A new method for rapidly and simultaneously decreasing serotonin and catecholamine synthesis in humans

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Objective: The administration of amino acid (AA) mixtures that are selectively deficient either in tryptophan or phenylalanine plus tyrosine can decrease serotonin or catecholamine synthesis, respectively. In the present study, we assessed whether a mixture that was simultaneously deficient in tryptophan, phenylalanine and tyrosine could induce sufficient protein synthesis that plasma levels of all 3 monoamine precursors would decrease. **Design:** Ten healthy volunteers (5 male, 5 female) were administered a tryptophan/phenylalanine/tyrosine-deficient AA mixture in an open-label study. Plasma concentrations of large neutral AAs were measured before and 5 hours after mixture ingestion. **Results:** The tryptophan/phenylalanine/tyrosine-deficient mixture lowered plasma concentrations of the 3 AAs by 67%, 78% and 77%, respectively ($p \leq 0.001$); their ratio to other large neutral AAs was decreased more, namely, by 87%, 90% and 90% ($p \leq 0.001$). Mood lowering was seen on 3 subscales of the bipolar Profile of Mood States, that is, elated–depressed, composed–anxious and clearheaded–confused, as well as 2 visual analog scales, bored and irritated ($p \leq 0.05$). **Conclusions:** Acute tryptophan/phenylalanine/tyrosine depletion may be a suitable new method for rapidly decreasing serotonin and catecholamine transmission simultaneously.

Objectif : L'administration de mélanges d'acides aminés (AA) comportant une carence sélective en tryptophane ou en phénylalanine et tyrosine peut réduire la synthèse de la sérotonine ou des catécholamines, respectivement. Au cours de cette étude, nous avons cherché à déterminer si un mélange comportant une carence simultanée en tryptophane, phénylalanine et tyrosine pourrait provoquer une synthèse de protéines suffisante pour réduire les taux plasmatiques des trois précurseurs des monoamines. **Conception :** Au cours d'une étude ouverte, on a administré à 10 volontaires en bonne santé (5 hommes, 5 femmes) un mélange d'AA comportant une carence en tryptophane, phénylalanine et tyrosine. On a mesuré les concentrations plasmatiques de gros AA neutres avant l'ingestion du mélange et cinq heures après. **Résultats :** Le mélange comportant une carence en tryptophane, phénylalanine et tyrosine a réduit les concentrations plasmatiques des trois AA de 67 %, 78 % et 77 %, respectivement ($p \leq 0{,}001$). Leur ratio par rapport à d'autres gros AA neutres a diminué davantage, soit de 87 %, 90 % et 90 % ($p \leq 0{,}001$). On a constaté une hypothymie sur trois échelles du Profile of Mood States bipolaire, soit excité-déprimé, calme-anxieux et

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lucide-confus, ainsi que sur deux échelles analogiques visuelles, ennuyé et irrité ($p \leq 0,05$). **Conclusions :** L'épuisement aigu du tryptophane, de la phénylalanine et de la tyrosine peut être un nouveau moyen convenable de réduire la transmission de la sérotonine et des catécholamines simultanément.

Introduction

Mood, anxiety and impulse-control disorders might be related to concurrent disturbances in dopamine (DA), norepinephrine (NE) and serotonin (5-HT) transmission.[1–3] At present, the only methods available to decrease the 3 monoamines simultaneously are administration of reserpine[4] and acute tryptophan depletion combined with the tyrosine hydroxylase inhibitor, α-methyl-*p*-tyrosine.[5] Each strategy has its disadvantages. α-Methyl-*p*-tyrosine can induce acute dystonic reactions[6] and crystalluria.[7] Reserpine administration is associated with few acute behavioural effects, whereas extended administration can trigger episodes of depression that do not remit without treatment.[4]

A third, not previously tested method entails the administration of an amino acid (AA) mixture deficient in all 3 monoamine precursors: tryptophan, phenylalanine and tyrosine. The primary mechanism by which monoamine-precursor-deficient mixtures produce AA depletion is through the induction of protein synthesis;[8,9] ingested AAs are incorporated into proteins, while AAs absent from the mixture are drawn from plasma and tissue. To ensure that sufficient protein synthesis would be induced by an AA mixture simultaneously deficient in tryptophan, phenylalanine and tyrosine, we measured, in a preliminary open-label study, the mixture's effect on plasma AA levels.

Methods

Ten healthy men and women (5 men, 5 women) whose mean age was 22.6 (standard deviation 3.3) years were recruited through newspaper advertisements. Exclusion criteria included a personal or first-degree relative's history of axis I psychiatric disorders, as assessed with the Structured Clinical Interview for DSM-IV[10] and family history research diagnostic criteria,[11] a Beck Depression Inventory[12] score above 10, a positive urine drug screen (Triage Panel for Drugs of Abuse [sensitive to phencyclidine, amphetamines, tetrahydrocannabinol, cocaine, opiates, barbiturates and benzodiazepines], Biosite Diagnostics, San Diego, Calif.) and a positive pregnancy test. None of the study subjects had significant medical diseases as determined by a physical examination and laboratory tests. All women were tested during their follicular phase (days 1–12); 3 were using oral contraceptives.

A mixture deficient in tryptophan/phenylalanine/tyrosine (TPT) was administered as an open-label procedure. The day before testing, all subjects ate a low-protein diet provided by the investigators and fasted from midnight. On the test day, subjects arrived at 8:30 am and had blood samples drawn to measure plasma AA concentrations. They then ingested the TPT-deficient mixture. The mixture's composition, preparation and administration were based on our tryptophan-deficient and phenylalanine/tyrosine-deficient mixtures.[13–15] After ingestion of the mixture, participants remained awake in a room with relatively neutral videos and reading material available to them.

Five hours after administration of the TPT-deficient mixture, a second blood sample was drawn to measure plasma AA concentrations. Plasma tryptophan levels were measured by isocratic reverse-phase high-performance liquid chromatography (HPLC) and fluorometric detection (FD). The other large neutral amino acids (LNAA: tyrosine, phenylalanine, leucine, isoleucine, valine) were measured using pre-column derivatization with o-phthalaldehyde and gradient reverse-phase HPLC-FD with aminoadipic acid as an internal standard.

Mood was assessed using the bipolar Profile of Mood States (POMS)[16,17] scale and 12 Visual Analog Scales (VAS)[18] labelled happy, bored, anxious, satisfied, excited, depressed, interested, angry, elated, restless, irritated and lively. Participants completed the mood scales immediately before ingesting the AA mixture and then hourly until 7 hours post ingestion. At the end of the test day, subjects were debriefed and given a tryptophan supplement plus a snack. Phenylalanine is available at high levels in protein, thus it is not necessary to administer it as a supplement over and above the snack. Tyrosine is formed from phenylalanine and is not an essential AA. Mood scales were inadvertently not administered to 1 subject.

Changes in plasma AA levels ($n = 10$) and mood (Δmax, $n = 9$) were assessed using repeated-measures analyses of variance (ANOVA).

The study was carried out in accordance with the Declaration of Helsinki and was approved by the McGill University Faculty of Medicine Institutional Review Board. All subjects gave informed written consent.

Results

The effect of the TPT-deficient mixture on plasma AA levels was assessed using sex × time ANOVAs. There were neither main effects of sex ($p > 0.25$) nor sex × time interactions ($p > 0.15$). In comparison, TPT depletion significantly lowered plasma concentrations of tryptophan ($F_{1,8} = 178.8$, $p \leq 0.001$), phenylalanine ($F_{1,8} = 104.2$, $p \leq 0.001$) and tyrosine ($F_{1,8} = 83.0$, $p \leq 0.001$). Compared with morning baseline, tryptophan, phenylalanine and tyrosine were reduced by 67%, 78% and

Table 1: Plasma concentrations of tryptophan, phenylalanine and tyrosine before and 5 hours after subjects* ingested the TPT-deficient mixture

	Mean plasma concentration (and SD)		
Amino acid	Morning baseline	5 h post ingestion	Decrease, %
Tryptophan, μmol/L	56.5 (9.1)	18.6 (6.5)†	67
Phenylalanine, μmol/L	40.5 (8.4)	8.8 (6.2)†	78
Tyrosine, μmol/L	43.1 (14.1)	9.8 (5.3)†	77
Tryptophan/LNAA	0.10 (0.02)	0.014 (0.01)†	87
Phenylalanine/LNAA	0.073 (0.02)	0.0075 (0.01)†	90
Tyrosine/LNAA	0.077 (0.02)	0.0072 (0.006)†	90

Note: TPT = tryptophan/phenylalanine/tyrosine; SD = standard deviation; LNAA = large neutral amino acids, i.e., tryptophan, phenylalanine, tyrosine, leucine, isoleucine and valine.
*n = 10.
†Compared with morning baseline, $p \leq 0.001$. Analysis of variance.

Table 2: Change in mood (Δmax) as assessed by the bipolar Profile of Mood States (POMS) and Visual Analog Scales (VAS) following ingestion of the TPT-deficient mixture

	Mean score (and SD)		
Scale	Morning baseline before ingestion	Score at peak change post ingestion	p value†
POMS*			
Elated–Depressed	55.6 (4.6)	46.9 (9.5)	0.006
Clearheaded–Confused	56.2 (8.5)	47.0 (13.1)	0.007
Composed–Anxious	63.3 (4.4)	52.3 (9.0)	0.04
Confident–Unsure	55.9 (8.1)	50.3 (11.0)	0.09
Energetic–Tired	51.8 (7.3)	46.4 (13.9)	0.22
Agreeable–Hostile	52.8 (7.3)	48.6 (13.9)	0.26
VAS			
Irritated	0.6 (1.0)	2.1 (2.1)	0.03
Bored	2.6 (1.6)	4.6 (2.6)	0.04
Interested	5.4 (2.3)	3.7 (2.2)	0.06
Satisfied	5.7 (1.9)	3.8 (2.8)	0.07
Depressed	0.2 (0.4)	1.2 (1.8)	0.07
Restless	1.1 (2.1)	3.4 (2.1)	0.08
Happy	5.8 (0.8)	4.8 (2.2)	0.18
Excited	3.9 (2.4)	2.9 (2.4)	0.38
Angry	0.4 (1.0)	0.7 (1.6)	0.45
Elated	3.6 (2.0)	2.9 (2.6)	0.54
Lively	4.4 (2.2)	4.0 (3.3)	0.65
Anxious	1.2 (1.4)	1.3 (2.1)	0.71

*The POMS data are normalized t scores. A change of 10 points corresponds to 1 standard deviation in the general population.
†Student's t test was performed comparing scores at pre-ingestion baseline and post-ingestion peak change.

77%, respectively. The effect of TPT depletion on the ratio of tryptophan ($F_{1,8}$ = 150.4, $p \leq 0.001$), phenylalanine ($F_{1,8}$ = 167.2, $p \leq 0.001$) and tyrosine ($F_{1,8}$ = 125.5, $p \leq 0.001$) to other LNAA was larger, –87%, –90% and –90%, respectively ($p \leq 0.001$) (Table 1).

Self-report scores suggest that TPT depletion was associated with lowered mood on the elated–depressed ($F_{1,7}$ = 15.24, p = 0.006), composed–anxious ($F_{1,7}$ = 8.72, p = 0.02) and clearheaded–confused ($F_{1,7}$ = 11.61, p = 0.01) POMS scales, as well as on VAS bored ($F_{1,7}$ = 5.11, p = 0.06) and irritated ($F_{1,7}$ = 7.76, p = 0.03) (Table 2). In contrast to reported findings for tryptophan depletion,[14] these effects appeared similar in men and women, as suggested by the absence of sex × time interactions ($p > 0.05$).

Discussion

The present pilot study indicates that TPT depletion can simultaneously decrease plasma levels of all 3 monoamine precursors, tryptophan, phenylalanine and tyrosine. The effect of TPT depletion on the ratio between the monoamine precursors and other LNAA that compete for active transport into the brain corresponded to an 87%–90% decrease. Neuroimaging, cerebrospinal fluid, microdialysis and postmortem tissue punch studies indicate that this magnitude of decline decreases brain 5-HT and catecholamine synthesis.[19–22] A mild mood-lowering response was also observed, though the mixture was administered in an open-label manner, so this should be interpreted cautiously. Together, the results suggest that omitting 2 essential AAs (tryptophan and phenylalanine) from an AA mixture induces protein synthesis to a similar extent as when only 1 is omitted. Acute TPT depletion may be a suitable new method of studying the effects of lowering all 3 biogenic amines at the same time.

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