Effect of Acute Tryptophan Depletion on the Response to Controllable and Uncontrollable Noise Stress

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Background: *Previous research provides evidence linking serotonin (5-hydroxytryptamine, 5-HT) with stress and depression. The controllable/uncontrollable (C/UC) stress paradigm aims to generate a state/condition, namely a feeling of lack of control in the context of a stressor, which might be an important factor in precipitating a negative mood state. Acute tryptophan depletion (ATD) is a technique that produces a decrease in central 5-HT levels in vivo. This study investigated the role of 5-HT in the behavioral response to a C/UC stress paradigm with ATD.*

Methods: *Healthy adult volunteers were randomly assigned to receive either a TRP-supplemented (*n = *15) or TRP-deficient (*n = *13) amino acid drink. At 5 hours postdrink, volunteers were subjected to sessions of controllable and uncontrollable noise stress (100-dB white noise). Subjective ratings of mood were obtained before and after the interventions.*

Results: *Participants who received the tryptophan-depleting drink had greater self-report ratings of negative mood on visual analogue scales and the Profile of Mood States after the uncontrollable stress than did participants who received the balanced drink.*

Conclusions: *The results suggest that 5-HT might play a role in providing resilience to uncontrollable stress. Additional studies with specific 5-HT pharmacologic probes will further clarify the results.*

Key Words: 5-HT, tryptophan depletion, controllable, uncontrollable, stress, resilience

Previous research suggests a link between serotonin (5-hydroxytryptamine, 5-HT) function and depression. Depressed patients exhibit decreased plasma free tryptophan (TRP) levels (Anderson et al 1990), a blunting of both the prolactin and growth hormone response to the probe L-tryptophan (Cowen and Charig 1987; Deakin et al 1990), and decreased uptake of [^{11}C]-5-HT in the frontal cortex (Agren and Reibring 1994), compared with healthy control subjects. In addition, depletion of central 5-HT causes a depressive relapse in patients who are maintained on selective serotonin reuptake inhibitors that resolves on replenishment of TRP levels (Delgado et al 1991).

The concept that aversive or stressful experiences might play a significant role in the onset of depression has existed for many years (Anisman and Zacharko 1982). More recently, it has been proposed that the 5-HT pathway originating in the median raphe nucleus (MRN) and projecting to the hippocampus functions as an adaptive system in times of chronic stress (Deakin and Graeff 1991) and that 5-HT$_{1A}$ receptors in the hippocampus mediate this resilience to stress (see Graeff et al 1996 for review). Failure of this system might therefore lead to the onset of depression. Consistent with this, both the number (Drevets et al 1999; Sargent et al 2000) and function (Deakin et al 1990) of 5-HT$_{1A}$ receptors have been shown to be reduced in unipolar depressed patients.

The controllable/uncontrollable (C/UC) stress paradigm aims to generate a state/condition, namely a feeling of lack of control in the context of a stressor, which might be an important factor in precipitating a negative mood state (Seligman 1972). Studies with C/UC stress paradigms, whereby a subject can prevent a certain stress (e.g., aversive noise) occurring on one occasion but not on another have lent some support to the learned helplessness theory (Hiroto 1974; Hiroto and Seligman 1975; Seligman 1972). Learned helplessness is the term used to describe the behavioral deficits evident in animals after exposure to UC stress, including a decrease in response initiation to escape further trauma and difficulty in learning response–escape associations (Seligman 1972; Seligman and Maier 1967). The theory proposes that feelings of helplessness produce both motivational and cognitive deficits and depressed affect (Seligman 1972). In humans, there is evidence for increased subjective and physiologic effects of UC stress compared with a similar exposure to C stress (Breier 1989; Breier et al 1987; Richell and Anderson 2004) but conflicting evidence for subsequent motivational deficits; Miller and Seligman (1975) found a deficit in anagram solving after UC stress, whereas studies by Breier and colleagues did not (Breier 1989; Breier et al 1987).

Animal studies have demonstrated that the relationship between acute stress and the 5-HT system is complex, and conflicting data exists on the role of 5-HT in mediating learned helpless behavior. Depletion of central 5-HT levels prevented the development of learned helpless behavior in rats exposed to UC shock (Edwards et al 1986), whereas a study by Petty et al (1992) suggested that maintaining neuronal 5-HT protects against the development of learned helplessness. The behavioral deficits of learned helplessness can be reversed by subchronic administration of antidepressants and 5-HT$_{1A}$ agonists (Graeff et al 1990; Joca et al 2003), and the escape deficits produced by inescapable shock can also be reversed by acute administration of a 5-HT reuptake inhibitor and a 5-HT$_{1A}$ agonist into the dorsal hippocampus (Joca et al 2003).

A wealth of evidence now supports the ability of acute TRP depletion (ATD) to reduce brain 5-HT function in man, with demonstration of decreased cerebrospinal fluid concentrations of TRP and 5-hydroxyindoleacetic acid (Carpenter et al 1998; Williams et al 1999) and decreased brain 5-HT synthesis, as assessed by positron emission tomography (Nishizawa et al 1997). The majority of studies in healthy male and female subjects have found no depressive mood change after TRP depletion (Benkelfat et al 1994; Miller et al 2000; Park et al 1994;

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Shansis et al 2000); however, certain individuals might be more vulnerable to the mood-altering effects of ATD, such as those with a family history of depression (Benkelfat et al 1994; Klaassen et al 1999).

There are much fewer data regarding the role that 5-HT plays in humans during stress. The aim of the present study was to investigate the effect of reducing 5-HT function through the technique of ATD on the psychological response to acute C/UC noise stress in healthy volunteers. On the basis of the animal studies described above (Graeff et al 1990; Joca et al 2003; Petty et al 1992), our hypothesis was that ATD would differentially increase the negative behavioral response to UC stress compared with C stress.

Methods and Materials

Participants and Study Design

Twenty-eight healthy volunteers (17 men, 11 women, aged 23.4 ± 5.1 years [mean ± SD]) were recruited by advertisement from the student and staff population of the University of Manchester. Participants were free from any serious medical disorder and had no present or past history of psychiatric disorder, as assessed by semistructured interview. They were required to be in good health, taking no medication apart from contraceptive pills, and to score less than 9 on the Beck Depression Inventory (Beck 1967). Before taking part, all participants had the study procedures explained to them, and their informed, written consent was obtained. Women were tested during the first half of their menstrual cycle or during the 3 weeks when they were taking contraceptive pills. The study was approved by the University of Manchester Committee on the Ethics of Research on Human Beings.

In a randomized, parallel group design, subjects received either an acute TRP-depleting drink (TRP−, $n = 15$, 7 male) or a control drink (TRP+, $n = 13$, 10 male) at approximately 9 AM. By chance, the gender ratios differed, although not significantly. At 5 hours after receiving the drink, they participated in a C/UC stress paradigm, which was given as part of a battery of tests investigating the effects of TRP depletion on mood and response to stress.

Acute Tryptophan Depletion

Acute TRP depletion was administered according to a standard protocol previously described (Miller et al 2000; Young et al 1985). Briefly, subjects ate a low-protein diet for 24 hours, fasted overnight, and were randomized (double-blind) to receive one of two amino acid drinks the following morning. The drinks consisted of approximately 100 g of amino acids and differed only in whether they lacked or contained 2.3 g TRP (TRP− and TRP+ drink, respectively). The powdered amino acids were mixed with 150 mL water, 100 mL chocolate syrup, and 2 tablespoons of artificial sweetener immediately before administration. Women received approximately 80% of the drink to reduce the risk of vomiting. Blood was taken for total and free TRP determination at baseline and 4.5 and 8 hours after the amino acid drink. Samples were immediately centrifuged at 2400 rpm for 10 min at 4°C and the plasma stored frozen at −75°C until analysis.

Mood Rating Scales

Mood states were assessed by self-report measures before and after each stress session and consisted of visual analogue scales (VAS) for "Happy," "Sad," "Helpless," "Anxious," "Stressed," and "Irritable" and three subscales of the Profile of Mood States (POMS) ("Depression–dejection," "Anxiety–tension," and "Anger–hostility"; McNair et al 1998). The VAS consisted of 100-mm lines on paper labeled "Not at all..." at the left end, and "Extremely..." at the right for each adjective. Subjects were asked to mark across the line at the point that indicated how they were feeling, with the distance of the mark from the left of the line taken as the score. The POMS adjectives were rated on a standard Likert-type scale. Subjects also rated how much control they had over the noise and its unpleasantness after each stress session. Participants were asked to give ratings on a 0–100-mm scale (0 = no control/not at all unpleasant, 100 = complete control/extremely unpleasant).

C/UC Stress Paradigm

In the C/UC paradigm, noise stress was used as an aversive stimulus, with two stress sessions. In the C stress session, the noise could be stopped by the subject, whereas in the UC stress session it could not be stopped and was terminated by the computer. Participants received the C stress first, followed by the UC stress, with an interval of 50 min separating the sessions. Both stress sessions consisted of the same total duration of noise. In our previous study (Richell and Anderson 2004), we balanced the order of administration of the two stress conditions but did not find any significant effect of order. Because a subject's responses determine the total duration of noise received during the C stress, having the UC stress second allows individual matching of the duration of noise on the two occasions.

The noise stimulus consisted of white noise (20–15,000 Hz) at 100 dB. The noise was generated by a computer and delivered through headphones. The duration of each train of noise (range 2–7 sec, mean 4.5 sec) and the internoise intervals (range 5–40 sec) were randomly determined by the computer. A console (12 cm × 15 cm) was attached to the computer, which contained two circular dome-lights, one red and one green, and a black push-button. During C stress the subject was able to stop the noise by pressing the black push-button four times, whereas during UC stress the noise could not be stopped. The push-button had a half-second stick in the down position (i.e., minimum duration of noise = 2 sec). Sixty trains of noise were administered during the C stress session. The number of noise trains in the UC stress session was adjusted to match the total noise duration actually received on the C stress session while maintaining a range of noise durations between 2 and 7 sec. The durations of the C and UC stress sessions were approximately 20 min and 15 min, respectively. Skin conductance was measured but showed no difference between type of stress, and we do not report the details here.

Subjects were given the same standardized instructions for each experiment. They were informed that "once the noise comes on there may be something that you can do to stop the noise" but no other instructions except that taking off the headphones was not an option. Feedback informing the participants of success or failure in terminating the noise was presented by the computer screen and console dome-lights (green for success, red for failure).

Analysis

Plasma total and free TRP were analyzed blind with high performance liquid chromatography with coulometric end point detection. The assay sensitivity was .01 μg/mL, and the intra- and interassay coefficients of variation were 1.83% and 6.26%, respectively, over the range of the standard curve.

Statistical analysis was carried out with SPSS for Windows, V.9 (SPSS, Chicago, Illinois). Data were analyzed by repeated-mea-