60 g)	APTD increased prolactin levels after 60 g. Decreased TRP levels No mood effects
		Ratio (TYR + PHE)/BCAA: 12.0 (10 g), 94.6 (30 g), 96.5 (60 g)		Increased reaction times on a spatial memory task after the 30 and 60 g mixture
Harrison *et al*[130]	*N*=13 (0 males), screened for positive family history. Within SS design: 86 g placebo/APTD/ATD	63.9	78.7	No effect of APTD and ATD on IL-6. Increased fatigue scores after ATD, no other mood effects. No effect on mood after APTD

[a]Estimated.TYR: tyrosine; PHE: phenylalanine; BCAA: branch chain amino acids; LNAA: large neutral amino acids.

formation of crystals in urine,[81] although probably only if individuals do not drink enough water. Another more practical point of concern is that the procedure is very time consuming. Finally, it is not quite clear as to what extent the AMPT procedure affects NE and DA. This will be further discussed below. Analogous to the development of Trp-free mixtures instead of PCPA, tyrosine/phenylalanine-free mixtures are now increasingly replacing AMPT.

Acute phenylalanine/tyrosine depletion

Mechanisms and procedure The synthesis of CAs can be influenced by deprivation of the precursor AAs, phenylalanine and tyrosine. APTD parallels the ATD paradigm, except that individuals ingest an AA mixture that lacks tyrosine and its precursor phenylalanine. The mixture stimulates protein synthesis, consuming circulating tyrosine and phenylalanine and rendering less of these available for transport to the brain. Furthermore, phenylalanine and tyrosine compete with other LNAAs (Trp, leucine, isoleucine and valine) at a relative disadvantage to cross the blood–brain barrier.[113] Placebo testing usually consists of a balanced mixture containing all (essential) AAs (Table 2).

Animal studies have shown that a tyrosine/phenylalanine-free mixture reduces levels of tyrosine and of the DA metabolites HVA and 3,4-dihydroxyphenylacetic in the caudate nucleus of the rat brain, but does not alter DA levels themselves.[113] In monkeys, APTD reduces plasma tyrosine levels, CSF-, MHPG- and HVA-levels in similar ways as ATD affects plasma TRP- and CSF 5-HIAA levels.[114] Ingestion of a tyrosine/phenylalanine-free mixture has also been associated with a reduction of the tyrosine hydroxylase rate.[115] In monkeys which voluntarily consume moderate or high levels of alcohol, APTD has been associated with reduced alcohol intake, whereas no behavioral effects were observed after ingestion of a balanced or tryptophan-free mixture.[114]

Effect of APTD on plasma levels in humans In one of the first studies in humans, five healthy males had two different strengths of a tyrosine/phenylalanine-free mixture (15.75 and 31.5 g essential AA), a control drink (including tyrosine and phenylalanine) and a fasting period.[116] The results suggested a dose–response relationship; 6 h after ingestion of the 31.5 g AA mixture, plasma tyrosine and phenylalanine levels were maximally reduced to 12.7 and 29.8% of the initial value. The 15.75 g AA mixture reduced plasma tyrosine and phenylalanine levels to 61.7 and 59.7%, whereas nonsignificant changes in plasma levels were observed after the control mixture and in the fasting condition. Although the exact composition and amount of AAs have varied, the minimum levels of 10–30% of initial plasma baseline values after APTD have been replicated.[117–119] This suggests a nonlinear dose–response relationship, with plasma CA precursor levels being maximally reduced after ingestion of at least a 30 g tyrosine/phenylalanine-free mixture. However, plasma tyrosine and phenylalanine were

Table 3 Composition of the amino-acid mixtures in APTD studies

Amino acids[a]	*(a)*	*(b)*	*(c)*	*(d)*	*(e)*
L-Alanine				5.6	
L-Arginine				4.9	
L-Cysteine				2.7	
L-Histidine				3.2	
L-Phenylalanine					
L-Isoleucine	13.3	16.6	16.6	8.0	30
L-Leucine	21.0	25	25	13.5	40
L-Threonine	9.5	11.1	11.1	6.9	
L-Methionine	21.0	5.6	5.6	3.0	
L-Proline				12.2	
L-Serine				6.9	
L–Lysine (HCL)	15.2	19.4	19.4	11.0	
L–Tyrosine					
L-Valine	15.2	19.4	19.4	8.9	30
Glycine				3.2	
L-Tryptophan	4.8	2.8	2.8	2.3	
Total amount[b]					
Active mixture (g)	15.75	45.0	90.0	100.0	30.0
	31.5	57.5			60.0
Control mixture (g)			115.0		10.0

[a]Amino acids expressed as a percentage from the total amount of tyrosine/phenylalanine-free amino-acid mixture. [b]Total amount reduced by 15–20% in women. (a) Moja *et al*.[116] (b) Sheehan *et al*.[120] (c) McTavish *et al*;[125,117] Harmer *et al*.[122] (d) Leyton *et al*;[118,119,124] Coupland *et al*;[127] Harrison *et al*;[130] Grevet *et al*.[123] (e) Gijsman *et al*.[14]

only moderately reduced to 50 and 40% of baseline values in a study that used a 45.0 g tyrosine/phenylalanine-free mixture of seven essential AAs.[120] As noted by the authors, these relatively moderate reductions may be related to the absence of an overnight fast prior to AA administration. Moreover, APTD was started in the late afternoon, whereas other studies usually start in the morning. Since there is evidence for diurnal variation of plasma AA levels,[121] this may have influenced the results. A 30 or 60 g mixture of branch chain AAs (isoleucine, valine, leucine) also reduced tyrosine levels by about 70%, but Trp levels were also decreased.[14]

To summarize, animal and human studies have shown that ingestion of a tyrosine/phenylalanine-free mixture temporarily reduces plasma tyrosine and phenylalanine levels within a 7 h time interval. The compositions of the AA mixture are more variable between studies than is the case for ATD (see Table 3). There may be a dose–response relationship, rendering it a promising method to investigate CA function experimentally.

Behavioral effects of APTD In healthy individuals, APTD does not induce depressive symptoms, as measured with the Hamilton scale. Unlike ATD, self-report ratings of depressed mood are also unaffected by APTD,[118,119,122,123] except when it is followed by a public speaking task.[118,119] Slight increases have been observed in 'anger', 'tension' or 'boredom' ratings using visual analogue scales; however, these findings are not consistent.[118,119,123]

As had been observed in monkeys, APTD but not ATD decreased voluntary alcohol ingestion (using a free choice 'taste' test) in healthy female social drinkers.[124] APTD also reduced the psychostimulant effects of amphetamine (indicated by self-report and by cognitive tests),[125,126] but did not influence pentagastrin-induced anxiety.[127] In addition, cognitive processes are affected by APTD. Some studies suggest that APTD specifically interferes with spatial short-term and working memory, while it has no effect on sustained attention or other memory processes.[14,122] However, another study found an impairment in the retrieval of words from long-term memory, whereas attention and memory for abstract figures remained unchanged.[123]

A recent study compared the cognitive effects of ATD and APTD in a double-blind, placebo-controlled crossover study in healthy volunteers. ATD selectively impaired memory consolidation, whereas APTD selectively impaired working memory performance.[128] A small study found that APTD did not induce changes in PET Raclopride D2 binding.[129] Prolactin levels are increased after APTD,[14,126] which is indicative of reduced central DA brain function. Secretion of melatonin levels remained unaffected by APTD,[120] nor does APTD affect immunological parameters (IL-6).[130] In a sample of 20 bipolar patients (acute manic phase), APTD was associated with a 35% decrease in manic symptoms.[117] No studies were found that investigated the effects in depressed patients or in other clinical samples.

To summarize, APTD does not induce significant mood changes in healthy individuals, although mood changes may occur if APTD is combined with experimental stress induction. Some studies excluded individuals with a psychiatric family history or included only women or only men,[118,119,130] but there is no evidence that factors like gender or family history could fully explain the inconsistent findings on mood. Cognitive effects may be more common in healthy samples, but again the results are not consistent. Methodological factors including the use of different AA mixtures, measurement instruments and investigated population may explain these differences.

Discussion

An overview of the main findings is presented in Table 4. The evidence suggests that all three interventions are neurochemically specific, in terms of their short-term effects on one or two neurotransmitter systems, rather than on brain protein metabolism in general. The AMPT procedure is somewhat less selective, affecting both the DA and NE systems. The behavioral effects of ATD and AMPT are remarkably similar. Neither procedure has an immediate effect on the symptoms of depressed patients, and both have an effect on recently recovered depressed patients, which is influenced by prior treatment: ATD affects a subgroup of SSRI-treated patients, whereas AMPT affects a subgroup of NRI-treated patients. Remission of SAD (both after light treatment and natural summer remission) is temporarily reversed by both ATD and AMPT. The response of patients who recovered on dual-acting medications (clomipramine or high-dose venlafaxine) has not been systematically investigated.

Patients who have been in remission for longer may also be vulnerable to ATD and AMPT. However, the relationship with prior treatment is much weaker. For these patients, individual vulnerability markers (such as chronicity, gender, history of suicidality, family history of psychiatric illness or genetic variability) are probably the more important predictors of depressive response to challenge procedures. These challenges are therefore not only useful to investigate the mechanism of action of antidepressants, but also as models of vulnerability to depression.

The rarity of placebo responses to each of the procedures is striking. Since the procedures are rather burdensome (in terms of time investment, side effects, expectancy of mood effects), one might expect high rates of negative mood during control sessions also, but that is not the case.

Despite the similarities, specific effects of 5-HT *vs* CA depletions do exist. The best examples of this are the dependency on prior treatment in recovered patients and the distinctive effects of ATD and APTD on different memory systems. A memory consolida-

Table 4 Comparison of the effects of ATD, AMPT and APTD

	ATD	*AMPT*	*APTD*
Biochemical effects			
Magnitude of reduction of precursor levels	70–90% (NB: plasma Trp/LNAA ratio better estimate of central effects than Trp (free/total))	Unknown	70–85% (tyrosine) 50% (phenylalanine)
Dose–response relationship	Probably nonlinear	Unknown	Probably nonlinear
Time course of reduction (maximum effect)	5–7 h after depletion	24–48 h after starting treatment	5–7 h after depletion
Decrease in metabolite levels (%)			
CSF 5-HIAA	34		
HVA		60–80	27.4
MHPG		50–60	26.9
Mood effects			
Depression			
Medication-free, symptomatic patients	No direct effect. Delayed effect (bidirectional) in some patients	No effect on mood	Unknown
Recently remitted, medicated patients	Lowered mood. 50–60% relapse. Treatment-specific (SSRI)	Lowered mood. 50–60% relapsed. Treatment-specific (NRI)	Unknown
Recovered depressed patients	Lowered mood. Partial relapse 40–50%. Full relapse rare. Predictors: chronicity, female gender, history of suicidality	Lowered mood. Relapse rate: 76%	Unknown
SAD patients			
Recently remitted from light therapy	Lowered mood	Lowered mood	Unknown
Summer remission	Lowered mood. Response to ATD predicts depression during next winter	Lowered mood	Unknown
Bipolar disorder patients			
In remission, stable at lithium	No effect	Delayed effect on hypomanic symptoms	Unknown
Other mental disorders			
OCD, unmedicated	No effect on OCD symptoms or mood	No effect on OCD symptoms or mood	Unknown
Schizophrenia	No effect on symptoms	Improvement of positive symptoms	Unknown
Cocaine users	Decreased feelings of 'high'	Decreased feelings of 'high'	Unknown
Bulimia nervosa	Small effect on mood and desire to binge	Unknown	Unknown
Panic disorder	Increases vulnerability to CO_2 and flumazenil challenge	Unknown	Unknown
Healthy volunteers			
Mood	Small mood changes in vulnerable individuals. No 'depression'	Small mood changes in some individuals. No 'depression'	Small mood changes in some individuals. No 'depression'

Table 4 Continued

	ATD	*AMPT*	*APTD*
Other effects			
Cognition	Impaired memory consolidation. Possible effect on working memory. Possibly improved attention. Effect on executive function (planning) depends on sample (no effect in normals)	Impaired attention, possibly due to sedation	Impaired spatial short-term and working memory
Immune system		Unknown	No effect on IL-6
Food/drink choices	No effect on alcohol intake or craving in alcoholics. Small increase of protein intake	Unknown	Decreased alcohol intake
Sleep	Decreased REM sleep. Inconsistent effect on other sleep measures	Decreased sleep latency. Sleepiness. Insomnia after stopping AMPT	Unknown
Melatonin	Decreased nocturnal melatonin secretion	Decreased melatonin secretion	No effect on melatonin
Prolactin	No effect	Increased	Increased
Receptor binding	Decreased 5-HT_2 receptor density	Increased D_2 receptor binding	Unknown
Methodological factors			
Specificity	5-HT	NA and DA	Mainly DA?
Response to 'placebo' testing	Mood effects very rare. NB: different biochemical effects of different 'placebo' procedures	Mood effects very rare	Mood effects very rare

See also Van der Does[5] for a more detailed overview of ATD studies up to July 1999.

tion deficit was induced by ATD and not by APTD, whereas a working memory deficit was induced by APTD and not ATD.[128] These findings are in accordance with the effects of 5-HT and DA enhancement,[12] which consisted of opposing influences on working memory. The effects of DA enhancement were positive, consistent with a positive linear association of DA and working memory performance. As for 5-HT, there was no association at low levels, whereas high levels impair working memory, possibly by interfering with DA neurotransmission. However, the observed improvement of focused attention after ATD may reflect improved working memory functions at low levels of 5-HT, which would again be in line with the known opposing influences of 5-HT and DA on working memory in the frontal cortex.[12,70] The specific memory consolidation deficit after ATD must then be based on a different mechanism. It has previously been suggested, on the basis of observations in ATD effects on regional lowering of 5-HT in the brain in animals, that this effect is specifically linked with low 5-HT induced disruption of hippocampal function.[23] The putative negative linear association of memory consolidation with 5-HT functioning predicts improved memory consolidation after 5-HT augmentation. This could be obscured at higher levels of 5-HT because working memory impairments automatically affect performance on long-term memory tasks. Further illustrations of selective neurochemical and clinical effects may be the association of ATD with appetite, especially in bulimia patients. The absence of DA effects on appetite remains to be demonstrated, however. Conversely, the review provides evidence for the specific association of DA and addictive/rewarding behaviors, whereas strong evidence for an association with 5-HT is lacking.

As for hormonal markers of 5-HT and DA manipulations, the available evidence seems to confirm that 5-HT and cortisol are positively associated, whereas DA and Prl are specifically negatively associated. Their combined use therefore would enable a 'read-out' of 5-HT/DA balance. However, it should be remembered that cortisol is a sensitive indicator of

5-HT augmentation rather than of 5-HT lowering. Furthermore, cortisol is a nonspecific indicator of HPA-axis activity, and there are many other neurochemical and physiological influences contributing to acute cortisol release. Finally, transient depressive symptoms in healthy samples seem to be more prominently induced by ATD than by APTD and AMPT, although AMPT may be sedative. Possibly this latter effect is specifically noradrenergically mediated.

A major methodological concern is the specificity of AMPT and APTD. It is not clear to what extent the effects are mediated by NE and DA brain function. There are reasons to suggest that AMPT affects DA and NE, whereas APTD mainly affects DA. In rats, administration of AMPT decreased baseline extracellular NE and inhibited the idaxozan-induced increase in extracellular NE activity.[131] After APTD, baseline NE levels remained unchanged and APTD could not change the idazoxan-evoked increase in NE levels.[131] Moreover, since APTD in humans did not attenuate the amphetamine-induced decreases of hunger or response sensitivity on a sustained attention task, these behaviors may largely be mediated by the NE rather than the DA system.[125] This may also be true for the reduced alcohol intake found in animals and humans.[114,124] AMPT and APTD both increased Prl levels, indicative of reduced DA function. Finally, human plasma melatonin levels, indirect indicators of central NE synthesis, are reduced after AMPT,[106] but not after APTD.[120] It should be noted, however, that the moderate reduction of plasma levels in the latter study may have influenced the results.

A limited number of studies compared the effects of APTD with ATD in the same individuals, which prevents inconsistencies due to chance variations between samples. Another argument for incorporating an active control condition is that comparing two active conditions and placebo requires only 1.5 times more data than one active condition and placebo. In other words, combining ATD *vs* placebo and APTD *vs* placebo experiments would save many placebo sessions and would increase scientific value in terms of specificity of the mechanisms of the experimental manipulation of monoamine function. An important caveat, however, is that the test–retest reliability of most challenges is completely unknown, as well as possible carryover or order effects.

The findings presented in Table 4 concerning the 'other psychiatric disorders' should be viewed with caution (particularly the AMPT and APTD findings) since some of these are based on only one study. The more detailed findings are described in the text and in Tables 1 and 2. The clinical significance of response to monoamine depletion procedures is a potentially fruitful area for research, since some encouraging data exist for ATD.[26,52] An area that remains completely unexplored is the relationship between these biological indices of depression vulnerability with psychological vulnerability measures. Recent research based on Beck's cognitive model of depression[132] has yielded a new measure of vulnerability, labeled cognitive reactivity (CR).[133] Research has shown that CR is higher in previously depressed but euthymic individuals than in never-depressed individuals.[134–136] Furthermore, CR is higher in remitted patients who were treated with medication than in those treated with cognitive therapy. The same study found that CR predicts future depressive episodes, irrespective of prior treatment.[136] It would be interesting to know how different biological and psychological treatments affect biological and psychological vulnerabilities, and whether and how these two types of vulnerability are related.

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