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The acute tryptophan depletion and loading tests: Specificity issues

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Abstract. The acute tryptophan (Trp) depletion (ATD) and loading tests are a powerful tool for examining the role of serotonin in normal subjects and in those with behavioral and other disorders. The question of how specific to serotonin the tests are, has, however, received little attention. In particular, two questions arise regarding the potential effects of the tests on catecholamine synthesis, which may confound interpretation of behavioral data, namely whether: (1) the absence of Trp or its addition alters the [Phe+Tyr]/[LNAA+Trp] ratio, thereby influencing the entry of tyrosine and phenylalanine into the brain; (2) the relatively larger amounts of the 3 large neutral amino acids (LNAA), namely Val, Leu and Ile, in the amino acid formulation, compared with those of Phe and Tyr, could actually decrease the above ratio. Furthermore, the [Phe+Tyr]/[LNAA+Trp] and/or the [Trp]/[CAA] ratio may also be altered in control balanced and/or in Trp-loaded amino acid formulations, given the disproportionate contents of these 6 competitors in the different formulations. We addressed these questions in 114 normal US subjects divided into 5 groups of 20–25 each receiving Trp-depleted, -loaded or -balanced amino acid formulations at two dose levels: 50 g and the traditional 100 g. As expected, the [Trp]/[CAA] ratio was decreased by ATD and increased after Trp loading. However, the [Phe+Tyr]/[LNAA+Trp] ratio was also decreased by both ATD and Trp loading at both dose levels of each formulation. In subjects receiving a 50 g balanced control amino acid formulation, both the [Trp]/[CAA] and the [Phe+Tyr]/[LNAA+Trp] ratios were decreased. In all cases, the above unwanted decreases were due to the relatively larger contents in the various

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formulations of the above 3 LNAA, compared with Phe and Tyr. Based on these results and on theoretical considerations, we suggest that by either decreasing the contents of the LNAA by up to ~30% or increasing those of Phe and Tyr by up to ~50%, the [Phe+Tyr]/[LNAA+Trp] ratio can be maintained at normal baseline levels after consumption of the depletion, loading or balanced formulation and the [Trp]/[CAA] ratio can also be kept unaltered in the latter control formulation. We believe that this approach could achieve a greater specificity for these Trp manipulations and thus enhance the validity of the ATD and loading tests and improve interpretation of behavioral and related data.

Keywords: Acute tryptophan depletion; Amino acids; Behavior; Catecholamines; Large neutral amino acids; Phenylalanine; Serotonin; Specificity; Tryptophan; Tryptophan loading; Tyrosine

1. Introduction

The acute tryptophan (Trp) depletion (ATD) test [1–4] is a powerful tool in psychopharmacological research and behavioral medical practice for assessing the role of the cerebral indolylamine 5-HT (5-hydroxytryptamine or serotonin) in normal subjects and in those with behavioral disorders. The test is based on a number of well-established principles. Firstly, as it involves consumption of a mixture of 15 amino acids devoid of Trp, the consequent stimulation of protein synthesis is thought to result in depletion of tissue stores of Trp leading to a decrease in its availability in the circulation. The little Trp available is further disadvantaged by the presence of relatively much larger amounts of competing amino acids (CAA), namely Val, Leu, Ile, Phe and Tyr, which share the same cerebral uptake mechanism with Trp, resulting in decreased entry of the latter into the brain, leading to inhibition of 5-HT synthesis. Secondly, the importance of brain Trp in 5-HT synthesis is emphasized by the fact that the rate-limiting enzyme, Trp hydroxylase, is unsaturated with its Trp substrate [5]. Consequently, peripheral factors controlling Trp availability to the brain play important roles in 5-HT synthesis. At the primary level of control is activity of hepatic Trp pyrrolase (Trp 2,3-dioxygenase) [6] and at the secondary, but more immediate, level, two other factors are important: (1) competition from the above 5 CAA [7] and Trp binding to albumin [8,9], since it is the small (5–10%) free (ultrafiltrable or diffusible) fraction of circulating Trp that is immediately available for uptake by tissues and organs. Thus, Trp availability to the brain is best expressed by the ratio of both free and total [Trp] to the sum of the above 5 competing amino acids [CAA], i.e. the [Trp]/[CAA] ratio, as it is the most accurate predictor of Trp entry into the brain and hence of the rate of 5-HT synthesis.

As the traditional 100 g dose of the ATD test's amino acid formulation is invariably associated with a low tolerability, we have compared it (and also a similar Trp-loading dose) with the smaller 50 g doses in a unique detailed time-course study, to be reported elsewhere, which showed that the 50 g depletion and loading formulations are better tolerated and possess broadly similar pharmacokinetic profiles to the 100 g doses. In the course of this detailed time-course study, we noted that both the depletion and loading formulations, irrespective of dose, and also a 50 g balanced control formulation, decreased the availability of tyrosine and phenylalanine to the brain by decreasing the [Phe+Tyr]/[LNAA+Trp] ratio. This latter ratio is also the most accurate predictor of Phe and Tyr entry into the brain and hence of brain catecholamine synthesis, because the rate-limiting enzyme of dopamine