Effect of acute tryptophan depletion on CO_2-induced anxiety in patients with panic disorder and normal volunteers*

H. E. J. MILLER, J. F. W. DEAKIN and I. M. ANDERSON

Background Uncertainties remain about the role of serotonin in the aetiology and treatment of panic disorder.

Aims To investigate the effect of reducing brain serotonin function on anxiety at rest, and following 5% CO_2 provocation in normal controls and patients with panic disorder.

Method Twenty drug-free patients with DSM–III–R panic disorder and 19 controls received a tryptophan-free amino acid drink on one occasion and a control drink on the other in a double-blind, balanced protocol. 5% CO_2 was given as a panic challenge after 270 minutes.

Results Plasma tryptophan fell by more than 80% both patients and controls after the tryptophan-free drink. Tryptophan depletion did not alter resting anxiety. In patients alone, tryptophan depletion caused a greater anxiogenic response and an increased rate of panic attacks (9 *v.* 2, $P < 0.05$) after 5% CO_2 challenge. No normal volunteers panicked.

Conclusions Serotonin may directly modulate panic anxiety in patients with panic disorder. This may underlie the efficacy of serotonergic antidepressants in treating panic disorder.

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On the basis of conflicting evidence about the role of serotonin (5-hydroxytryptamine, 5-HT) in anxiety (Charney *et al*, 1987; Deakin & Graeff, 1991), we have suggested that 5-HT reduces unconditioned anxiety but increases conditioned anxiety and that this may relate to its effects in panic and anticipatory/generalised anxiety, respectively (Deakin & Graeff, 1991). Normal volunteer studies have been consistent with this 'dual' role for 5-HT in human anxiety (Deakin *et al*,1994). A previous small study in patients with panic disorder had reported that reducing 5-HT function using acute tryptophan depletion had no effect on resting anxiety (Goddard *et al*, 1994). In this study we investigated the effect of tryptophan depletion in a larger group of patients with panic disorder and in controls receiving a panic challenge. Our hypothesis was that tryptophan depletion would decrease resting anxiety while exacerbating anxiety responses to 5% CO_2 inhalation.

MATERIALS AND METHODS

Subjects

Twenty patients with DSM–III–R (American Psychiatric Association, 1987) panic disorder, diagnosed by the Structured Clinical Interview for DSM–III–R (SCID; Spitzer *et al*, 1990*a*), were recruited by referral or newspaper advertisement (Dratcu & Bond, 1993). Patients were required to report at least weekly panic attacks, confirmed by diary-keeping, and to be without comorbid psychiatric diagnoses (except secondary major depression) or alcohol/drug misuse within the last two years. Nineteen controls with no history of psychiatric disorder were recruited by advertisement and screened using the non-patient version of the SCID (Spitzer *et al*, 1990*b*). Exclusion criteria for all subjects included any significant medical disorder, taking regular medication or other drugs (within two weeks for patients and two months for volunteers) and drinking more than 20 units of alcohol a week. Benzodiazepine use was asked about but not directly screened for. All subjects had normal physical examination, electrocardiogram and biochemistry, including thyroid function. Baseline measures before the first test consisted of the Spielberger Trait Anxiety Inventory (Spielberger *et al*, 1979), the Hamilton Rating Scale for Anxiety (Hamilton, 1969), the Hamilton Rating Scale for Depression (Hamilton, 1960) and the Beck Depression Inventory (Beck *et al*, 1961). Subject details are given in Table 1. The study was approved by the local Research Ethics Committee and all subjects gave written informed consent.

Experimental procedures

Subjects were tested on two occasions separated by at least four days; for females this occurred during the first 14 days of the menstrual cycle or in the contraceptive pill-free week. On arrival, a forearm vein was cannulated and kept patent using heparinised saline. After 45 minutes the subjects received either a tryptophan-free 100 g amino acid drink or a control drink containing 2.3 g of tryptophan at 09.00 (time zero). Each test session lasted a further 330 minutes. A 5% CO_2 panic challenge (see below) was administered 270 minutes after the amino acid drink on both test days. Psychological ratings and blood samples were taken before the amino acid drink and then hourly until 240 minutes, followed by half-hourly until 330 minutes. The subjects sat in a quiet testing room and were not allowed to sleep, eat or smoke but could drink water and read emotionally neutral material. A meal was provided at the end of the experiment.

Acute tryptophan depletion

Oral administration of a tryptophan-free amino acid load leads to a profound reduction in plasma tryptophan concentration, thus reducing its availability for brain 5-HT synthesis (Reilly *et al*, 1997). In humans there is preliminary evidence for reduced 5-HT synthesis (Nishizawa *et al*, 1997) and reduced cerebrospinal fluid tryptophan and 5-hydroxyindoleacetic acid (Carpenter *et al*, 1998) after tryptophan depletion. Following a standard protocol (Young *et al*, 1985; Benkelfat *et al*, 1994), subjects ate a low-protein diet (20 g) for 24 hours, fasted overnight and received one of two amino acid drinks, double-blind, in a balanced order in the morning.

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Table I Subject characteristics

	Patients	Controls
Number of subjects (Male:Female)	10:10	11:8
Age (years)	38.4 (9.9)**	29.1 (8.4)
Agoraphobia	17	–
Comorbid DSM–III–R major depression	7	–
Family history of panic disorder	8	0
Number of panic attacks per weeks	2 (1–3.75)	–
Spielberger Trait Anxiety Inventory score	52.0 (13.9)***	30.8 (7.8)
Hamilton Rating Scale for Anxiety score	19.1 (7.9)***	1.1 (1.6)
Hamilton Rating Scale for Depression score (17-item)	14.3 (7.6)***	1.1 (1.6)
Beck Depression Inventory score	21.7 (11.7)***	3.2 (3.9)

Values are means (s.d.) except panic attacks per week, which is median (interquartile range); **$P < 0.01$ and ***$P < 0.001$.

The drinks differed only in whether they lacked or contained 2.3 g of tryptophan (tryptophan-free drink and control drink, respectively). The other amino acids were: L-alanine, 5.5 g; L-arginine, 4.9 g; L-cysteine, 2.7 g; glycine, 3.2 g; L-histidine, 3.2 g; L-isoleucine, 8 g; L-leucine, 13.5 g; L-lysine monohydrochloride, 11 g; L-methionine, 3 g; L-phenylalanine, 5.7 g; L-proline, 12.2 g; L-serine, 6.9 g; L-threonine, 6.9 g; L-tyrosine, 6.9 g; and L-valine, 8.9 g. Methionine, cysteine and arginine were encapsulated because of their unpleasant taste. Females received 80% of the above amounts because of their lower body weight. The powdered amino acids were mixed with 150 ml of water, 100 ml of chocolate syrup and two desert spoons of sugar immediately before administration. Subjects drank the mixture quickly through drinking straws and then chewed sugar-free gum to minimise the unpleasant taste.

Panic challenge with 5% CO_2

The CO_2 challenge is a widely used panic challenge, with evidence for increased sensitivity to its anxiogenic action in patients with panic disorder (Sanderson & Wetzler, 1990) and those at genetic risk for panic disorder (Perna *et al*, 1995). This may reflect derangement of a brainstem suffocation-alarm system (Klein, 1993). Using the method of Roth *et al* (1992), compressed air (21% oxygen and 79% nitrogen from the British Oxygen Company) was administered for 10 minutes through a positive pressure mask before switching to continuous 5% CO_2 (5% CO_2, 21% oxygen and 74% nitrogen; British Oxygen Company) for 20 minutes. Single breaths of 5% CO_2 were given at two and seven minutes to maximise blindness. The gas cylinders were in an adjacent room and connected to the subject through a Y-valve and CO_2-impervious tubing, with a reservoir bag connected to the tubing just before the mask. End-tidal CO_2 pressure was monitored. To minimise psychological effects on panic rates (Sanderson *et al*, 1989), the experimental conditions were standardised by familiarising the subjects with the procedure on the first visit, providing oral and written information on the effects of breathing 5% CO_2 on both test days and ensuring that the experimenter was visible during the challenge. A prior decision was made to exclude subjects from analysis of the effects of panic challenge if they panicked before CO_2 was administered.

Psychological measurements

Rating scales consisted of the Visual Analogue Scales (VAS: sad, anxious, panicky, lightheaded, happy, drowsy, nauseated, irritable) on a 100 mm line (0 mm=not at all; 100 mm=extremely), the Spielberger State Anxiety Inventory (STAI–S; Spielberger *et al*, 1979) and Profile of Mood States (POMS; McNair *et al*, 1971), given in that order. Before and after the 5% CO_2 challenge, subjects completed a modified version of the Acute Panic Inventory (API; Dillon *et al*, 1987), consisting of 24 items formed from the 13 DSM–III–R panic symptoms and apprehension rated on a five-point severity scale (0=not present; 4=extremely severe). After CO_2 challenge, subjects were asked to rate the peak severity that occurred during the challenge and also the similarity to their usual panic attacks. Because of the difficulties in assessing laboratory panic attacks (Sanderson & Wetzler, 1990), we applied two definitions of panic attack. First, the patients' own report of a panic attack rated at least 'quite similar' to their usual attacks. Second, an increase over baseline of four DSM–III–R panic symptoms rated at least moderately severe on the API, together with an increased Anxiety VAS or Panic VAS of 15 mm or greater.

Biochemical measurements

Blood samples were taken into lithium heparin tubes and centrifuged within an hour for 10 minutes at 2400 rpm and 4°C. The separated plasma was frozen and stored at −20°C before analysis. Plasma was assayed for total and free tryptophan (at 0, 240 and 300 minutes) and for cortisol (at 270, 300 and 330 minutes). Plasma tryptophan concentration was measured by a semi-automated high-performance liquid chromatography with fluorescence endpoint detection. Intra- and interassay coefficients of variation were 8% and 13%, respectively, and the limit of detection was 1.3 pg/ml. Cortisol was analysed by standard radioimmunoassay. Intra- and interassay coefficients of variation were 4.3% and 5.6%, and the limit of detection was 0.1 μg/100 ml.

Analysis

Two time periods were analysed using SPSS for Windows Release 6 (SPSS Inc., Chicago, IL): the pre-challenge period (0–270 minutes), reflected a resting period; and the CO_2 challenge period (270–330 minutes). The principal analysis for continuous data was by repeated measured analysis of variance (ANOVA) using the Huynh–Feldt correction with a between-subjects factor (group) and two within-subject factors of occasion (tryptophan-free or control drink) and time. Controls and patients were analysed separately by ANOVA following significant interactions by group or by occasion. *Post hoc t*-tests were used to aid interpretation of data. Categorical data were analysed using McNemar's test (Armitage & Berry, 1987) and correlations using Spearman's correlation coefficient. Data are presented as mean (s.d.) values.

RESULTS

The control and tryptophan-free drinks were indistinguishable by the subjects and experimenter (H.E.J.M.) and generally well

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Table 2 Effect of control and tryptophan-free drinks on total and free plasma tryptophan

	Control drink			Tryptophan-free drink		
	0	240 min	300 min	0	240 min	300 min
Total tryptophan						
Controls	9.8 (1.6)	25.0 (7.6)***	21.7 (7.8)***	10.5 (2.5)	2.0 (1.2)***	1.6 (1.2)***
Patients	11.3 (3.4)	31.8 (14.2)***	24.4 (11.6)***	12.4 (4.2)	2.5 (1.8)***	2.2 (1.5)***
Free tryptophan						
Controls	0.74 (0.45)	1.37 (0.66)**	1.34 (0.57)***	0.74 (0.65)	0.22 (0.36)***	0.19 (0.21)***
Patients	0.77 (0.46)	1.26 (0.86)**	1.22 (0.70)***	0.69 (0.44)	0.34 (0.4)**	0.26 (0.18)***

Values are means (s.d.) in μg/ml; **$P<0.01$ and ***$P<0.001$ compared to baseline.

tolerated, although drowsiness, nausea and abdominal fullness did occur up to 120 minutes. No order effects were detected in the results.

Tryptophan measures

There were no significant differences in baseline measures (Table 2). Significant occasion × time interactions for total tryptophan ($F(2,74)=174.23$, $P<0.001$) and free tryptophan ($F(2,74)=4.123$, $P<0.001$) occurred, with plasma tryptophan decreasing on the tryptophan depletion occasion (total tryptophan: patients, 83% and volunteers, 85%; free tryptophan: patients, 62% and volunteers, 74%) and increasing on the control occasion (total tryptophan: patients, 283% and volunteers, 256%; free tryptophan: patients, 164% and volunteers, 185%).

Pre-challenge period (time 0–270 minutes)

Caseline behavioural ratings

Patients had significantly higher anxiety and depression ratings than controls (group effect on ANOVA $P<0.01$ for all measures; Fig. 1).

Effect of tryptophan depletion on behavioural ratings

Anxiety scores, apart from Panic VAS, fell during the pre-challenge period (Figs 1a–d). There were no significant interactions, indicating no effect of tryptophan depletion and no difference between patients and controls. Anxiety increased between 240 and 270 minutes (Fig. 1), suggesting acute anticipatory anxiety before the CO_2 challenge. Analysis of variance of these two time points showed increases in STAI–S (time: $F(1,37)=5.43$; $P=0.025$) and POMS Anxiety-Tension (time: $F(1,37)=4.00$; $P=0.053$). Patients, but not controls, had increased Anxiety VAS ratings (group × time: $F(1,37)=5.89$; $P=0.02$) with a similar but non-significant pattern with Panic VAS (Fig. 1). For Panic VAS alone there was a greater increase on the tryptophan depletion occasion compared with the control occasion (occasion × time: $F(1,37)=4.37$; $P=0.043$).

Depression-related ratings fell during this period but this was only significant for POMS Depression. There were no significant interactions between group, time or occasion. No significant effect of tryptophan depletion was found in separate analyses of patients with panic disorder according to the presence ($n=7$) or absence ($n=13$) of current major depression or a past history of major depression in the absence of current depression ($n=8$) (results not shown).

Other behavioural ratings generally showed a maximal effect at 60 minutes

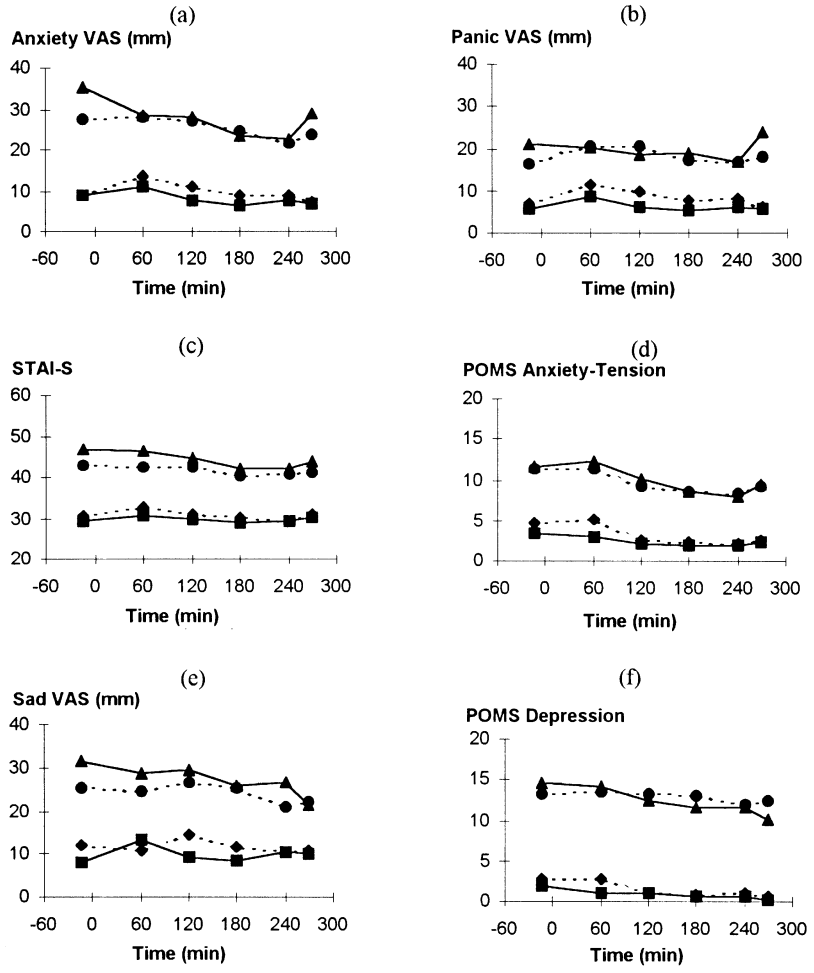

Fig. 1 Effect of tryptophan depletion on psychological ratings during the resting phase: (▲) patients with panic disorder on tryptophan depletion occasion; (●) patients with panic disorder on control occasion; (■) normal volunteers on tryptophan depletion occasion; (◆) normal volunteers on control occasion. (a) Anxiety VAS, time: $F(5,185)=4.27$; $P=0.003$. (b) Panic VAS, time: $F(5,185)=1.29$; $P=0.28$. (c) STAI–S, time: $F(5,185)=5.73$; $P<0.001$. (d) POMS Anxiety-Tension, time: $F(5,185)=8.70$; $P<0.001$. (f) POMS Depression, time: $F(1,37)=4.60$, $P=0.007$.

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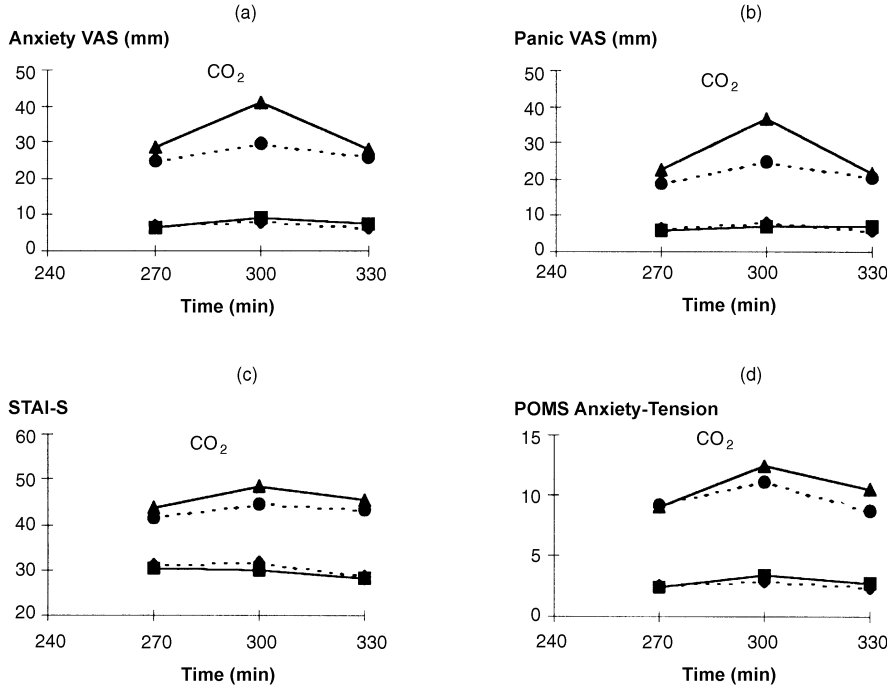

Fig. 2 Effect of tryptophan depletion on psychological ratings during 5% CO_2 challenge. For meaning of symbols, see legend to Fig. 1. (a) Anxiety VAS, time: $F(1,35)=16.87$, $P<0.001$; group time: $F(2,70)=4.35$, $P=0.017$. (b) Panic VAS, time: $F(1,35)=11.78$, $P<0.001$; group × time: $F(2,70)=7.93$, $P=0.001$. (c) STAI–S, time: $F(1,35)=28.89$, $P<0.001$; group × time; $F(2,70)=10.09$, $P<0.001$. (d) POMS Anxiety-Tension, time: $F(1,35)=25.12$, $P<0.001$; group × time: $F(2,70)=5.23$, $P=0.008$.

due to the drink, returning towards baseline by the end of the resting period. There were significant effects of time for Nausea VAS, Lightheaded VAS, Drowsiness VAS and POMS Vigour (all $P<0.001$) but not POMS Fatigue or POMS Confusion. There were no significant differences over time between patients and volunteers or occasions.

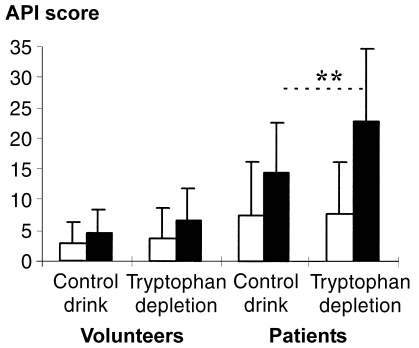

Fig. 3 Effect of tryptophan depletion on Acute Panic Inventory (API) scores before and after 5% CO_2 challenge: (□) pre-CO_2 challenge; (■) post-CO_2 challenge. Values are means with standard deviations; $^{**}P<0.001$ for occasion × time interaction for patients with panic disorder.

Carbon dioxide challenge period (270–330 minutes)

One patient discontinued the gas inhalation before receiving CO_2 (control occasion) and one panicked at the time of putting on the mask (tryptophan depletion occasion), leading to exclusion. One control subject was excluded from analysis of API scores only because of a very high pre-inhalation API score inconsistent with all other anxiety ratings.

Cortisol measures

Plasma samples from four controls were missing at 330 minutes and results were therefore available from 32 subjects. There were no significant effects of CO_2 inhalation and no difference between patients and controls or occasion. Patients who had a panic attack (by either definition) did not differ from those not panicking (results not shown).

Psychological measures

As noted for other anxiety ratings (see above), patients had significantly higher API scores at challenge baseline (270 minutes) than controls (see Fig. 3; group: $F(1,34)=18.62$; $P<0.001$). Carbon dioxide challenge caused increases in all ratings of anxiety (Fig. 2). Significant group × time interactions occurred for all anxiety ratings due to patients' ratings increasing more than controls (Fig. 2). Panic VAS showed a significant group × occasion × time interaction ($F(2,70)=5.69$; $P=0.005$) due to a greater increase following tryptophan depletion in patients but not controls. When controls and patients were considered separately, the anxiogenic effect of CO_2 was small in controls and only Anxiety VAS increased significantly ($F(2,36)=4.36$; $P=0.024$); indeed, STAI–S decreased throughout the period ($F=(2,36)9.27$; $P=0.001$). Tryptophan depletion had no effect on anxiety ratings in controls. In contrast, patients showed consistent increases in anxiety ratings following CO_2 (Fig. 2), with greater responses seen on the tryptophan depletion occasion for Panic VAS ($F(2,34)=6.14$; $P=0.005$) and a trend for Anxiety VAS ($F(2,34)=3.07$; $P=0.060$). Increases in POMS Anxiety-Tension and STAI–S were non-significantly greater on the depletion occasion.

Carbon dioxide inhalation increased API scores particularly in patients and on the tryptophan depletion occasion (group × occasion × time: $F(1,34)=7.90$; $P=0.008$; Fig. 3). In controls analysed separately there was a modest CO_2-induced increase in API scores (time: F $(1,17)=6.54$; $P=0.021$) but no effect of tryptophan depletion (occasion × time: F $(1,17)=0.46$; $P=0.508$). In patients there was a robust effect of CO_2 inhalation (time: $F(1,17)=46.08$; $P<0.001$) and a marked effect of tryptophan depletion (occasion × time: $F(1,34)=23.06$; $P<0.001$).

Panic attacks in patients were more frequent on the tryptophan depletion occasion for both subjective panic attacks (eight *v.* two, $P<0.05$) and DSM–III–R panic attacks (nine *v.* two, $P<0.05$). Patients commented that the security of the experimental situation had reduced cognitive symptoms and severity compared with their usual panic attack. This is reflected in relative low mean panic attack similarity ratings, which were higher on the tryptophan depletion occasion (1.39 (s.d.=0.85) *v.* 0.78 (s.d.=0.65), $P=0.012$).

On the tryptophan depletion occasion, patients with a DSM–III–R panic attack had higher mean anxiety scores immediately before CO_2 inhalation (270 minutes) than non-panickers; this was statistically significant for API scores (11.9

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(s.d.=10.9) *v.* 3.8 (s.d.=2), P=0.049), STAI–S (49.2 (s.d.=7.9) *v.* 38.4 (s.d.=9.2), P=0.017) and POMS Anxiety-Tension (12.1 (s.d.=5.0) *v.* 6.0 (s.d.=4.1), P=0.012). However, there were no significant differences at 240 minutes on any anxiety measures, suggesting that those who experienced greater acute anticipatory anxiety were more likely to panic when challenged by 5% CO_2.

Analysis of the two respiratory items on the API separately showed no significant effect of tryptophan depletion (increases on control *v.* tryptophan depletion occasion: 2.0 (s.d.=1.7) *v.* 2.6 (s.d.=2.0), P=0.165 in patients and 0.8 (s.d.=1.5) *v.* 1.4 (s.d.=1.2), P=0.127 in volunteers).

Correlations

In patients, anxiety responses to 5% CO_2 (the difference score between 270 and 300 minutes) correlated with a number of values at baseline (time zero) and 270 minutes on the tryptophan depletion occasion. Anxiety VAS responses to 5% CO_2 tended to correlate with: Anxiety VAS values at time zero (rho=0.43, P=0.078) and 270 minutes (rho=0.44, P=0.065); Panic VAS values at time zero (rho=0.57, P=0.050) and 270 minutes (rho=0.51, P=0.031); and STAI–S at time zero (rho=0.56, P=0.016). Panic VAS responses also correlated with STAI–S at time zero (rho=0.49, P=0.039). No significant correlations between baseline ratings and anxiety responses to 5% CO_2 challenge were seen on the control occasion or in volunteers.

There were no consistent patterns of association between bichemical measures and anxiety responses to 5% CO_2.

DISCUSSION

Our main finding was that acute tryptophan depletion increased anxiety responses induced by inhalation of 5% CO_2 in drug-free patients with panic disorder. In contrast, resting levels of anxiety and other mood measures were not affected by tryptophan depletion. We saw no effects in normal volunteers apart from a mildy anxiogenic effect of 5% CO_2 inhalation.

Tryptophan depletion and resting anxiety and depression

We only measured resting anxiety up to 270 minutes, which could be too short to detect an effect because maximum plasma tryptophan reduction does not occur until about 300 minutes (Young *et al*, 1985; Delgado *et al*, 1990; see Table 2). However, our results agree with those of Goddard *et al* (1994) who also found little effect of tryptophan depletion in a small study with eight drug-free patients with panic disorder assessed up to 420 minutes. They are also consistent with findings in other psychiatric conditions where tryptophan depletion does not alter anxiety (e.g. Delgado *et al*, 1994; Aronson *et al*, 1995; Benkelfat *et al*, 1995), unless there is exacerbation of the primary disorder (e.g. Delgado *et al*, 1990; Menkes *et al*, 1994; Weltzin *et al*, 1994). The lack of effect of tryptophan depletion on anxiety in normal volunteers in our study is also consistent with most other studies (e.g. Young *et al*, 1985; Smith *et al*, 1987; Weltzin *et al*, 1994; Goddard *et al*, 1995; Koszycki *et al*, 1996).

Our findings appear to conflict with evidence suggesting that tryptophan depletion should be anxiolytic. Reducing 5-HT function is anxiolytic in a number of paradigms in animals (Coplan *et al*, 1992) and ritanserin, a 5-HT_2 antagonist, is anxiolytic in a human model of generalised anxiety and in patients with mixed anxiety and depression (Deakin *et al*, 1992). Conversely, increasing 5-HT functioning using *m*-chlorophenylpiperazine (Charney *et al*, 1987) and fenfluramine (Targum & Marshall, 1989) challenge causes anxiety in patients with panic disorder, as can initial treatment with clomipramine and selective serotonin reuptake inhibitors (e.g. Ramos *et al*, 1993). These data are difficult to reconcile but the present study suggests that the increased levels of anxiety generally seen in patients with panic disorder are not simply due to tonically increased 5-HT function.

Our lack of effect of tryptophan depletion on depressive symptoms is consistent with the previous study in patients with panic disorder (Goddard *et al*, 1994), whereas in other patient groups there have been variable findings with regard to induction of depression (Delgado *et al*, 1990, 1994; Barr *et al*, 1994; Smith *et al*, 1997), possibly related to diagnosis and drug status. Similarly variable results in lowering mood have been seen in normal volunteers studies (e.g. Young *et al*, 1985; Oldman *et al*, 1994; Weltzin *et al*, 1994; Goddard *et al*, 1995; Koszycki *et al*, 1996).

Tryptophan depletion and provoked anxiety

We found 5% CO_2 inhalation to be panicogenic in patients but only mildly anxiogenic in controls. Like Roth *et al* (1992), we found that patients who panicked had higher anxiety levels immediately before challenge than non-panickers. They argued that this could be explained by anticipatory anxiety increasing above a threshold of tolerance (i.e. situational panic). In contrast, other studies have found no relationship between baseline anxiety and subsequent panic attacks (e.g. Gorman *et al*, 1988; Sanderson & Wetzler, 1990), suggesting a model of spontaneous panic attacks. To what extent prior anxiety may predict panic to CO_2 challenge therefore remains unresolved, but our results suggest that it may act as a factor.

Tryptophan depletion increased acute anticipatory and 5% CO_2-induced anxiety in patients and it is interesting to relate this to the effect of treatment with 5-HT-enhancing antidepressants, which have been shown to reduce panic patients' hypersensitivity to CO_2 challenge (Bertani *et al*, 1997; Gorman *et al*, 1997). Our result supports increased 5-HT neurotransmission playing an important part in the anti-panic effect of these drugs. The lack of effect that we found in volunteers appears to contrast with a recent report of increased anxiety ratings and some panic symptoms following 35% CO_2 inhalation after tryptophan depletion (Klaassen *et al*, 1998). Although methodological differences, including the weak anxiogenic response in our study, may explain this, there have been conflicting results in normal volunteers using other anxiety challenges that do not simply appear related to the severity of the anxiogenic challenge. Tryptophan depletion has been reported to enhance anxiety after yohimbine administration (Goddard *et al*, 1995) but not after cholecystokinin challenge (Koszycki *et al*, 1996) or in response to simulated public speaking (Mortimore *et al*, 1997). It therefore remains unclear whether 5-HT modulates stimulated anxiety in normal subjects.

It is of interest that the measures specifically relating to panic (Panic VAS and API) appeared to be more robustly affected than general anxiety ratings in patients. We cannot, however, distinguish between situational panic related to anticipation of a proximal threat (imminent or actual CO_2 challenge) and panic produced by a biological effect of CO_2. Although the results are generally consistent with the hypothesis that 5-HT acts to restrain panic, a distinction between its role in spontaneous panic compared to acute anticipatory anxiety is

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difficult to sustain and it may be that the important distinction is between other aspects of anxiety, such as proximal versus distal threat or conditioned versus unconditioned anxiety.

The lack of effect of 5% CO_2 challenge on plasma cortisol is generally consistent with the literature (Carr *et al*, 1986; Klein, 1993). Why patients with panic disorder lack a stress hormone response when panicking is unknown, but it could relate to an adaptive change in the hypothalamus–pituitary axis related to chronic anxiety or stress (Gray, 1987), or simply a low threshold for reporting panic attacks. In addition, the anxiety responses to 5% CO_2 were relatively mild and may have been insufficient to stimulate a stress response.

Methodological considerations

We found a lower rate of panic following 5% CO_2 inhalation on the control occasion than that suggested by the literature (about 10% *v*. 50%; Sanderson & Wetzler, 1990), possibly because the setting of the experiment and careful explanation minimised the panic rate through cognitive factors (Clark, 1986). We think it unlikely that the control drink suppressed the panic rate, because although it increased both total and free plasma tryptophan, the plasma tryptophan to large neutral amino acid ratio and hence tryptophan entry into the brain would still be expected to be reduced, although much less than following the tryptophan-free drink (Weltzin *et al*, 1994).

The successful blinding of the experimental occasions argues against cognitive factors directly affecting the result. However, there is a theoretical possibility that tryptophan depletion stimulated respiratory function and hence, indirectly, the panic rate through the occurrence of somatic symptoms. In rats, 5-HT depletion stimulates respiration (Olson *et al*, 1979) and a similar tendency was seen with tryptophan depletion in humans (Kent *et al*, 1996). However, arguing for a direct effect on anxiety, tryptophan depletion enhanced acute anticipatory anxiety and there was no significant effect on the respiratory item scores of the API. Unfortunately we did not have direct measures of respiratory effort or tidal volume for a direct answer to the question.

Implications

Our study suggests that 5-HT directly inhibits panic anxiety and that this may help to explain the anti-panic effects of antidepressants such as selective serotonin reuptake inhibitors. The lack of effect of tryptophan depletion on resting anxiety is consistent with 5-HT playing a different role in different types of anxiety, although the results did not suggest a direct role in the maintenance of high resting levels of anxiety in our patients with panic disorder. This implies that the anxiolytic effect of antidepressants in generalised anxiety and non-panic anxiety associated with depression may involve a different mechanism to the anti-panic effect. Studies using different manipulations of 5-HT function and alternative anxiety challenges in patients with anxiety disorders and healthy volunteers are needed in order to shed further light on the role of 5-HT in human anxiety.

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CLINICAL IMPLICATIONS

- Acute reduction in brain 5-HT function appears to enhance anxiety provoked by 5% CO_2 inhalation in patients with panic disorder.
- Direct enhancement of 5-HT neurotransmission may explain the efficacy of some antidepressants in treating panic disorder.
- The lack of effect of acute tryptophan depletion on 5% CO_2-induced anxiety in normal volunteers may indicate different mechanisms operating in pathological and normal anxiety.

LIMITATIONS

- Reduction in brain 5-HT function as a consequence of acute tryptophan depletion could only be inferred.
- Anxiety induced under laboratory conditions as a consequence of 5% CO_2 inhalation may not be a valid analogue of naturally occurring panic attacks.
- The lack of effect of acute tryptophan depletion in normal volunteers may reflect their weak anxiogenic response to 5% CO_2 inhalation in our study.

H. E. J. MILLER, MD, Department of Psychiatry, Manchester Royal Infirmary, Manchester; J. F. W. DEAKIN, PhD, I. M. ANDERSON, MD, Department of Psychiatry, University of Manchester, Manchester

Correspondence: Dr I. M. Anderson, University of Manchester Department of Psychiatry, Room 9809, Stopford Building, University of Manchester, Oxford Road, Manchester M13 9PT, UK. Tel: 0161 276 5396; Fax: 0161 273 2135; e-mail: ian.anderson@man.ac.uk

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