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An effective dietary method for chronic tryptophan depletion in two mouse strains illuminates a role for 5-HT in nesting behaviour

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ABSTRACT

Physiological depletion of tryptophan, the precursor to serotonin has been shown to alter mood and cognition in both humans and rodents. Few studies have investigated the neurochemical and behavioural effects associated with tryptophan depletion in mice. Given that BALB/c and C57BL/6J mice differ in tryptophan hydroxylase (TPH) functionality, serotonin levels and behavioural phenotype, we hypothesised that a differential strain response to chronic dietary tryptophan manipulations would be observed. Therefore, the effects of four chronic dietary tryptophan manipulations were investigated, the diets include a depleted diet (0% tryptophan, TRP^{-}), a deficient diet (0.25% tryptophan, $TRP^{-/+}$), an enhanced diet (1.25% tryptophan, TRP^{+}) and a control diet (0.7%). Diet-induced alterations in peripheral and central tryptophan levels and brain serotonin turnover were determined by high performance liquid chromatography. In addition, dietary-induced alterations in behaviour were assessed in several commonly used tasks. Peripheral and central tryptophan levels and consequently central serotonergic turnover were significantly decreased by the TRP^{-} diet in both strains, however, no effect of tryptophan supplementation was observed on tryptophan or serotonin levels. Dietary tryptophan manipulation induced pronounced behavioural effects, particularly in nesting behaviour where a reduction in nesting was observed following depletion and an increase in nesting behaviour was observed with enhanced tryptophan in both strains. Additionally, depletion produces an anxiolytic-like effect and did not impede locomotion. This study demonstrates significant alterations in the levels of tryptophan, serotonin turnover and behaviour following chronic dietary tryptophan depletion.

1. Introduction

Acute tryptophan depletion (ATD) is a widely implemented pharmacological tool used to investigate the role of serotonin (5-hydroxytryptamine, 5-HT) in a variety of behavioural processes including cognition, mood regulation and anxiety in both healthy controls and individuals with psychiatric disorders (Evers et al., 2005a, 2007, 2006; Horacek et al., 2005; Roiser et al., 2008; Rubia et al., 2005; van der Veen et al., 2007, 2006). L-Tryptophan has a number of fates in vivo, protein syntheses, catabolism in the kynurenine pathway and 5-HT biosynthesis. Approximately 1% of total circulating tryptophan crosses the blood brain barrier (BBB) into the central nervous system (CNS) (Ruddick et al., 2006), as such the availability of tryptophan in the periphery greatly affects the synthesis of 5-HT centrally. Therefore, peripheral depletion of the essential amino acid should theoretically reduce serotonin levels in the brain. This is the principal basis of ATD. Delgado and colleagues provided empirical evidence in the 1990s that peripheral depletion of tryptophan stores reduces central serotonin levels and induces depressive symptoms in susceptible individuals (Delgado et al., 1994, 1991). The majority of ATD studies opt to deplete tryptophan using either amino acid loading or through the administration of a tryptophan-free diet/drink (Bell et al., 2005; Hood et al., 2005). These studies to date have been predominantly conducted in humans or rat models. Few studies have investigated the potential of using such a pharmacological tool in inbred mouse strains.

Abbreviations: BALB/c, BALB/cOlaH; C57BL/6J, C57BL6JOlaHsd; TPH, tryptophan hydroxylase; 5-HT, 5-hydroxytryptamine, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; FST, forced swim test; TST, tail suspension test; EPM, elevated plus maze; TRP^{-}, tryptophan depleted diet; $TRP^{-/+}$, tryptophan deficient diet; TRP^{+}, tryptophan enhanced diet.

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Currently the efficacy of ATD in rodents is limited; although the central neurochemical and cognitive effects of ATD are been shown in rats, the behavioural alterations have not always reproducible (Blokland et al., 2002; Cahir et al., 2007; Jans et al., 2007; Lieben et al., 2004a,b; Olivier et al., 2008). Furthermore, ATD has proven rather difficult to establish in mice with limited central depletion of tryptophan and no behavioural effects observed following ATD in Swiss and C57BL/6J mice (van Donkelaar et al., 2010). Conversely, chronic administration of a tryptophan limited diet in C57BL/6N mice caused alterations in contextual fear-memory and anxiety-provoking tasks (Uchida et al., 2005, 2007). Chronic dietary tryptophan depletion has previously and reproducibly induced neurochemical and behavioural alterations in rats (Cahir et al., 2007). However, given the burgeoning use of mice in dissecting the genetic basis of many psychiatric disorders (Cryan and Holmes, 2005) it is thus logical to optimise and characterise chronic dietary tryptophan manipulations in mice.

C57BL/6J and BALB/c mice are two widely used strains in behavioural pharmacology. These strains differ markedly in their behavioural profiles; BALB/c mice are a more stress sensitive strain and thought to be a model of pathological anxiety (Belzung and Griebel, 2001; Jacobson and Cryan, 2007; Savignac et al., 2011). These strains also differ in neurotransmitter levels and tryptophan hydroxylase (TPH) function. TPH (Enzyme Commission number, EC 1.14.16.4) is the rate limiting enzyme in the synthesis of central serotonin stores (Siesser et al., 2010; Walther et al., 2003; Zhang et al., 2004). BALB/c mice harbour a loss of function SNP in their *Tph2* gene (C1437G), similar to a loss of function polymorphism (C1463A) found in human *Tph2* (Zhang et al., 2005). Although this particular SNP in humans has not been reproduced in other studies, the presence of the C1437G SNP in both BALB/c and DBA/2J mice is of interest and has been suggested to be an underlying factor impeding the efficacy of a number of antidepressants such as paroxetine (Calcagno et al., 2007; Cervo et al., 2005; Guzzetti et al., 2008). This SNP manifests as a region-specific decrease in serotonergic levels compared to C57BL/6J mice which carry the C/C allele of the functional *Tph2* gene.

Given the differences in TPH function and 5-HT levels, we hypothesised that BALB/c and C57BL/6J mice would display a differential response in terms of serotonin turnover and behaviour following chronic tryptophan manipulation. We chose a method of dietary manipulation that allowed us to investigate the impact of control (0.7% Trp), depleted (0% Trp) deficient (0.25% Trp) and enhanced (1.25% Trp) diets on peripheral and central tryptophan levels, central serotonergic turnover and performance in several behavioural tasks.

2. Materials and methods

2.1. Animal maintenance

40 male BALB/c and 40 male C57BL/6J mice aged 8 weeks were obtained from Harlan UK. Animals were allowed 1 week to acclimate to the facility and maintained under a 12 h light/dark cycle (lights on at 07:00 h/off at 19:00 h) with room temperature of 21 ± 1 °C. Food and water was provided *ad libitum*. Mice were singly housed 5 days prior to the beginning of the experiment. Diets were weighed and bodyweight of each mouse was recorded daily. All procedures were carried out in accordance with the European Communities council Directive of 24 November 1986 (86/609EEC) and approved by the Animal Experimentation Ethics Committee of University College Cork.

2.2. Diets

Diets were obtained from Harlan™ Teklad, where each diet was formulated with varying amounts of tryptophan, (Table 1). Fig. 1 illustrates the timeline of manipulations and behavioural tests. All behavioural tests were carried out during the light phase of the light/dark cycle. The control diet contained the normal amount of tryptophan available in the regular chow provided to the animals (0.7%), which is based on the required levels of amino acids necessary for optimal health.

Table 1
This table indicates the amount of tryptophan and other constituents of the diet formulated in Harlan Teklad. Tryptophan was the only amino acid that modified in these diets. The table shown is an example of the control diet which contains 0.7% of the diet. The following are the percentages of tryptophan in the remaining diets, TRP^{-} (0% tryptophan), $TRP^{-/+}$ (0.25% tryptophan), and TRP^{+} (1.25% tryptophan).

Diet components	g/kg
Sucrose	648.75
Corn oil	100.0
Zein	20.0
Gelatin	30.0
Mineral mix, AIN-76 (170915)	35.0
Calcium phosphate, dibasic $CaHPO_4$	4.5
Vitamin mix, AIN-76A	10.0
Choline chloride	1.5
Ammonium citrate, dibasic	23.4
Glycine	30.0
L-Lysine	16.35
L-Histidine	4.2
DL-Methinonine	5.3
L-Phenylalanine	7.1
L-Leucine	14.2
L-Isoleucine	6.2
L-Threonine	9.7
L-Valine	11.1
L-Tyrosine	7.0
L-Arginine HCL	8.7
L-Tryptophan	7.0

Tryptophan protein content is the lowest of all the essential amino acids in the body. It has been shown that the amount of tryptophan ingested is simply enough to replace the amount lost to catabolism and in general this is less than the daily recommended allowance (Lazaris-Brunner et al., 1998; Mahan and Shields, 1998). Furthermore, during periods of growth, tryptophan levels are deficient (Sawadogo et al., 1997). Dietary tryptophan deficiency induces a slight reduction in the tryptophan levels available for metabolism and favours protein synthesis, these effects of the deficiency can be reversed by adequate supply of tryptophan levels, whereas the absence of tryptophan as is the case in the TRP^{-} diet will lead to a significant reduction in TRP availability which may have detrimental outcomes in relation to both TRP catabolism and protein synthesis. A series of pilot experiments were carried out to determine the appropriate TRP^{-} doses to use both in terms of efficacy and tolerability (data not shown). Based on these preliminary studies we determined that chronic administration of the TRP^{-} diet induced ~70% depletion of tryptophan in plasma and brainstem of both strains. The dietary manipulations investigated were as follows, tryptophan depleted (TRP^{-}, 0%), tryptophan deficient ($TRP^{-/+}$, 0.25%) tryptophan enhanced (TRP^{+}, 1.5%), and control (0.7%). On day 32 mice were cervically dislocated, brains were rapidly microdissected; the hypothalamus, prefrontal cortex, frontal cortex, striatum, hippocampus, amygdala and brainstem were frozen on dry ice and stored at −80 °C until analysed. Trunk blood was collected in heparin tubes, centrifuged at 6000 rpm at 4 °C for 15 min using a MIKRO 22 R refrigerated centrifuge. The plasma was then collected and stored at −80 °C for analysis. As a significant decrease in bodyweight may be indicative of illness, the mice were carefully monitored throughout the experiment for any other signs of illness or symptoms of wasting diseases. The mice did not display any overt signs of illness, faecal output did not differ between groups and general sickness behaviours such as pilorection, hunching, and lack of activity were not evident in mice receiving the TRP^{-} diet. As approved by the ethical review process substantial weight loss was set at 25%, at which point mice must be euthanised in accordance with these regulations.

2.3. Behavioural tests

2.3.1. Light-dark box

Mice actively avoid brightly lit and potentially dangerous open areas and will preferentially occupy darker, more sheltering environments (Crawley, 2000). These innate and conflicting behaviours allow the researcher to analyze the anxiolytic effect of certain compounds on these behaviours. The dimensions of the light compartment are 45 × 22 × 22 cm, in which the dark compartment (21 × 14 × 21.5 cm) is inserted. The light compartment is brightly illuminated by a 1000 Lux from a height of 60 cm above the box floor. The procedure is based on that previously described (Cryan et al., 2003; O'Mahony et al., 2010). Mice were placed into the light compartment, facing away from the dark compartment entrance. Latency to first entry, total number of entries and time spent in light compartment were recorded for the 10 min duration of the test. The more time spent in dark compartment and the lower the number of transitions indicates an