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**BEHAVIORAL, CARDIOVASCULAR AND NEUROENDOCRINE INDICES OF
CHOLECYSTOKININ TETRAPEPTIDE INDUCED PANIC SYMPTOMS
IN HEALTHY VOLUNTEERS FOLLOWING ACUTE ADMINISTRATION
OF A TRYPTOPHAN DEFICIENT DIET**

by

Diana Koszycki

**A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfilment of
the requirements for the degree of**

**Doctor of Philosophy
Department of Psychology**

**Carleton University
Ottawa, Ontario
October, 1994**

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ABSTRACT

Data from experimental animals suggest that serotonin (5-HT) mechanisms may mediate the anxiogenic effects of cholecystokinin (CCK)-related peptide. Accordingly, the present study investigated the effects of lowering plasma tryptophan to the elicitation of behavioral, cardiovascular and hormonal changes in healthy volunteers challenged with the tetrapeptide CCK agonist, CCK-4. Forty males with no personal or family history of psychiatric disorders were randomly assigned to either a tryptophan deficient amino acid diet, which decreases central 5-HT by inhibition of 5-HT synthesis, or a control diet. Five hours after administration of the diets, all subjects received a single intravenous bolus injection of 50 μ g CCK-4. .

Injection of CCK-4 was profoundly anxiogenic, as demonstrated by significant increases in anxiety symptoms, cardiovascular arousal and release of stress hormones, including ACTH, cortisol, prolactin and growth hormone. Panic attacks occurred in 60% of subjects. Panickers were more sensitive to the behavioral, cardiovascular and neuroendocrine effects of CCK-4 than were nonpanickers. This enhanced sensitivity to CCK-4 may stem from a disruption of the interaction between peripheral (CCK_A) and central (CCK_B) CCK receptors, favoring an overactive CCK_B system. Surprisingly, there were no robust effects of tryptophan depletion on behavioral or cardiovascular sensitivity to CCK-4, despite depletion of plasma tryptophan by 92%. By contrast, acute tryptophan depletion potentiated the stimulatory action of CCK-4 on prolactin and

ACTH/cortisol secretion. These results suggest that behavioral and cardiovascular responses to CCK-4 may occur independently of 5-HT receptor activity, but that 5-HT availability within the hypothalamic-pituitary axis is involved, at least in part, in CCK-4 induced activation of stress hormones.

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Panic disorder is an anxiety syndrome whose salient characteristic is the repeated occurrence of discrete episodes of intense anxiety which appear to occur spontaneously, are perceived to be uncontrollable and are accompanied by alarming somatic sensations such as palpitation, chest pain, dyspnea, choking, sweating, tremors, faintness and paresthesia, and cognitive symptoms such as depersonalization and fear of losing control, going crazy or dying (American Psychiatric Association, 1987). Immediate symptom exacerbation accompanies a panic attack which ordinarily persists for only a few minutes and intense fatigue is frequently reported at the conclusion of the episode. Stress or stressful life events may precipitate panic attacks in vulnerable individuals, although many individuals experience recurrent spontaneous attacks of panic anxiety during nonstressful periods (Faravelli, 1985).

Panic disorder invariably imparts anticipatory fear that these attacks of panic anxiety will recur unexpectedly (Klein & Klein, 1989). Further, in the majority of cases, panic disorder is associated with avoidance of a wide range of situations in which escape might be difficult or where help might be unavailable in the event of a panic attack. Indeed, agoraphobic avoidance behavior develops in about 70% of panic patients (Robins & Regiers, 1990). In any event, panic disorder with or without agoraphobia is chronic (Wittchen, 1990; Noyes, Reich, Christiansen, Suelzer, Pfohl et al, 1990), punctuated with pervasive social alterations (Markowitz, Weissman, Ouellette, Lish & Klerman, 1989; Noyes et al, 1990; Wittchen, 1990), health risks (Stewart, Linet &

Celentano, 1989; Walker, Roy-Byrne & Katon, 1990; Bowen, D'Arcy & Orchard, 1991) and comorbid psychiatric disturbances, particularly depression (Stein & Uhde, 1988; Wittchen, 1990), increased risk for suicide (Weissman, Klerman, Markowitz & Ouellette, 1989; Noyes, 1991; Johnson, Weissman & Klerman, 1990), and substance abuse (Cox, Norton, Swinson & Endler, 1990; George, Nutt, Dwyer & Linnoila, 1990).

The prevalence of panic disorder in the general population is approximately 1.5%, its incidence is evenly distributed between males and females in the absence of agoraphobia, typically emerges in young adulthood and is reduced following age 65 (Robins & Regier, 1990). Panic disorder appears to be familial (Noyes, Clancy, Crowe, Hoenk & Slyman, 1978; Crowe, Noyes, Pauls & Slyman, 1983; Noyes, Crowe, Harris, Hamra, McChesney et al, 1986; Crowe, 1990) and the data derived from twin studies, although relatively scarce, are compatible with a genetic transmission hypothesis for the disorder (Torgerson, 1983).

NEUROBIOLOGY OF PANIC DISORDER

In recent years there has been increasingly greater attention devoted to an assessment of the neurobiology of panic disorder and the central neurotransmitters norepinephrine (NE), serotonin (5-HT), gamma aminobutyric acid (GABA) and adenosine in particular (see Hoehn-Saric, 1982; Charney, Woods, Krystal & Heninger, 1990a; Nutt, 1990 for review). Recent demonstration of colocalization mosaics in the central nervous system and the

neuromodulatory role of various neuropeptides (Hökfelt, Johansson & Goldstein, 1984; Van Ree & DeWied, 1987; Hökfelt, 1991) have suggested a complex neurochemical interaction in promoting behavioral pathology. For example, the neuropeptide cholecystokinin (CCK), which promotes NE (Kendrick, Leng & Higuchi, 1991), 5-HT (Raiteri, Maura & Paudice, 1992) and GABA (Nakamura, Matsumoto, Hirano & Uchimura, 1990) turnover and induces anxiety in human (Bradwejn & Koszycki, 1992) and infrahuman subjects (Harro & Vasar, 1991a) is colocalized with these amines in brain sites which may underlie expression of some of the symptoms associated with panic disorder (Hökfelt, Bean, Ceccatelli, Dagerlind, Elde et al, 1992). Cholecystokinin also enhances dopamine (DA) turnover (Markstein & Hökfelt, 1984; Crawley, 1991) and is colocalized with DA in brain regions associated with the expression of affect and where synthesis and utilization of DA are also influenced by Met- and Leu-enkephalin, as well as the tachykinins, substance P and K (neurokinin A) (Hökfelt, Rehfeld, Skirboll, Ivemark, Goldstein et al, 1980; Crawley, 1991; Hökfelt et al, 1992). Such multiple neuromodulatory processing has also been demonstrated for NE, 5-HT and GABA, among other neurotransmitter systems (see Hökfelt, Johansson, Holets, Mesiter & Melander, 1987 for review).

The central sites underlying such a multiple neurotransmitter interface include the dorsal and ventral noradrenergic bundles as well as the mesocortical, mesolimbic and raphe systems (Gorman, Liebowitz, Fyer & Stein, 1989; Charney et al, 1990a). These catecholamine and indoleamine containing

pathways associated with such exemplary functions as mood, motivation, vigilance, attention, arousal, learning, memory and the control of hormonal output also interact with brainstem and spinal nuclei involved with respiratory and cardiopulmonary regulation (Gorman et al, 1989). Accordingly, both brain and spinal mechanisms appear to interact with multiple and putative neurotransmitter systems in provoking a pattern of physiological and behavioral alteration compatible with some of the symptoms of panic disorder.

In this respect, some of the research strategies which have been adopted in investigating panic disorder have involved analysis of (a) plasma and urinary metabolite concentrations associated with some of the behavioral concomitants of the disorder (b) hormonal release profiles (e.g., prolactin, cortisol, growth hormone, adrenocorticotrophic hormone (ACTH)) accompanying panic induction as an indicant of hypothalamic pituitary axis dysfunction and central NE and/or 5-HT receptor alteration (c) peripheral receptor alterations (e.g. NE, 5-HT) associated with the disorder (d) pharmacological interventions (e.g., benzodiazepines, antidepressants) which are effective in treating anxiety in general and panic disorder in particular (e) cerebral blood flow profiles in panic patients and (f) various panicogenic challenges (e.g., carbon dioxide, sodium lactate, yohimbine, CCK) in inducing a symptom profile reminiscent of the disorder.

The procedural difficulties encountered in attempting to isolate naturally occurring panic attacks have led to the use of panicogenic challenges, of which

the most frequently employed have been sodium lactate (Cowley & Arana, 1990) and carbon dioxide (Woods, Charney, Loke, Goodman, Redmond et al, 1986; Gorman, Papp, Martinez, Goetz, Hollander et al, 1990). The mechanism by which these agents precipitate anxiety and panic however is ill defined and procedural difficulties may compromise and confound data interpretation. For example, the use of carbon dioxide is complicated by the necessity of introducing a mask, the discernible odour and audible release of the agent and the rapid disappearance of the behavioral manifestations of panic. In the case of sodium lactate, the volume of the agent infused and the concentration required to provoke panic may induce volume overload and diffuse metabolic change. Moreover, the relatively protracted infusion interval may impart considerable anticipatory anxiety (Cowley & Arana, 1990). Other panicogens, such as isoproterenol (Rainey, Ettedgui, Pohl, Balon, Weinberg et al, 1984a), caffeine (Uhde, 1990), m-chlorophenylpiperazine (mCPP) (Kahn & Weltzer, 1991) and yohimbine (Charney & Heninger, 1985a), although capable of provoking panic attacks in vulnerable individuals, remain plagued by their inability to predict onset latency and/or difficulty in crossing the blood brain barrier.

It is noteworthy that the tetrapeptide form of CCK, CCK-4, has been reported to produce a reliable, dose dependent profile of symptoms reminiscent of spontaneous panic attacks in patients with panic disorder (Bradwejn, Koszycki & Meteressian, 1990; Bradwejn, Koszycki, Annable, Coueteux du

Tertre, Reine & Karkanas, 1992a). The latency to effect symptoms of panic with CCK-4 is rapid and predictable, permitting blood sampling during the interval associated with peak panic symptoms. Cholecystokinin is also simple to administer in a low volume intravenous bolus injection (i.e., in 5 seconds). Finally, CCK-4 is a selective agonist of central CCK receptors, producing behavioral and physiological alterations consistent with the symptom profile of panic disorder (Bradwejn & Koszycki, 1992). Taken together, CCK-4 appears to satisfy operationally defined criteria for an ideal panicogenic agent (Bradwejn, Koszycki & Payeur, 1991a; Bradwejn & Koszycki, 1992; Bradwejn, Koszycki, Coueteux du Tertre, Bourin, Palmour & Ervin, 1992b; Bradwejn & Koszycki, 1994a).

It should be emphasized that consideration of any unitary neurochemical hypothesis of anxiety related dysfunction in general and panic disorder in particular is overly simplistic and potentially counterproductive. The diverse behavioral and neurochemical profile associated with panic disorder and the considerable interindividual variability which have been described in the clinical literature may well be associated with region specific central alterations which vary as a function of the duration and severity of the illness, the influence of comorbidity as well as genetic and organismic variables. Although current analysis of the neurochemical basis of panic disorder have focused on the potential dysfunction of NE and 5-HT, there is considerable evidence to suggest that these amines are functionally interdependent (Friedman, Cooper & Dallob,

1983; Stockmeier, Martino & Keller, 1985) and under the neuromodulatory influence of various neuropeptides including CCK (Hökfelt et al, 1992). Moreover, variations in colocalization mosaics within the catecholamine and indoleamine systems as well as inter- and intraregional neurochemical variability within these respective pathways suggest that multiple neurotransmitter systems may contribute to the induction of panic disorder.

Central Norepinephrine Activity and Panic Disorder

The potential relationship between panic disorder and central NE activity has received considerable attention (see Gorman, Fyer, Liebowitz & Klein, 1987 for review). In this respect, it is well documented that central NE is derived principally from the dorsal noradrenergic bundle (DNB) and the locus coeruleus in particular. Noradrenergic innervation of various forebrain sites by the DNB, notably the hippocampus, amygdala and the cortex, is considerable while input to the hypothalamus, although demonstrable, is rather limited (Moore & Bloom, 1979). Such innervation profiles may be relevant to a consideration of specific symptom profiles in panic disorder or to the emergence or exacerbation of particular behaviors. In addition, the DNB provides NE input to various hindbrain areas including the spinal cord, specific brainstem nuclei and the cerebellum (Dahlstrom & Fuxe, 1964). Taken together, it appears that the DNB is well suited by its innervation pattern to influence cognitive functions including memory (Sara & Devauges, 1988; Decker & McGaugh, 1991), learning and anticipatory responses (Everitt, Robbins & Selden, 1990) as well as to integrate

the effects of sensory stimulation delivered to the periphery with the central nervous system (Rasmussen & Aghajanian, 1989).

Some of the symptoms identified in human subjects during panic attacks (e.g., tachycardia, sweating, tremor) are suggestive of increased sympathetic nervous system activation which has been shown to interface with specific sites in the brain and the spinal cord. For example, the paraventricular nucleus of the hypothalamus contributes to limbic control of autonomic function by influencing the dorsal motor vagus nucleus. In addition, descending locus coeruleus afferents terminate in portions of the medullary region (Aston-Jones, Foote & Bloom, 1984), where parasympathetic cardiac regulatory neurons can be identified and retrograde horseradish peroxidase and wheat germ agglutinin labelling have revealed projections to the locus coeruleus in both dorsal and ventral planes (Ter Horst, Toes & Van Willigen, 1991). In this anatomical configuration, activation of the paraventricular nucleus of the hypothalamus elicits tachycardia, presumably through vagal inhibition and concomitant sympathetic excitation. The locus coeruleus has been argued to contribute to this disinhibitory process by virtue of its innervation of the paraventricular nucleus (Miyawaki, Kawamura, Komatsu & Yasdugi, 1991). It should also be considered that the nucleus tractus solitarius is a site of cardiopulmonary reflex integration (Jordan & Spyer, 1986). In addition to the influence of the nucleus tractus solitarius on phrenic nerve activity in effecting breathing, the nucleus tractus solitarius also receives and integrates sensory information from the

carotid and aortic chemo- and baroreceptors as well as stretch, mechanoreceptive and irritant receptors in the lungs and upper respiratory tract. Taken together, it would appear that environmental stimuli can influence the locus coeruleus directly and peripheral activation can be integrated with the function of the locus coeruleus (Amaral & Sinnamon, 1977; Foote, Bloom & Aston-Jones, 1983; Alreja & Aghajanian, 1991).

The source of the NE variations associated with panic attacks has been routinely attributed to variations of neurochemical activity in the DNB and the locus coeruleus in particular. In this respect, neuronal activity within the locus coeruleus appears to be regulated by somato-dendritic α_2 NE and conceivably α_1 autoreceptors (Nakamura, Sakaguchi, Kimura & Aioki, 1988). Consideration of the functional significance of the latter receptor type has only recently been introduced and firm conclusions are not currently available. In contrast, there are considerable experimental data which describe the effects of α_2 autoreceptor activation to neurochemical, electrophysiological and behavioral change (Cederbaum & Aghajanian, 1976; 1977; Engberg, Elam & Svensson, 1982, Starke & Altmann, 1973; Anden, Grabowska, Strombom, 1976; Svensson, Bunney & Aghajanian, 1975). In particular, agonists of the α_2 receptor site (e.g., clonidine) suppress locus coeruleus firing and lead to marked reductions in central NE availability. Animal behavioral studies have suggested that the α_2 -receptor agonist clonidine possesses anxiolytic properties (Davis, Redmond & Barabon, 1979; Handley & Mithani, 1984a; Sepinwall & Cook,

1981; Soderpalm & Engel, 1988), although the lack of such an effect (Gardner & Piper, 1982; Johnston, Koenig-Berard, Cooper & File, 1988) and the induction of anxiety (Pellow & File, 1987) have also been observed. In addition to its influence on central NE activity, clonidine has been reported to influence central DA (Grenhoff & Svensson, 1988), opioid (Chang, Dillard & Dixon, 1989), 5-HT (Aslanian & Renaud, 1989) and GABA activity (Czyzewaska-Szafran, Jastrzebski, Remiszewska & Wutkiewicz, 1991). Accordingly, some of the behavioral effects of α_2 receptor agonists, including clonidine, might be attributed to the cascading influence of such agents on transmitter systems other than or in addition to that of NE.

In contrast to the observation that α_2 agonists diminish neural activity in the locus coeruleus, it is well documented that the relatively selective α_2 receptor antagonist yohimbine increases the spontaneous activity of locus coeruleus neurons and NE availability (Cerderbaum & Aghajanian, 1976). Administration of yohimbine and other α_2 receptor antagonists (e.g., piperoxan, idazoxan, atipamexole) elicit anxiogenic responses in rodents (Davis et al, 1979; Handley & Mithani, 1984a; Handley & Mithani, 1984b; Pellow, Chopin & File, 1985; Soderpalm & Engel, 1989). In habituated chair-restrained rhesus monkeys, activation of the locus coeruleus with intravenous yohimbine or piperoxan produced a profile of behavioral alterations (e.g., hand wringing, self-clasping, startle) reminiscent of fear and anxiety (Redmond, 1977; Redmond & Huang, 1979; Redmond, Huang, Baulu & Gold, 1979). These behavioral

effects, which were analogous to those manifested by monkeys during electrical stimulation of the locus coeruleus (Redmond, Huang, Snyder & Maas, 1976; Redmond & Huang, 1979), were antagonized by the α_2 -NE receptor agonist clonidine (Redmond & Huang, 1979; Redmond, 1987). Oral yohimbine produced a significant increase in fear-like behaviors in unrestrained bonnet macaques (Rosenblum, Coplan, Friedman & Bassoff, 1991; Coplan, Rosenblum, Friedman, Bassoff & Gorman, 1992), although these behaviors appeared to depend on baseline anxiety levels (Rosenblum et al, 1991) as well as rearing conditions (Coplan et al, 1992). Nevertheless, there are some data to suggest that the anxiogenic effects of yohimbine may be mediated by the activation of neurotransmitter systems in addition to that of NE. For example, yohimbine appears to influence 5-HT, benzodiazepine and DA receptor activity (Scatton, Zivokovic, Dedek, 1980; Lal, Shearman, Bennet & Horvat, 1983; Pettibone, Pfleuger & Totaro, 1985). Moreover, yohimbine and more selective α_2 receptor antagonists, such as idazoxan and atipamezole, have been found to produce little, if any, effect on behavior (File, 1987; Treit, 1990) or to reduce anxiety (Gower & Tricklebank, 1988) in only some animal models of anxiety. Despite some of the aforementioned shortcomings with selective α_2 receptor antagonists and the neurochemical heterogeneity of yohimbine, it is relevant that the behavioral and the neurochemical alterations associated with yohimbine challenge in rats were attenuated by the α_2 receptor agonist clonidine (Grenhoff & Svensson, 1988). Taken together, these animal data

suggest that an α_2 coupled receptor mechanisms in the DNB and the locus coeruleus in particular may be instrumental in provoking at least some of the symptoms associated with panic disorder.

Clinical evidence for the potential involvement of central NE in the pathophysiology of panic disorder is supported indirectly by the efficacy of agents which affect NE reuptake, unit activity of central NE neurons or NE receptor sensitivity and/or density (e.g., tricyclic antidepressants, monoamine oxidase inhibitors, benzodiazepines) in the treatment of this condition (Klein, 1964; Zitrin, Klein & Woerner, 1978; Rifkin, Klein, Dillon & Levitt, 1981; Pohl, Berchou & Rainey, 1982; Zitrin, Klein, Woerner & Ross, 1983; Ballenger, Burrows, DuPont, Lesser, Noyes et al, 1988), although at least one study reported a lack of efficacy of the selective NE reuptake inhibitor, maprotiline, in the treatment of panic disorder (den Boer & Westenberg, 1988). In this respect, evaluation of basal plasma and urinary MHPG concentrations have been used as potential indices of central NE activity associated with panic disorder. However, the data describing circulating basal plasma or urinary MHPG concentrations have been variable. For example, some investigators have reported lowered concentrations of the NE metabolite (Hamlin, Lydiard, Martin, Dackis, Pottash et al, 1983; Edlund, Swann & Davis, 1987), others have demonstrated a significant enhancement (Garvey, Tollefson & Orsulak, 1987; den Boer & Westenberg, 1988), while still others failed to detect demonstrable variations in MHPG levels between panic patients and control subjects

(Charney, Heninger & Breier, 1984a; Nesse, Cameron, Curtis, McCann & Huber-Smith, 1984; Charney & Heninger, 1985a; Charney & Heninger, 1985b; Carr, Sheehan, Surman, Coleman, Greenblatt, et al, 1986; Pohl, Ettedgui, Bridges, Lycaki, Jimerson et al, 1987; Woods, Charney, McPherson, Gradman & Heninger, 1987; Uhde, Joffe, Jimerson & Post, 1988; Gurguis, Mefford & Uhde, 1991; Woods, Krystal, Nagy & Heninger, 1992). Moreover, Garvey and associates (Garvey, Noyes, Cook & Tollefson, 1989; Garvey, Noyes & Cook, 1990) reported a tendency for basal 24 hour urinary MHPG values to be elevated in panic patients relative to control subjects but patients with low 24 hour urinary MHPG were more likely to experience both major and "limited symptom" spontaneous panic attacks than patients with high 24 hour urinary concentrations of the metabolite. Interestingly, patients with high 24 hour levels of MHPG were more likely to report increased severity ratings for a number of items on the Hamilton Anxiety Scale including fears, impaired concentration and memory, depressed mood, genitourinary symptoms and behavioral manifestations of anxiety in comparison with patients exhibiting low MHPG concentrations. Accordingly, patients with high or low MHPG levels may be representative of functionally different panic disorder subtypes.

In addition to the variability in the data describing basal NE activity of panic patients as revealed by plasma or urinary MHPG levels, the influence of situational exposure or clinically induced panic attacks have also been variable. For example, Ko, Elsworth, Roth, Rifkin, Leigh et al (1983) reported that panic

attacks occurring during exposure to situations that previously resulted in a panic attack produced robust increases in plasma MHPG levels in a small sample of females with panic disorder with agoraphobia. Such elevated metabolite concentrations were positively correlated with subjective and somatic anxiety. Conversely, other investigators failed to observe increases in plasma MHPG levels during situationally provoked panic attacks (Woods et al, 1987) or during spontaneous panic attacks (Cameron, Lee, Curtis & McCann, 1985; Cameron, Lee, Curtis, McCann, 1987).

A reduction in basal MHPG concentrations has been reported in panic patients following chronic treatment with antipanic agents such as alprazolam and diazepam (Charney & Heninger, 1985b; Garvey et al, 1989), although at least one study (Sheehan, Coleman, Greenblatt, Jones, Levine et al, 1984) failed to report any change in baseline urinary MHPG concentrations following chronic alprazolam therapy. Chronic administration of imipramine, a frequently employed agent in the treatment of panic disorder, also decreased pretreatment baseline MHPG levels (Charney & Heninger, 1985a; Ballenger, Peterson, Laraia, Hucek, Lake et al, 1986). The time course of the NE metabolite variations in response to such pharmacological intervention has also been variable. For example, Nutt and Glue (1991) reported that plasma MHPG levels decreased during the first week of imipramine treatment but returned to pretreatment levels by the eighth week of treatment. The behavioral significance of these neurochemical alterations remain to be determined, although Nutt and Glue

(1991) have argued that down-regulation of the α_2 NE autoreceptor may be associated with the re-emergence of the MHPG elevations.

A few cautionary notes should be introduced in this discussion. While it is well established that MHPG is a major metabolite of brain NE (Elsworth, Redmond & Roth, 1982), the percentage of such excretion actually derived from the central nervous system is unclear, much less the specific brain area(s) giving rise to such accumulation (Kopin, 1984). It is also conceivable that the dynamics of transmitter biosynthesis and degradation in clinical populations are fundamentally different from those evident in healthy individuals. The evaluation of NE neuronal activity may also be dependent on circadian rhythmicity and on several clinical variables such as age at onset and duration of illness, severity of panic attacks, codiagnosis and family history of the illness. The influence of such variables to the considerable intersubject variability apparent in panic disorder remains to be verified.

Another widely used strategy to investigate central NE activity in panic disorder has been to pharmacologically manipulate α_2 receptor activity. In healthy human subjects, yohimbine and the relatively selective α_2 -receptor antagonist, idazoxan, produced significant increments in anxiety, autonomic reactivity, blood pressure and plasma free MHPG concentrations (Charney, Heninger & Sternberg, 1982a; Charney, Heninger & Redmond, 1983; Krystal, McDougle, Woods, Price, Heninger & Charney, 1992). These pharmacologically induced effects were antagonized by clonidine administration (Charney et al,

1983). It has also been demonstrated that antagonism of α_2 -NE autoreceptors with yohimbine yields differential mood, cardiovascular and neuroendocrine effects in patients and normal controls. In particular, panic patients are more likely to experience panic following yohimbine challenge and typically report greater increases in anxiety, autonomic symptoms and blood pressure relative to control subjects (Charney et al, 1984a; Charney, Woods, Goodman & Heninger, 1987a; Gurguis & Uhde, 1990a; Albus, Zahn & Brier, 1992; Charney et al, 1992). Further, patients with a history of frequent panic attacks appear to be more sensitive to the effects of yohimbine compared with patients with less frequent attacks (Charney et al, 1984a). There is some evidence that yohimbine sensitivity is specific to panic disorder. Other diagnostic groups including those exhibiting generalized anxiety disorder, obsessive compulsive disorder, major depression or schizophrenia do not display panicogenic responses to yohimbine (Charney, Woods & Heninger, 1989a; Charney, Woods, Price, Goodman, Glazer et al, 1990a). Although patients with posttraumatic stress disorder have been reported to be vulnerable to the panicogenic effects of yohimbine (Southwick, Krystal, Morgan, Johnson, Nagy et al, 1993), sensitivity to yohimbine was markedly increased in patients with a coexisting panic disorder (Southwick et al, 1993).

Evaluation of some of the neurochemical correlates of yohimbine challenge has revealed that panic patients display only modest increases in plasma MHPG, for example, compared to controls (Charney et al, 1984a,

Charney et al, 1987a; Gurguis & Uhde, 1990a), suggesting that disturbances in NE activity may not be critical to the generation of panic attacks. Nevertheless, alterations in NE turnover following yohimbine challenge have been observed in a subset of panic patients. Charney et al (1984a) noted that patients with a history of frequent panic attacks displayed greater increases in MHPG concentrations following yohimbine challenge than either healthy volunteers or patients with relatively infrequent panic attacks. In addition, significant increments in MHPG have been observed in patients who displayed a panicogenic response to yohimbine challenge compared to normal controls and patients who did not panic (Charney et al, 1992). Yohimbine challenge has also been reported to produce higher levels of plasma cortisol in panic patients in comparison with control subjects (Charney et al, 1987a; Gurguis & Uhde, 1990a; Charney et al, 1992; Albus et al, 1992). These data have been interpreted by several groups to suggest dysfunction of central postsynaptic α_2 -NE receptor function in panic disorder. Yohimbine-induced anxiety was not associated with significant alterations in plasma homovanillic acid (HVA) in either panic disorder or normal subjects (Gurguis & Uhde, 1990b), suggesting that yohimbine-induced panic attacks are not associated with concomitant activation of central DA receptors.

The use of brain imaging studies of yohimbine-induced anxiety have revealed reductions in cerebral blood flow (e.g., frontal cortex) in panic disorder but not in healthy subjects (Woods, Koster, Krystal, Smith, Zubal et al, 1988a).

This observation is consistent with the hypothesis of hyperactivity of the locus coeruleus in panic disorder and the observation that cortical neuronal activity and cerebral blood flow are diminished by locus coeruleus stimulation in infrahuman subjects (Charney et al, 1990a). Moreover, pharmacological interventions (e.g., alprazolam) which block panic attacks and influence NE turnover have been reported to attenuate the effects of yohimbine. Nevertheless, pretreatment with diazepam antagonized yohimbine-induced anxiety in healthy subjects, although it failed to reduce increases in blood pressure, autonomic symptoms and plasma MHPG concentrations, suggesting that diazepam can reduce anxiety in the absence of alterations in peripheral NE metabolites (Charney et al, 1983). In contrast, Charney and Heninger (1985a) reported that chronic treatment with alprazolam blunted yohimbine-induced increases in anxiety, plasma MHPG and systolic blood pressure in panic patients, although Albus et al (1992) found no effect of chronic alprazolam on yohimbine-induced anxiety in panic disorder. Consistent with the data of Holmberg and Gershon (1961), Charney and Heninger (1985b) demonstrated that long-term imipramine therapy produced a modest potentiation of yohimbine-induced increases in blood pressure in panic patients and failed to attenuate anxiety and increases in plasma MHPG levels. Some of these findings are at variance with an NE hypothesis of panic disorder and inconsistent with data that tricyclic antidepressants reduce locus coeruleus firing rate and NE turnover in rats (Nyback, Walters & Aghajanian, 1975; Svensson & Usdin,

1978; Keith, Howe & Salama, 1986).

It is interesting to note that plasma MHPG levels have not been found to be significantly elevated in panic patients relative to values detected in healthy controls following either sodium lactate (Carr et al, 1986; Pohl et al, 1987; Den Boer, Westenberg, Klompmakers & van Lint, 1989), isoproterenol (Pohl, et al, 1987), caffeine (Charney, Galloway & Heninger, 1984b) or carbon dioxide (Woods, Charney, Goodman & Heninger, 1988) challenge, despite clear indications of anxiety induction. Further, no significant correlations between anxiety ratings and MHPG levels were found in any of these studies. Although it is conceivable that NE activity may contribute to the expression of panic symptoms, it should also be considered that the nature of the challenge agent may also be instrumental in determining the specific transmitter systems activated. In fact, NE alterations may be sufficient but not necessary to the provocation of a panic attack. Finally, it should be considered that several subtypes of the α_2 NE receptor have been identified in the locus coeruleus (Wamsley, Alburges, Hunt & Bylund, 1992). The functional significance of such receptor activation remains to be determined and the sensitivity of such receptor sites to the various panicogenic agents employed is presently unclear.

A number of laboratories have employed clonidine as a clinical probe of α_2 -adrenoceptor function in panic disorder. In healthy human subjects, clonidine produces a decrease in concentrations of plasma cortisol and MHPG, increases growth hormone release and provokes hypotension and sedation (Nutt &

Molyneux, 1986; Charney, Woods, Krystal, Nagy & Heninger, 1992). In panic patients, acute clonidine challenge enhances blunting of growth hormone release, suggesting that the sensitivity of postsynaptic NE receptors may be decreased in panic disorder (Charney & Heninger, 1986a; Uhde, Vittone, Siever, Kaye & Post, 1986; Nutt, 1989; Uhde, Stein, Vittone, Siever, Boulenger et al, 1989; Abelson, Glitz, Cameron, Lee, Bronzo et al, 1992a; Charney et al, 1992; Tancer, Stein, Black & Uhde, 1993). Nevertheless, at least one study has failed to detect abnormalities of growth hormone response (Schittecatte, Charles, Depauw, Mesters & Wilmotte, 1988) and a blunted hormonal response is not specific to panic disorder since a comparable effect has been reported in patients with major affective (Matussek, Ackenhil, Hippus, Muller, Schroder et al, 1980; Charney, Heninger, Sternberg, Hafstead, Giddings et al, 1982b; Siever, Uhde, Silberman, Jimerson, Aloï et al, 1982; Uhde et al, 1986) and obsessive-compulsive (Siever, Insel, Jimerson, Lake, Uhde et al, 1983) disorders. Although the possibility of altered growth hormone secretion in panic patients has been investigated, other physiological indices have not been found to reliably differentiate panic patients from control subjects. Clonidine has been found to produce larger decreases in MHPG levels and blood pressure in panic patients as compared to normal controls (Charney & Heninger, 1986a; Nutt, 1989; Charney et al, 1992) but these results have not been uniformly replicated (Uhde et al, 1989; Abelson et al, 1992; Charney et al, 1992). Further, although the anxiolytic effects of clonidine were more pronounced in

panic patients than in control subjects (Nutt, 1989, Uhde et al, 1989), these effects may be attributable in part to elevated baseline levels of anxiety in some patients.

While acute pretreatment with clonidine has been shown to attenuate yohimbine-induced increases in self-rated anxiety, blood pressure and MHPG levels in healthy volunteers (Charney et al, 1983), clonidine has been found to have little effect in blocking CO₂-induced increases in anxiety, pulse rate and ventilatory response despite significant sedative and hypotensive effects (Woods, Krystal, Heninger & Charney, 1989). Coplan, Liebowitz, Gorman, Fyer, Dillon et al (1992) observed that clonidine infusion in patients with panic disorder significantly lowered blood pressure prior to lactate challenge but failed to decrease baseline anxiety. In addition, clonidine infusion antagonized the panicogenic effects of sodium lactate in only 40% of patients.

In contrast to the behavioral and neurochemical consequences of acute clonidine administration, chronic treatment with clonidine has been found to reduce symptoms of panic to a negligible degree. Although some investigators (Hoehn-Saric, Merchant, Keyser & Smith, 1981) found clonidine to be superior to placebo in decreasing panic attacks and psychic anxiety, other laboratories (Liebowitz, Fyer, McGrath & Klein, 1981; Uhde et al, 1989) have failed to demonstrate reliable decreases in anxiety ratings. Moreover, Ko et al (1983) found a chronic clonidine regimen to be only marginally superior to placebo in reducing MHPG levels following exposure to phobic stimuli. It might be relevant

to add at this juncture that electrophysiological data suggest that locus coeruleus neurons develop tolerance to the effects of repeated clonidine administration. Indeed, release of inhibitory influence and normalized electrophysiological activity in the locus coeruleus have been reported following chronic drug treatment (Engberg et al, 1982; Nestler, Terwilliger & Beitner, 1989). Accordingly, the lack of efficacy of chronic clonidine therapy in panic disorder may be attributable, at least in part, to variations in membrane excitability of locus coeruleus neurons.

The observation that challenge doses of yohimbine and clonidine produce behavioral and neurochemical alterations which are largely consistent with an NE hypothesis of panic disorder, has led to attempts to employ peripheral markers as potential indices of central NE activity in panic disorder by focusing on the α_2 NE receptor in blood platelets. In some respects however, the significance of platelet receptor alteration for panic disorder is unclear. For example, Cameron, Smith, Hollingsworth, Nesse and Curtis (1984) reported that the density of peripheral binding sites for tritiated clonidine was comparable in panic disorder and control subjects, although a subsequent study from this laboratory reported that binding density was reduced in panic patients (Cameron, Smith, Lee, Hollingsworth, Hill et al, 1990). In several other investigations, panic patients were found to exhibit a decreased platelet α_2 NE receptor binding (yohimbine) relative to normal subject, depressed patients, or those with generalized anxiety disorder (Cameron et al, 1984; Cameron et al,

1990). Perhaps not unexpectedly, however, some investigations also failed to identify variations in H^3 -yohimbine (Nutt & Frazer, 1987; Charney, Innis, Duman, Woods & Heninger, 1989b) or 3H -rauwolscine (Norman, Kimber, Judd, Burrows & McIntyre, 1987) binding between patients with panic disorder and age and sex matched control subjects.

In a few instances, evidence for increased α_2 NE receptor affinity (higher K_d) in panic disorder has been detected (Norman et al, 1987; Charney et al, 1989b), but these findings have not been reliable (Nutt & Frazer, 1987). In other cases, some investigators have suggested that panic patients can be differentiated from patients with major affective disorder, generalized anxiety disorder, obsessive compulsive disorder or schizophrenia (Charney et al, 1990b) with respect to α_2 NE receptor activity, but not necessarily from controls subjects. However, some investigators have argued that an analysis of peripheral α_2 receptor activity in the assessment of central NE function is inadequate. This proposal has relied on arguments which relate fundamentally to differences in receptor proliferative capacity between the brain and periphery, platelet turnover, transmitter availability from peripheral tissues and autoreceptor capability (Hamilton & Reid, 1982). Methodological variations including the effect of blood collection, the nature of the anticoagulant employed as well as the temperature of blood collection have also been shown to influence binding profiles (Norman, Burrows & Judd, 1990).

In addition to the potential involvement of α NE activity to the

provocation or exacerbation of panic disorder, some investigators have proposed that the β -NE receptor system is also involved in the pathophysiology of panic disorder. Tricyclic antidepressants which have demonstrated therapeutic effects in the treatment of panic disorder down-regulate β -NE receptors (U'Prichard & Enna, 1979) and decrease β -NE cyclic adenosine monophosphate (cAMP) production (Wolfe, Harden, Sporn & Molinoff, 1978). This decrease in β -NE receptor density and sensitivity during antidepressant therapy parallels the onset of clinical improvement (Charney & Heninger, 1985b; Butler, O'Halloran & Leonard, 1992). Moreover, many of the peripheral physiological indices of panic attacks, including palpitations or tachycardia, dyspnea and tremor are suggestive of β -NE receptor hyperactivity (Nutt, 1990). Although change in heart rate has not been found to be a reliable measure during spontaneous panic attacks (Taylor, Sheikh, Agras, Roth, Margraf et al, 1986; Shear, Kligfield, Harshfield, Devereux, Polan et al, 1987; Gaffney, Fenton, Lane & Lake, 1988), there is no a priori reason to suspect that peripheral measures of β -NE activity (e.g., sensitivity, density) are fundamentally different from those in evidence in the central nervous system (see Stone, 1987 for review).

Some investigators have employed basal heart rate and blood pressure as potential indices of central β -NE activity. In general, basal heart rate and/or blood pressure have been reported to be elevated in panic patients relative to control subjects (Nesse et al, 1984; Liebowitz, Gorman, Fyer, Levitt, Dillon et

al. 1985; Aronson, Carasiti, McBane & Whitaker-Azmitia, 1989), but these data have not been reliably reproduced (Cowley, Hyde, Dager & Dunner, 1987; Gaffney et al, 1988). Moreover, Freedman, Ianni, Ettedgui and Nandakumaran (1985) and Shear et al (1987) failed to detect significant differences between panic disorder and control subjects in mean 24-hour heart rate and blood pressure. In a more recent study, cardiovascular assessment of medication-free recovered panic patients revealed that such individuals exhibited higher resting systolic blood pressure but lower resting pulse rate relative to normal control subjects (Middleton, 1990). Finally, it should be considered that basal plasma or urinary epinephrine (E) concentrations have been used to evaluate β -NE activation in panic disorder. These data have also been found to be equivocal. Whereas a number of investigations have reported basal E levels to be significantly elevated in panic patients relative to healthy control subjects (Appleby, Klein, Sachar & Levitt, 1981; Ballenger et al, 1984; Cameron et al, 1984; Nesse et al, 1984; Nesse, Cameron, Buda, McCann, Curtis et al, 1985; Carr et al, 1986; Villacres, Hollifield, Katon, Wilkinson & Veith, 1987), others have failed to replicate these data (Cameron et al, 1984; Liebowitz et al, 1985; Carr et al, 1986).

The selective β -NE agonist isoproterenol has been widely used to probe central β -NE receptor sensitivity in panic disorder. Nevertheless, the results are difficult to interpret because of the difficulty with which isoproterenol crosses the blood brain barrier. In healthy human subjects, isoproterenol increases

subjective ratings of anxiety and elicits autonomic symptoms associated with panic including dyspnea and rapid or pounding heart rate (Rainey et al, 1984a). Patients with panic disorder have been reported to experience more frequent panic attacks following continuous infusion of isoproterenol relative to healthy subjects (Rainey et al, 1984a; Rainey, Pohl, Williams, Knitter, Freedman, Ettedgui, 1984b; Freedman, Ianni, Ettedgui, Pohl & Rainey, 1984; Pohl, Rainey, Ortiz, Balon, Singh et al, 1985; Pohl, Yeragani, Balon, Rainey, Lycaki et al, 1988a) and to display greater increases in skin conductance, heart rate and state anxiety, and lower finger temperatures (Freedman et al, 1984). The behavioral and cardiovascular effects of continuous isoproterenol infusion were antagonized by chronic treatment with tricyclic antidepressants in panic patients (Rainey et al, 1984; Pohl, Yeragani & Balon, 1990).

In contrast to continuous isoproterenol administration, Nesse et al (1984) reported that rapid infusion of isoproterenol failed to precipitate panic attacks in either patients or healthy controls. Further, although panic patients ordinarily display elevated growth hormone and cortisol levels relative to control subjects in response to isoproterenol infusion, a blunted cardiovascular response was elicited. These results suggested that panic disorder might be associated with decreased rather than increased β -NE receptor sensitivity, possibly due to chronic activation by high levels of endogenous catecholamines. This hypothesis has been supported by the observation that panic patients, relative to control subjects, exhibited decreased basal peripheral lymphocyte cAMP

(Lima & Turner, 1983; Charney et al, 1989b) and decreased cAMP response to isoproterenol challenge (Lima & Turner, 1983). Further, pretreatment with the β -NE antagonist, propranolol, but not diazepam, increased peripheral lymphocyte cAMP response to isoproterenol in patients (Lima & Turner, 1983). Several other studies have reported that basal plasma lymphocyte β -NE receptors are decreased in panic patients relative to healthy subjects (Brown, Charney, Woods, Heninger & Tallman, 1988; Aronson et al, 1989; Albus et al, 1986), however this could not be confirmed in a study of Butler et al (1992) which found that basal plasma lymphocyte β -NE receptor density was increased in panic patients relative to controls. Interestingly, basal plasma lymphocyte β -NE receptors was reported to correlate negatively with trait but not state anxiety scores in panic patients (Aronson et al, 1989). This latter finding suggested that some panic patients might be in a relative state of β -NE hyperactivity, perhaps secondary to chronic anticipatory anxiety or generalized anxiety, resulting in a compensatory down regulation of β -NE receptors. In this respect, it has been demonstrated that propranolol, a non-specific β -NE antagonist, readily crosses the blood brain barrier and exerts anxiolytic effects in human (Birkett & Tyrer, 1990; Noyes, 1982) and infrahuman subjects (Durel, Krantz & Barrett, 1986; Audi, de Oliveira, de Oliveira & Graeff, 1990; Audi, de Oliveira & Graeff, 1991). However, in addition to its effects on central NE activity, propranolol also effects a relatively selective antagonism of 5-HT autoreceptors (Middlemiss, Blakeborough & Leather, 1977; Audi et al, 1991).

Accordingly, the anxiolytic effects of β blockers may be attributable, at least in part, to their action of 5-HT neurotransmission.

Finally, it should be considered that some of the evidence describing the pharmacological efficacy of propranolol in reducing panic attacks is ambiguous. An early uncontrolled study of Heiser and Defrancisco (1976) found that propranolol was ineffective in blocking panic attacks in patient with a chronic history of panic disorder with agoraphobia but appeared to effective in patients with a more recent onset of the illness. Although Kathol, Noyes, Slymen, Crowe, Clancey et al (1980) found propranolol to be superior to placebo in reducing both somatic and psychic symptoms of anxiety, patients with agoraphobia were more responsive to propranolol than patients with panic disorder alone. Ravaris, Friedman, Hauri & McHugo (1991) reported that propranolol was equally effective as alprazolam in suppressing panic attacks and reducing avoidance behavior in patients with panic disorder. By contrast, other placebo or reference-controlled studies have suggested that propranolol does not constitute an effective treatment for panic attacks (Noyes, Anderson, Clancy, Crowe, Slymen et al, 1984; Munjack, Crocker, Cabe, Brown, Usigli et al, 1989). It might also be added parenthetically that Gorman, Levy, Liebowitz, McGrath, Appleby et al (1984) and Aronson et al (1989) observed no effect of propranolol on lactate-induced anxiety and increases in heart rate in panic patients. Accordingly, it has been argued that activation of central β -NE receptors is not relevant to the demonstration of a sodium lactate-induced

anxiogenic response. These findings have prompted some investigators to debate the issue of central β -NE receptor participation in the provocation of panic attacks in general (Gorman et al, 1984; Aronson et al, 1989).

In summary, the potential contribution of NE to the promotion of some of the symptoms of panic disorder have been well documented. Several research strategies have been employed in evaluating the role of NE activity in panic disorder, including platelet and lymphocyte binding of α and β NE receptors, peripheral MHPG levels and behavioral response to drugs which enhance or decrease α - and/or β -NE receptor activity. The variable plasma MHPG data derived from situational and spontaneous panic attacks and from paradigms employing agents which provoke panic other than yohimbine indicate that increases in central and peripheral NE function do not necessarily accompany panic attacks. Available data to date have revealed that alterations of transmitter systems in addition to that of NE may influence the course, severity and symptom appearance in panic disorder.

Central Serotonin Activity and Panic Disorder

In addition to the data suggesting that alterations of central NE activity may contribute to the expression of at least some of symptoms of panic, a role for 5-HT in the induction of anxiety is likely (Kahn, van Praag, Weltzler, Asnis & Barr, 1988; Marsden 1989). Serotonin containing cell clusters derived principally from the midbrain raphe system innervate specific sites within the dorsal NE bundle, the DA containing mesolimbic/mesocortical and the

nigrostriatal systems as well as the spinal cord (Azmitia, 1987; Molliver, 1987). Such afferent input is conducive to the influence of 5-HT on efferent outflow and to the potential neuromodulatory action of the indoleamine on various neurotransmitters localized in these pathways. Of the various neurotransmitter interfaces, the 5-HT-NE colocalization verified in the locus coeruleus deserves emphasis (Lechin, van der Dijs, Amat & Lechin, 1989). Indeed, the respective contributions of NE and 5-HT to the provocation of panic attacks are not mutually exclusive. It will be recalled that there are considerable data to suggest that variations in NE and 5-HT turnover and receptor sensitivity induced by environmental stimuli and pharmacological challenge are functionally interdependent. The relatively dense 5-HT innervation of the locus coeruleus appears to be derived from several raphe nuclei including the raphe dorsalis, centralis and pontis which project from rostral-caudal and medio-lateral positions (Morgane & Jacobs, 1979; Leger, McRae-Degueurce & Pujor, 1980). In addition to the provision of 5-HT input to the locus coeruleus from the raphe system, intrinsic 5-HT perikarya have also been identified in the locus coeruleus (Steinbusch, 1991). Nevertheless, the influence of GABA (Belin, Nanopoulos, Didier, Aguera, Steinbusch et al, 1983; Strahlendorf, Lee & Strahlendorf, 1991) substance P (Lovick & Hunt, 1983), thyrotropin releasing hormone (Bowker, Westlund, Sullivan, Wilber & Coulter, 1983), galanin (Meiander, Hökfelt, Rofaeus, Cuello, Oertel et al, 1986), other peptides (McFadzean, Henderson & Hill, 1984) and E (Kachidian, Astier, Renaud & Bosler, 1990; Liaw, He &

Barraclough, 1992) cannot be dismissed.

The nature of the NE 5-HT interaction in the locus coeruleus has been elucidated to some degree by the data derived from ablation and stimulation paradigms. In the former instance, electrolytic or 5,7 dihydroxytryptamine lesions of the raphe dorsalis and centralis enhance tyrosine hydroxylase activity and availability (McRae-Dugueurce, Dennis, Leger & Scatton, 1985) in the locus coeruleus and elevate 3-methoxy-4-hydroxyphenylethyleneglycol (MOPEG) and 3,4 dihydroxyphenylacetic acid (DOPAC) concentrations in both the hippocampus and the cerebral cortex. These data suggest that 5-HT ordinarily exerts a tonic inhibitory influence over locus coeruleus neurons, although the absence of functional 5-HT contacts suggests that the influence of 5-HT may be one of neuromodulation. Accordingly, these data suggest that 5-HT may not only alter neuronal activity of the locus coeruleus but may also exert paracrine effects in brain regions distal to the locus coeruleus (Hökfelt et al, 1992).

It has been hypothesized that alterations of central 5-HT activity contribute to the pathophysiology of panic disorder (Evans & Moore, 1981; Koczkas, Holmberg & Wedin, 1981; Humble & Wistedt, 1992; Coplan, Gorman & Klein, 1992). In this respect, the data from open and placebo- or reference-controlled studies have shown that selective 5-HT reuptake inhibitors including fluoxetine, fluvoxamine, zimelidine and citalopram are particularly effective in blocking panic attacks (Evans, Best, Cox & Moore, 1980; Koczkas et al, 1981; Evans, Kenardy, Schneider & Hoey, 1986; den Boer, Westenberg, Kamerbeek,

Verhoeven & Kahn, 1987; Gorman, Liebowitz, Fyer, Goetz, Campeas et al, 1987; Schneider, Liebowitz, Davies, Fairbanks, Hollander et al, 1990; Humble & Wistedt, 1992). It is also well established that antidepressant agents which inhibit both NE and 5-HT reuptake, notably imipramine and clomipramine, have potent antipanic effects (Klein, 1964; Zitrin et al, 1978; Sheehan, Ballenger & Jacobsen, 1980; Gloger, Grunhaus, Birmacher & Tgroudart, 1981; Pohl et al, 1982; Zitrin et al, 1983; Lydiard & Ballenger, 1987; Fahy, O'Rourke, Brophy, Schazmann & Sciascia, 1992). Moreover, clomipramine, which is a more potent 5-HT reuptake inhibitor relative to imipramine (Richelson & Pfenning, 1984), has been found in at least one study to be more therapeutically effective than imipramine in treating panic attacks (Modigh, Westberg & Eriksson, 1992). The elimination of panic attacks has also been observed following treatment with the monoamine oxidase inhibitor, phenelzine (Lydiard & Ballenger, 1987), and the reversible monoamine-A inhibitors, brofaromine and moclobemide (Bakish, 1992; Garcia-Borreguero, Lauer, Ozdaglar, Wiedemann, Holsboer et al, 1992; Bakish, Saxena, Bowen & D'Souza, 1993; Van Vliet, Westenbert & den Boer, 1993). In addition, chronic treatment with the 5-HT precursor, L-hydroxytryptophan (5-HTP), has been found to reduce anxiety and to block panic attacks (Kahn & Westenbert, 1985; Kahn, Westenbert, Verhoeven, Gispen de Wied & Kamerbeek, 1987).

The therapeutic effects of drugs which influence specific 5-HT receptor subtypes have not been examined extensively, although there are some data

to suggest that these agent do not possess antipanic properties. For example, buspirone, a 5HT_{1a} partial agonist, although effective in treating generalized anxiety disorder (Rickels, Weisman & Norstad, 1982), had no effect in preventing panic attacks (Frazer & Lapierre, 1987; Robinson, Shrotriya, Alms, Messina & Andary, 1989; Sheehan, Raj, Harnett-Sheehan, Soto & Knapp, 1993), although two studies demonstrated that this agent effectively reduced anticipatory anxiety (Frazer & Lapierre, 1987; Robinson et al, 1989). In other patients, buspirone exacerbated panic symptoms (Frazer & Lapierre, 1987; Chignon & Lepine, 1989), an effect that is likely attributable to α_2 NE receptor antagonism in the locus coeruleus. Similarly, the 5-HT₂ receptor antagonist ritanserin, which displayed anxiolytic action in generalized anxiety disorder (Ceulemans, Hoppenbrouwers, Gelders & Reyntjens, 1985), was less effective than fluvoxamine in reducing the frequency of panic attacks, self- and clinician-rated symptoms of anxiety and phobic avoidance (den Boer & Westenberg, 1990). Furthermore, relative to imipramine and alprazolam, trazadone, a potent 5-HT₂ receptor antagonist, was ineffective in treating panic attacks (Charney, Woods, Goodman, Rifkin, Kinch et al, 1986), although a recent open trial employing a higher dose of the agent reported more positive therapeutic effects (Mavissakalian, Perez, Bowler & Dealy, 1987). There are presently no available data on the antipanic action of 5-HT₃ antagonists.

It is noteworthy that in contrast to depressed patients, a significant proportion of panic patients initially respond to tricyclic antidepressants with

characteristic overstimulation, jitteriness and paradoxical increases in anxiety (Zitrin et al, 1978; Pohl, Yeragani, Balon & Lycaki, 1988b). Such transient behavioral deterioration was attributed to increased NE activity (Pohl et al, 1988b). It is notable, however, that these effects have also been observed with the 5-HT precursor, 5-HTP (Kahn et al, 1987), and with more selective 5-HT reuptake inhibitors including fluoxetine (Gorman et al, 1987), fluvoxamine (den Boer et al, 1987) and citalopram (Humble & Wistedt, 1992). A notable exception in this respect is the selective NE reuptake inhibitor, maprotiline (den Boer et al, 1987). Eriksson and Humble (1990) speculated that the exacerbation of anxiety accompanying chronic treatment with 5-HT reuptake inhibitors may be due to a transient reduction of 5-HT neurotransmission during early stages of drug therapy. In fact such an argument is strengthened by electrophysiological studies which demonstrated that enhancement of 5-HT neurotransmission following 5-HT reuptake inhibition does not appear for at least two weeks following the initiation of drug treatment and that acute administration of such agents decreases rather than increases 5-HT activity (e.g., Blier, de Montigny & Chaput, 1987).

A description of the 5-HT NE interaction in the locus coeruleus and the functional significance of such a neurochemical interface to behavior in general and anxiety in particular have been complicated with the identification of multiple 5-HT receptors; specifically 5-HT_{1a-1d}, 5-HT₂, 5-HT₃ and 5-HT₄ (Bonate, 1991; Petroutka, 1991). In this respect, Gorea and Adrien (1988) recently

reported that both the non-selective 5-HT agonists, quipazine, and the 5-HT₂ agonist, 4-bromo-2,5-dimethoxyamphetamine (DOB), attenuated unit activity in the locus coeruleus; effects which were eliminated by the 5-HT₂ antagonist, kitanserin. Interestingly, activation of the 5-HT_{1b} receptor site with RU24969 (the principal 5-HT receptor identified in the locus coeruleus) produced variable results. In some cases basal activity of locus coeruleus neurons was unaffected by RU24969 while in other cases a gradual induction of activity was achieved. Although it is conceivable that different populations of locus coeruleus neurons are affected by such treatment, the possibility that RU24969 provoked alterations distal to the locus coeruleus and may have also activated 5-HT_{1a} raphe autoreceptors cannot be excluded. Taken together, these data tentatively suggest that 5-HT₂ receptors participate in mediating the inhibitory influence of 5-HT neurons on NE activity in the locus coeruleus, although such a role does not appear to be exclusive and the influence of other 5-HT receptor subtypes to the modulation of locus coeruleus activity remains to be resolved. Indeed, the 5-HT_{1a}, 5-HT₂ and 5-HT₃ receptors have also been implicated in the promotion of anxiety in infrahuman subjects (Petroutka, Sleight, McCarthy, Pierce & Schmidt, 1989). It remains to be determined if a role of these receptor subtypes in panic disorder can also be established.

The postsynaptic 5-HT_{1c} receptor agonist mCPP, which displays robust anxiogenic and neuroendocrine effects in rodents (Kennett, Whitton, Shah & Curzon, 1989; Whitton & Curzon, 1990), nonhuman primates (Aloi, Insel,

Mueller & Murphy, 1984; Heninger, Charney & Smith, 1987) and healthy human subjects (Mueller, Murphy & Sunderland, 1986; Kalus, Wetzler, Kahn, Asnis & van Praag, 1992; Kahn, Wetzler, Asnis, Kling, Suckow & van Praag, 1990) has been used to challenge 5-HT receptors in panic disorder. Oral administration of mCPP was associated with an exacerbation of anxiety and panic symptoms and increased cortisol secretion in panic patients in comparison with either control subjects or patients with major depression (Kahn, Asnis, Wetzler & van Praag, 1988). However, intravenous mCPP treatment induced comparable increases in anxiety and panic responses as well as prolactin, cortisol and growth hormone release in patients and normal controls (Charney, Woods, Goodman & Heninger, 1987b), suggesting that while 5-HT activity may contribute to the induction of anxiety, such an effect may be distinguishable from the propensity of 5-HT to induce panic attacks. These apparently contradictory results with mCPP conceivably reflect differences in dose and route of administration. The intravenous dose employed by Charney et al (1987b) may have produced a ceiling effect and obscured potential differences in 5-HT receptor sensitivity between panic patients and normal control subjects. While these data are provisionally suggestive of a relationship between panic disorder and 5-HT receptor sensitivity (Kahn & van Praag, 1990), interpretation is difficult. For example, mCPP shows considerable heterogeneity in 5-HT receptor binding and also influences α_2 -NE and DA receptors as well (Smith & Suckow, 1985; Schoeffler & Hoyer, 1989; Hamik

& Peroutka, 1989; Kahn & Wetzler, 1991).

Other researchers (Lesch, Wiesmann, Hoh, Muller, Disselkamp-Tietz et al (1992) have used ipsapirone, a selective partial 5-HT_{1A} receptor agonist, in investigating 5-HT_{1A}-related serotonergic dysfunction in panic disorder. Although ipsapirone produced minimal somatic complaints it did cause a modest increase in symptoms of anxiety in patients and drowsiness in control subjects. Further, compared to controls, thermoregulatory and neuroendocrine (e.g., ACTH/cortisol) responses to ipsapirone were attenuated in panic patients, suggesting subsensitivity of both the post- (ACTH and cortisol) and presynaptic (hypothermia) response to 5-HT_{1A} stimulation.

Assessment of 5-HT activity in panic disorder has also relied on behavioral and neuroendocrine responses to 5-HT precursors. In this regard, Charney and Heninger (1986b) administered tryptophan intravenously and found a comparable profile of prolactin release in patients with panic disorder relative to healthy controls. Although Charney and Heninger (1986b) concluded that postsynaptic 5-HT activity is not abnormal in panic disorder, it is unclear to what extent tryptophan-induced alterations of prolactin levels are due to enhanced 5-HT output. The rather large tryptophan load employed may have also decreased levels of the central catecholamine precursor, tyrosine, resulting in NE- or DA-induced increases in prolactin. Westenberg and den Boer (1989) as well as den Boer and Westenberg (1990) reported that infusion of 5-HTP also effected increases in plasma cortisol and β -endorphin concentrations in

panic patients and control subjects. Behaviorally, 5-HTP had no effect in panic patients but depressed mood in some control subjects. While these results also suggest that panic patients have normal postsynaptic 5-HT receptors, a major limitation of this study is that the majority of normal subjects and 9 of the 20 patients vomited during the procedure. Consequently, it is likely that cortisol release may have been stress induced rather than the result of 5-HTP-induced adrenocorticotrophic hormone release. Finally, it was reported that oral administration of fenfluramine, which causes release of serotonin, elicited an enhanced prolactin and cortisol response in patients with panic disorder compared to healthy volunteers (Targum & Marshall, 1989; Targum, 1990; Apostolopoulos, Judd, Burrows & Norman, 1993) and patients with major depression (Targum & Marshall, 1989; Targum, 1990). Moreover, Targum & Marshall (1989) reported that fenfluramine provoked panic attacks in six of nine patients with panic disorder. In five of these patients, the attacks were described as "waves" of anxiety that persisted for protracted intervals (e.g. 6-8 hours) after fenfluramine challenge.

The use of blood platelets binding has been employed to arrive at a peripheral marker of potential alterations of central 5-HT activity in panic disorder, although concerns have been raised regarding the validity of platelet models of 5-HT function (Gershon & Tamier, 1983). In general, most investigations have focused on platelet ^3H -imipramine binding as a putative marker of the 5-HT reuptake site. In this respect, Lewis, Noyes, Coryell and

Clancey (1985) reported that imipramine platelet binding was significantly decreased in panic disorder compared to healthy control subjects. However, other researchers have failed to report abnormal ³H-imipramine binding characteristics in panic disorder patients (Roy-Byrne & Uhde, 1985; Innis, Charney & Heninger, 1987; Nutt & Frazer, 1987; Pecknold, Chang, Fleury, Koszycki, Quirion, et al, 1987; Schneider, Munjack, Severson & Palmer, 1987; Uhde, Berrettini, Roy-Byrne, Boulenger & Post, 1987; Norman, Sartor, Judd, Burrows, Gregory & McIntyre, 1989a). The investigation of platelet 5-HT levels or platelet 5-HT reuptake dynamics have also produced variable results. For example, while platelet 5-HT levels have been reported to be lower in panic disorder in comparison to normal control subjects (Evans et al, 1986; Butler et al, 1992), others studies have failed to detect variations in platelet 5-HT levels (Balon, Pohl, Yeragani, Rainey & Oxenkrug, 1987). Several investigations by Norman and his colleagues (Norman, Judd, Gregory, James, Kimber & McIntyre, 1986; Norman, Judd, Burrows & McIntyre, 1989b; Norman, Gregory, Judd, Burrows & McIntyre, 1988; Norman et al, 1989a) have revealed that patients with panic disorder exhibit an increase in rate of platelet 5-HT uptake relative to control subjects, although at least one study (Pecknold, Suranyi-Cadotte, Chang & Nair, 1988) reported a reduction of platelet 5-HT uptake in panic patients as compared to controls while still another investigation (den Boer & Westenberg, 1990) found no difference between panic patients and control subjects in 5-HT uptake. Overall, the results of these paradigms are

inconsistent.

In summary, the 5-HT hypothesis of panic disorder is supported by pharmacological data which indicate that patients with panic disorder respond preferentially to agents which influence 5-HT reuptake. The apparent effectiveness of chronic treatment with 5-HT reuptake inhibitors contrasts with the relative lack of efficacy of 5-HT_{1a} receptor agonists (e.g., buspirone) and 5-HT₂ receptor antagonists (e.g., ritanserin and trazadone). The therapeutic efficacy of 5-HT reuptake inhibitors over the 5-HT₂ receptor antagonist, ritanserin, has prompted some investigators to suggest that panic disorder may be associated with a deficiency in central 5-HT activity. This hypothesis is strengthened by the observation that enhancing 5-HT activity with tryptophan or 5-HTP does not alter in mood in panic patients. Although an enhanced anxiogenic response to mCPP and fenfluramine has been reported in panic disorder patients, such increased sensitivity may reflect alterations of presynaptic 5-HT activity.

Central GABA Activity and Panic Disorder

Benzodiazepine receptors are widely distributed in the central nervous system with high densities apparent in limbic regions and lower densities revealed in the hippocampus, striatum and spinal cord (Mohler & Okada, 1977; Squires & Braestrup, 1977; Hoehn-Saric, 1982; Muller, 1987). It is well established that benzodiazepine receptor agonists, including diazepam, lorazepam, alprazolam and clonazepam, possess antianxiety effects (Enna,

1984; Sanger, 1985) and inhibit stress-induced increases in hormonal secretion (Roy-Byrne, Risch & Uhde, 1988; de Souza, 1990; Roy-Byrne, Cowley, Hommer, Ritchie, Greenblatt et al, 1991). It has been well documented that the clinical effects of benzodiazepine receptor agonists are largely mediated by the propensity of these agents to bind with high affinity sites on the GABA_A receptor, which effects increased GABA activity in the central nervous system (Paul, Marango & Skolnick, 1981; Ross, Waszczak, Lee & Walters, 1982; Haefely, 1990; 1991; Costa & Guidotti, 1991). Biochemical and electrophysiological data also suggest that benzodiazepines potentiate the effects of GABA in a variety of neuronal system (Tallman, Paul, Skolnick & Gallager, 1980) and that benzodiazepine binding affinity is increased by GABA and by ligands which act as GABA receptor agonists (Tallman et al, 1980). Nevertheless, it is unlikely that the anxiolytic effects of benzodiazepines are exclusively mediated by GABA receptors. Indeed, there is evidence that non-GABAergic mechanisms, including adenosine and 5-HT, may well underlie some of the therapeutic effects of benzodiazepine agonists (Shephard, 1986; Polc, 1988; Petrouka, 1992).

A second class of benzodiazepine ligands, the inverse agonists, exert their effects on central benzodiazepine receptors by effecting a pharmacological response opposed to that of the benzodiazepine agonists. Such actions presumably shift the GABA dose-response curve to the right and attenuate the influence of GABA (Biggio, Concas, Mele & Corda, 1987; Haefely, 1990;

1991). It is well documented that inverse agonists, which include the β -carbolines (e.g., FG 7142, DMCM, β -CCM, noreleagnine), display robust anxiogenic effects in mice, rats and cats (Ongini, Barzaghi & Marzanatti, 1983; Prado de Carvalho, Greksch, Chapouthier & Rossier, 1983; File, Pellow & Bastrup, 1985; Hindley, Hobbs, Paterson & Roberts, 1985; File & Baldwin, 1987; Novas, Wolfman, Medina & De Robertis, 1988; Rgo, Kiivet, Harro & Pold, 1988; Ademec, 1991; Cutler & Aitken, 1991; Hill, Fontana, McCloskey, Randall & Commissaris, 1992). These anxiogenic effects have been reported to be antagonized by benzodiazepine receptor agonists (File et al, 1982; File & Pellow, 1984; Novas et al, 1988), as well as by benzodiazepine antagonists (i.e., flumazenil) (File & Pellow, 1984; Hill et al, 1992; File, Lister & Nutt, 1982).

In nonhuman primates, anxiety and concomitant increases in blood pressure, heart rate and cortisol have also been observed following β -CCE challenge (Ninan, Insel, Cohen, Cook, Skolnick et al, 1982; Insel, Ninan, Aloï, Jimerson, Skolnick et al, 1984; Skolnick, Ninan, Insel, Crawley & Paul, 1984; Crawley, Ninan, Pickar, Chrousos, Linnoila et al, 1985), although the behavioral effects of β -CCE appear to depend on dose, experimental setting, social status and rearing (Insel, Scanlan, Champoux & Suomi, 1988). The behavioral, cardiovascular and neuroendocrine effects of β -CCE were antagonized by pretreatment with benzodiazepine agonists (Insel et al, 1984) and antagonists (Ninan et al, 1982) as well as by clonidine (Crawley et al, 1985), suggesting

that the anxiogenic action of β -CCE might be linked to hyperactivity of central NE systems. This latter hypothesis has been supported by the data of Ida, Elsworth and Roth (1991) who reported that treatment with FG 7142 significantly increased MHPG in the hippocampus, amygdala, thalamus and cortex; effects which were antagonized by clonidine pretreatment. Sudden attacks of severe anxiety and concomitant increases in blood pressure, pulse rate, cortisol, prolactin and growth hormone levels have also been detected in healthy volunteers following administration of FG 7142 (Dorow, Horowski, Paschelke, Amin & Braestrup, 1983). Taken together, studies on the effects of β -carbolines provide compelling evidence that benzodiazepine receptors contribute to the induction of anxiety in human and infrahuman subjects.

Considerable clinical support for a role of the GABA-benzodiazepine receptor complex in panic disorder is derived from investigations employing the high potency benzodiazepines, such as alprazolam and clonazepam, which are particularly effective antipanic agents (Chouinard, Annable, Fontaine & Solyom 1982; Beaudry, Fontaine, Choinard & Annable, 1986; Liebowitz, Fyer, Gorman, Campeas, Levin et al, 1986; Pollack, Tesar, Rosenbaum & Spier, 1986; Spier, Tesar, Rosenbaum & Woods, 1986; Ballenger et al 1988; Pollack & Rosenbaum, 1988; Rickels, Schweizer, Csanalosi, Case & Chung, 1988; Uhlenhuth, Matuzas, Glass & Easton, 1989; Lydiard, Lesser, Ballenger, Rubin, Laraia & DuPont, 1992; Schweizer, Rickels, Weiss & Zavodnick, 1993). In comparison, conventional benzodiazepines (e.g., diazepam) have been found to

be equally effective in blocking panic attacks when administered at relatively high doses (Noyes et al, 1984; Pollack & Rosenbaum, 1988; Schweizer, Fox, Case & Rickels, 1988). Non-benzodiazepine-related GABA agonists, notably carbamazepine and valproate, have also recently received attention as putative antipanic interventions (Primeau & Fontaine, 1988; Tondo, Burrai, Scamonatti, Tocca Fondi, Poddigh et al, 1989; Keck, McElroy & Friedman, 1992; Keck, Taylor, Tugrul, McElroy & Bennet, 1993), although one placebo-controlled study reported a lack of effect of carbamazepine in the treatment of panic disorder (Uhde, Stein & Post, 1989).

There is also evidence that the GABA-benzodiazepine receptor may be an important site of action of diverse panicogenic agents. Woods et al (1989) reported that oral alprazolam reduced carbon dioxide-induced increases in ratings of anxiety in healthy humans subjects. Furthermore, in panic patients, the anxiogenic effects of carbon dioxide inhalation were markedly reduced following chronic treatment with clonazepam (Beckett, Fishman & Rosenbaum, 1986; Pols, Zandberger, de Loof & Griez, 1990) and alprazolam (Woods et al, 1986). Chronic alprazolam (Carr et al, 1986; Cowley, Dager, Roy-Byrne, Avery et al, 1991) and valproate (Keck et al, 1993) therapy and intravenous diazepam (Aronson et al, 1989) have been reported to attenuate panic attacks induced with sodium lactate infusion, and to modestly attenuate lactate-induced increases in plasma MHPG concentrations (Carr et al, 1986). It has also been recently demonstrated that plasma GABA levels significantly decrease during

infusion of sodium lactate in healthy subjects, suggesting that sodium lactate decreases available GABA and makes people anxious (Balon, Petty, Yeragani, Kramer & Pohl, 1993). Caffeine-induced panic attacks were also reported to be blocked by chronic alprazolam therapy (Uhde, 1990). However, it will be recalled that the effects of alprazolam on yohimbine-induced panic have been variable (Charney et al, 1986; Albus et al, 1992).

Considerable effort has been devoted to identifying an endogenous modulator of benzodiazepine receptors. The compound β -CCE, which was extracted from human urine by Braestrup and associates (Braestrup, Nielsen & Olsen, 1980; Braestrup & Nielson, 1980), was originally presumed to be an endogenous benzodiazepine receptor ligand, although it was subsequently verified that β -CCE was an artifact of the extraction procedure. However, a number of studies have suggested that diazepam binding inhibitor (DBI), abundant in cortical and limbic regions, the cerebellum and brainstem (Ferrero, Santi, Conti-Tronconi, Costa & Guidotti, 1986), may function as the endogenous anxiolytic ligand at the benzodiazepine-GABA receptor complex (Barbaccia, Costa, Ferrero, Guidotti, Roy, et al, 1986; Ferrero et al, 1986). Payeur, Lydiard, Ballenger, Laraia, Fossey et al (1992) recently examined cerebrospinal (CSF) concentrations of DBI in patients with panic disorder. Although no significant differences in CSF concentrations of DBI were evident between panic disorder and control subjects, covariance of CSF levels of DBI with CSF corticotropin-releasing hormone was only evident in panic patients,

suggesting a possible influence of DBI in the regulation of the HPA axis. In addition, elevated levels of tribulin, a MAOI/central benzodiazepine receptor binding inhibitor has been detected during lactate-induced panic attacks (Clow, Glober, Weg, Walker, Sheehan et al, 1988).

In addition to the evaluation of potential endogenous anxiolytic agents in the provocation of anxiety, there are also data which suggest a relationship between central benzodiazepine receptors and anxiety. For example, some investigators have reported that "emotional" (i.e., reactive) strains of mice or rats exhibit demonstrably lower densities of whole brain benzodiazepine receptors than do "nonemotional" (i.e., non-reactive) strains (Robertson, Martin & Candy, 1978; Robertson, 1979). Moreover, Rgo et al (1988) and Rgo, Adojaan, Harro and Kiivet (1991) have observed that a decreased density of both GABA and benzodiazepine receptors in the cerebral cortex was associated with lowered levels of exploratory behavior in the elevated plus maze. In addition, a reduced density of peripheral benzodiazepine binding sites in platelets and lymphocytes was detected in these same animals. In effect, both peripheral and central benzodiazepine receptors appear to associated with anxiety in rodents.

The data describing the genetic variations in benzodiazepine receptor are paralleled by clinical investigations (Weizman, Tanne, Granek, Karp, Golomb et al, 1987; Rocca, Ferrero, Gualerzi, Zanalda, Maina et al, 1991) which have revealed that platelet benzodiazepine binding sites were reduced in patients

with generalized anxiety relative to healthy subjects and that chronic treatment with benzodiazepine agonists increased peripheral binding density. Patients with panic disorder have been reported to exhibit a reduced number of peripheral benzodiazepine receptors as compared to both healthy controls and patients with obsessive compulsive disorder (Marazziti, Rotondo, Martini, Giannaccini, Lucacchini et al, 1994), although at least one study reported that panic patients exhibited peripheral benzodiazepine density comparable to that of healthy control subjects (Rocca et al, 1991). Roy-Byrne, Lewis, Villacres, Diem, Greenblatt et al 1989 and Roy-Byrne, Cowley, Greenblatt, Shader and Hommer (1990) employed benzodiazepine-induced changes in saccadic eye movements as a peripheral index of central benzodiazepine receptor sensitivity in panic disorder. These data presumably reflect benzodiazepine receptor function in the superior colliculus/pons of the brainstem. This investigation revealed that panic disorder patients but not those with generalized anxiety disorder exhibited a reduction in saccadic eye movement following diazepam compared with normal subjects. Such behavioral data were related to a functional subsensitivity of the GABA-benzodiazepine supramolecular complex in brainstem areas controlling saccadic eye movement.

To further explore the possibility of reduced benzodiazepine sensitivity in panic disorder Roy-Byrne et al (1991) also determined whether cortisol, growth hormone and ACTH response to acute intravenous diazepam would be attenuated in patients with panic disorder and generalized anxiety disorder

relative to normal control subjects. Although acute diazepam reduced ACTH and cortisol levels and increased growth hormone levels, there was no evidence that the neuroendocrine effects of diazepam administration differentiated patients with anxiety disorders from normal control subjects. More recently, it was reported that there was an overall tendency for plasma GABA to decrease following acute administration of diazepam compared with placebo but there were no differences between patients with either panic disorder or generalized anxiety disorder and normal controls (Roy-Byrne, Cowley, Hommer, Greenblatt, Kramer et al, 1992). The diazepam-induced reduction in GABA was interpreted as reflecting a homeostatic adjustment in presynaptic GABA levels to compensate for enhancement of GABAergic neurotransmission by diazepam.

Finally, the possibility that panic disorder may be associated with a shift in the benzodiazepine receptor "set-point" should be considered, since there is evidence that panic patients, but not healthy control, exhibit an anxiogenic response to the benzodiazepine receptor antagonist, flumazenil (Nutt, Glue & Lawson, 1990; Nutt, Smith, Bennett & Jackson, 1992). Flumazenil has also been reported to have weak anxiogenic effects in several rodent tests of anxiety (File et al, 1982; File & Pellow, 1986; 1986; Nishimura, Ida & Tanaka, 1990). Interestingly, Wilson, Glue and Nutt (1992) reported that the benzodiazepine antagonist, flumazenil, also reduced saccadic eye movement in panic patients and normal controls, although no clear differences between patients and controls in sensitivity to these effects were observed.

Nevertheless, the observation that flumazenil had agonist-like action on saccadic eye movement but partial inverse agonist effects on mood in panic patients was interpreted as suggesting that the paradoxical responses to flumazenil may be due to different receptor subtypes controlling these variables (Wilson et al, 1992).

In summary, there is considerable evidence that alterations in the GABA-benzodiazepine receptor complex may be linked to the generation of panic attacks. It is well established, for example, that benzodiazepine receptor agonists attenuate panic attacks while benzodiazepine receptor inverse agonists exert anxiogenic effects. Animal models of anxiety have raised the possibility that pathological anxiety is associated with decreased benzodiazepine receptor density although preliminary data have revealed that such alterations are evident in patients with generalized anxiety disorder rather than panic disorder. Nevertheless, there are some clinical data suggesting that panic disorder may be associated with functional subsensitivity of the GABA-benzodiazepine supramolecular complex in brainstem regions. More direct assessment of benzodiazepine receptor systems in panic disorder is required before any firm conclusions can be reached.

Central Adenosine Activity and Panic Disorder

Adenosine is a central neuromodulator with potent inhibitory effects on neurotransmission and the spontaneous firing rate of neurons in a variety of brain regions including the cerebral cortex, hippocampus, and locus coeruleus

(Phillis & Kostopoulos, 1975; Arushanian & Belozertsev, 1978; Dunnwiddie, Hoffer & Fredholm, 1981; Greene, Haas & Hermann, 1985; Jackisch, Strittmatter, Kasakov & Hertting, 1984; Popoli, Sagratella & Scotti de Carolis, 1987). In particular, adenosine appears to modulate release of NE (Enero & Saidman, 1977), GABA (Phillis & Wu, 1981; Stone, 1981) and 5-HT (Phillis & Wu, 1981; Stone, 1981). Administration of adenosine and adenosine-like derivatives produce sedation (Dunnwiddie & Worth, 1982; Crawley, Patel & Marangos, 1983), muscle relaxation (Baird-Lambert, Marwood, Davies & Taylor, 1980) and anticonvulsant activity (Dunnwiddie & Worth, 1982). These behavioral effects are reversed by methylxanthines, such as caffeine and theophylline, which act as adenosine receptor antagonists (Snyder, Katims, Annau, Bruns & Daly, 1981).

Caffeine is a widely used psychostimulant which produces its effects via its action as an adenosine A_1 and A_2 receptor antagonist (Uhde, 1990). Moderate doses of caffeine enhance arousal, concentration and attention and relieve the effects of fatigue and boredom on performance (Griffith, Evans, Heishman, Preston, Sannerud et al, 1990; Zwuyghuizen-Doorenbos, Roehrs, Kipschutz, Timms & Roth, 1990). However, at relatively higher doses, caffeine can elicit symptoms of anxiety in healthy human subjects (Gilliland & Andress, 1981; Charney et al, 1984b; Uhde, Boulenger, Jimerson & Post, 1984; Baldwin & File, 1989; Orlikov & Ryzov, 1991), psychiatric patients (Greden, Fontaine, Lubetsky & Chamerlain, 1978) and experimental animals (File & Hyde, 1979;

File, Baldwin, Johnston & Wilks, 1988). Toxic doses of caffeine are proconvulsant (Curatolo & Robertson, 1983). In addition to these central nervous system effects, caffeine has well characterized cardiorespiratory actions (Dobmeyer, Stine, Leier, Greenbert & Schaal, 1983; Lane & Manus, 1989).

The potential involvement of adenosine in the pathogenesis of panic disorder was initially inferred from retrospective self-report surveys of caffeine consumption which revealed a significant association between caffeine intake and self-rated anxiety for patients with panic disorder but not for normal control subjects (Boulenger & Uhde, 1982; Boulenger, Uhde, Wolff & Post, 1984; Uhde et al, 1984; Lee, Flegel, Greden & Cameron, 1988). Patients who are extremely sensitive to the anxiogenic effects of caffeine have typically been observed to abstain from caffeine because of its unwanted psychological effects (Boulenger et al, 1984; Lee et al, 1988). Furthermore, there is evidence that adherence to a caffeine-free diet markedly reduces the frequency of panic attacks (Lee et al, 1988; Bruce & Lader, 1989).

The suspected propensity of caffeine to induce anxiety in panic patients prompted experimental analysis of the effects of acute caffeine challenge in this patient population. Charney, Heninger & Jatlow (1985) for example reported that 10 mg/kg of oral caffeine (average 700 mg) produced greater increases in self-rated anxiousness, fearfulness and nervousness, autonomic reactivity, and diastolic blood pressure in panic patients than in healthy control subjects. These

behavioral and physiological effects were correlated with plasma caffeine levels in panic patients. In addition, caffeine induced panic attacks in 71% of patients but only 10% of controls. Uhde (1990) reported that a single oral dose of 480 mg of caffeine provoked panic attacks in 38% of panic patients but not in control subjects. Moreover, caffeine induced greater increases in lactate, glucose and cortisol concentrations in patients relative to controls. A comparison of orally administered caffeine (480 mg) and mCPP (0.5 mg/kg) revealed that caffeine and mCPP were equipotent in eliciting panic attacks and increases in plasma cortisol concentration in patients with panic disorder, although only mCPP produced increases in plasma prolactin concentrations (Klein, Zohar, Geraci, Murphy & Uhde, 1991). Patients with panic disorder have also been reported to be more sensitive than control subjects to low dose caffeine challenge (i.e., 250 mg) (Cameron, Modell & Hariharan, 1990; Beck & Berisford, 1992), although at least one study reported few notable behavioral changes in panic patients (Mathew & Wilson, 1990). Physiological responses to low dose caffeine challenge have not been found to reliably discriminate between panic disorder and control subjects (Cameron et al, 1990; Mathew & Wilson, 1990; Beck & Berisford, 1992). Brain imaging studies of caffeine-induced anxiety have revealed significant reductions in cerebral blood flow in panic patients and healthy volunteers, although no consistent correlation between cerebral blood flow and subjective anxiety and peripheral physiology have been observed (Cameron et al, 1990; Mathew & Wilson, 1990). These

findings led Mathew and Wilson (1990) to conclude that caffeine-induced changes in cerebral blood flow is largely caused by the vascular effect of the drug rather than by alterations in mood.

Although the likely mechanism of action of caffeine is the antagonism of the adenosine receptor (Synder et al, 1981; Daly, Bruns & Snyder, 1981; Barraco, Coffin, Altman & Phillis, 1983; Daly, Butts-Lamb & Padgett, 1983), it should be emphasized that caffeine has complex pharmacological effects, and some of the physiological and behavioral effects of this purine have been ascribed to its action on the benzodiazepine receptor (Marangos, Paul, Parma, Goodwin, Syapin & Skolnick, 1979; Polc, Bonnetti, Pieri, Cumin, Angioi et al, 1981; Matilla, Palva & Savolainen, 1982; File, 1982; File, Bond & Lister, 1982; Kaplan, Tai, Greenblatt & Shader, 1990). In this respect, it has been demonstrated that caffeine binds to benzodiazepine receptors in vitro (Marangos et al, 1979; Marangos, Martino, Paul & Skolnick, 1981) and can provoke upregulation of benzodiazepine receptors following chronic administration in rats (Fredholm, 1982; Boulenger, Pael, Post, Parma & Marangos, 1983; Wu & Coffin, 1984). It has also been documented that chronic alprazolam therapy significantly attenuated the anxiogenic effects of caffeine challenge in panic disorder patients (Uhde, 1990). An interaction between caffeine and NE has also been suggested (Berkowitz, Tarver & Spector, 1970; Olpe, Jones & Steinmann, 1983; Galloway & Roth, 1983), however, clinical studies have failed to detect significant increases in plasma

MHPG following caffeine administration (Charney et al, 1984b; 1985; Uhde et al. 1984b). Caffeine has also been reported to modify brain levels of 5-HT and 5-HIAA in mice (Berkowitz & Spector, 1971; Valzelli & Bernasconi, 1974). Moreover, Orlikov and Ryzov (1991) reported that concentrations of kynurenine, a neuroactive tryptophan metabolite, increased during caffeine-induced anxiety in healthy volunteers, suggesting a possible mediating role of 5-HT in the anxiogenic effects of caffeine.

It has been demonstrated that chronic caffeine administration causes marked increases in adenosine receptor density in human (Boulenger, Patel & Marangos, 1982) as well as in rat and mouse cortex (Fredholm, 1982; Murray, 1982; Boulenger et al, 1983; Marangos, Boulenger & Patel, 1984). Such variation in receptor density do not appear to be accompanied by significant modification in affinity (Fredholm, 1982; Murray, 1982; Boulenger et al, 1983). There is some preliminary data suggesting that panic patients have either an increased number of A₁ adenosine receptors or that these receptors may exhibit increased affinity for caffeine. For example, DeMet, Stein, Tran, Chicz-DeMet, Sangdahl et al (1989) used a gustatory caffeine challenge test to examine the sensitivity of oral adenosine receptors in patients with panic disorder. The gustatory response to caffeine, which is believed to reflect A₁ adenosine receptor activity, was significantly greater in patients with panic disorder relative to both normal control and post-traumatic stress disorder subjects. Apfeldorf and Shear (1993) also recently reported that panic disorder patients

exhibited a heightened caffeine-mediated taste enhancement relative to normal control subjects. Finally, Stein, Black, Brown and Uhde (1993) reported that the adenosine reuptake inhibitor, dipyridamole, was ineffective in the treatment of panic disorder, suggesting that adenosinergic dysfunction may not be a central component of the pathophysiology of panic disorder.

In summary, it is postulated that adenosine may play a role in the pathophysiology of panic disorder. This hypothesis is derived mainly from the observation that patients with panic disorder may be hypersensitive to the anxiogenic effects of caffeine relative to healthy volunteers. Additional studies are clearly needed to further elucidate adenosine function in panic disorder.

Central Cholecystokinin Activity and Panic Disorder

Cholecystokinin (CCK) availability is conspicuous in the cerebral cortex, striatum, amygdala, hippocampus and the brainstem, with putative neurotransmitter or neuromodulator function (Rehfeld, Goltermann, Larsson, Emson & Lee, 1979; Vanderhaeghen, Lotstra, Vierendeels, Giles, Deschepper et al, 1981; Beinfeld, 1983). Molecular forms of varying amino acid lengths of CCK have been isolated in the brain (Sauter & Frick, 1983; Miller, Jardine, Weissman, Go & Sneicher, 1984; Morley, Rehfeld & Emson, 1984). Of the various fragments identified, the C-terminal octapeptide (CCK-8) in the sulphated (CCK-8S) and unsulphated form (CCK-8US) appear to be most abundant (Larsson & Rehfeld, 1979; Goltermann, Rehfeld & Roigaard-Petersen, 1980). Moreover, two principal CCK receptor types have been identified

(Moran, Robinson, Goldrich & McHugh, 1986; Dourish & Hill, 1987; Hill, Campbell, Shaw & Woodruff, 1987). The CCK_A ("alimentary") receptor is found in the viscera and in some specific brain regions notably the area postrema, nucleus tractus solitarius and interpeduncular nucleus, and the CCK_B ("brain") receptor which is more widely distributed in the brain.

A role for CCK has been offered in the promotion of schizophrenia (Hommer, Pickar, Roy, Ninan, Boronow et al, 1984; Nair, Lal & Bloom, 1986), satiety (Smith & Gibbs, 1975; Geraciotti & Liddle, 1988) and pain (McRoberts, 1986), but only recently has a link to panic disorder been drawn. The hypothesis that CCK might be a mediator of anxiety originated from electrophysiological experiments which demonstrated that systemically administered low dose benzodiazepine receptor agonists attenuated the excitatory influence of CCK-8S on hippocampal pyramidal neurons in rats (Bradwejn & de Montigny, 1984; Bradwejn & de Montigny, 1985a; Bradwejn & de Montigny, 1985b). These studies provided the first evidence that anxiolytic benzodiazepines could antagonize the central action of a neuropeptide and it was proposed that benzodiazepine-mediated antagonism of CCK-induced excitation might be an important mechanism by which benzodiazepines exert their clinically relevant action. More importantly, the observation that an anxiolytic could block the excitatory action of CCK raised questions about whether CCK might be an endogenous anxiogen.

Several recent clinical studies are compatible with the hypothesis that

CCK neurotransmission may be related to panic disorder. In particular, Bradwejn et al (1990) initially observed that CCK-4, a CCK_B receptor agonist, had profound panicogenic effects in patients with panic disorder. These investigators reported that CCK-4 precipitated a panic attack, as defined by DSM-III criteria and patient self-report, within one minute following administration of 50 μ g of CCK-4 in 11 trial patients studied whereas none of the patients panicked following placebo. In addition, CCK-4 elicited an average of 12 symptoms per patient, the most common being dyspnea, palpitations/rapid heart, chest pain/discomfort, faintness, dizziness, paresthesia, hot flushes/cold chills, nausea/abdominal distress, anxiety/fear/apprehension and fear of losing control. In a subsequent placebo-controlled study, Bradwejn, Koszycki and Shriqui (1991b) attempted to determine whether patients with panic disorder displayed enhanced sensitivity to the anxiogenic properties of two doses of CCK-4 (25 and 50 μ g) relative to healthy control subjects. The frequency of panic attacks was markedly higher in patients than in controls following injections of 25 μ g (91% versus 17%) and 50 μ g (100% versus 47%) of CCK-4. Interestingly, Bradwejn et al (1991b) noted that the number and intensity of symptoms as well as the symptom profile were remarkably similar in both patients and normal subjects who panicked with 50 μ g of CCK-4, suggesting that the enhanced response to CCK in panic patient could not be readily attributed to a tendency to overendorse symptoms. Concurrently, Abelson and Nesse (1990) observed that pentagastrin, a central CCK agonist

which incorporates the identical 4 amino acid sequence of CCK-4, provoked DSM-III-R panic attacks in patients with panic disorder but not in healthy individuals.

Bradwejn et al (1992a) recently completed a double-blind dose response study of CCK-4 (25, 20, 15 and 10 μ g of CCK-4 and placebo) in patients with panic disorder. The frequency of panic attacks following the CCK-4 challenge was 17% (10 μ g), 64% (15 μ g), 75% (20 μ g) and 75% (25 μ g). None of the patients panicked with placebo. Further, the behavioral effects of CCK-4 were associated with marked and dose-related increases in heart rate and blood pressure. In contrast to panic patient, healthy subjects required larger concentrations of CCK-4 to panic. Bradwejn, Koszycki and Bourin (1991c) noted that lower concentrations (9 and 25 μ g) of CCK-4 had minimal panicogenic effects, precipitating panic attacks (as defined by DSM-III-R criteria plus anxiety of at least moderate intensity) in only 11% and 17% of subjects respectively. Conversely, the 50 μ g dose of CCK-4 promoted panic attacks in about 50% of controls, which is somewhat lower than the 64% panic rate observed in panic patients who received 15 μ g. Doses of 10 μ g or lower of CCK-4 appear to have little panicogenic effects in either panic patients or control subjects (Bradwejn et al, 1991c; Bradwejn et al, 1992a).

In order to determine whether the behavioral effects of CCK-4 could be replicated in the same individual, Bradwejn, Koszycki, Payeur, Bourin and Borthwick (1992c) administered 25 μ g of CCK-4 to 11 patients with panic

disorder on two separate occasions in the absence of intervening treatment. The vulnerability of panic patients to the anxiogenic effects of CCK-4 was not diminished with repeated challenge. The panic frequency following the initial and subsequent challenge was 82% and 73%, respectively. In addition, the number and intensity of symptoms remained constant with rechallenge. This property of CCK-4 was regarded as having important implications in relation to testing the effectiveness of antipanic drugs in blocking CCK-4-induced panic.

A comparison of CCK-4-induced panic with that in evidence following exposure to a single inhalation of 35% carbon dioxide was recently reported in panic patients (Bradwejn & Koszycki, 1991). In particular, an intravenous CCK-4 challenge dose of 25 μ g was more effective in eliciting panic attacks than a single inhalation of 35% carbon dioxide (91% versus 45%), although the profile of symptoms which emerged in response to either agent was similar in subjects who reported a panic attack (Bradwejn & Koszycki, 1991). Interestingly, the concentrations of CCK-4 and carbon dioxide employed were equipotent in promoting panic attacks in healthy subjects (17% versus 21%) (Koszycki, Bradwejn & Bourin, 1991). These data have suggested that perhaps CCK-4 and carbon dioxide act on comparable neural systems in the promotion of panic or on separate systems with a final common pathway. For example, carbon dioxide is presumed to exert its effects via NE and perhaps multiple non-NE neurotransmitter systems (Woods et al, 1986).

There is scant information on the biochemical correlates of CCK-induced

anxiety. Several investigators have observed an effect of the CCK_B receptor agonists pentagastrin (Degli Uberti, Trasforini, Margutti, Rotola & Pasini, 1983; 1989; Abelson, Nesse & Vinik, 1991; Calogero, Nicolasi, Moncada, Coniglione, Vicari et al, 1993; Abelson, Nesse & Vinik, 1994a) and CCK-4 (de Montigny, 1989) on ACTH and/or cortisol release in humans, suggesting an important interaction between CCK and the hypothalamic-pituitary-adrenal axis. However, Abelson et al (1994a) demonstrated that the ability of pentagastrin to increase hypothalamic-pituitary-adrenal activity was unrelated to its anxiogenic effects, and that panic disorder and normal control subjects exhibited comparable increases in ACTH and cortisol. In addition to modulating the hypothalamic-pituitary-adrenal axis, CCK agonists have been reported to stimulate release of growth hormone and prolactin in humans (Almonte, Zoli, Mirone, Ghirlanda, Bertolt, Manna et al, 1986; de Montigny, 1989; Calogero et al, 1993), although results have not been consistent (Lal, Nair, Eugenio, Thavundayil, Lizondo et al, 1983; Nair, Lal, Eugenio, Lizondo, Thavundayil, et al, 1986; Abelson et al, 1994). Peripherally administered pentagastrin has also been shown to transiently increase plasma E in panic disorder and control subjects (Abelson et al, 1994).

Some potential neuroanatomical correlates of CCK-4 challenge in healthy volunteers were recently provided by Benkelfat, Bradwejn, Meyer, Ellenbogen, Milot et al (1992). Using positron emission tomography, analysis of CCK-4 minus placebo cerebral blood flow images revealed significant extracranial

cerebral blood flow increases bitemporally in both panickers and non-panickers and cerebral blood flow increases in the anterior cingulum, left and right putamen and vermis in non-panickers only. This latter finding has led to speculation that panic disorder might be associated with a lack of CNS compensatory mechanisms to the anxiogenic effects of CCK-4.

The behavioral effects of systemically-administered CCK agonists in clinical paradigms appear to be paralleled by the data describing the effects of CCK agonists in animals. For example, intra-amygdaloid administration of CCK-8S produced an anxiogenic effect in rats (Csonka, Fekete, Nagy, Szanto-Fekete, Feledy et al, 1988). Likewise, intraventricular or systemic administration of the caerulein, a nonselective CCK_A/CCK_B receptor agonist, dose-dependently produced anxiogenic effects in rats or mice, as revealed by reductions of exploratory behavior in the elevated plus maze (Harro, Pold & Vasar, 1990a). Intraventricular administration of the selective CCK_B receptor agonist, pentagastrin, also had potent anxiogenic effects in the rat elevated plus maze, the mouse light/dark box and the rat conflict test (Singh, Field, Hughes, Menzies, Oles et al, 1991a). Likewise, peripheral administration of the selective CCK_B agonists, CCK-4 and BC 264, provoked anxiety-related behaviors in mice and rats (Harro & Vasar, 1991b; Roques, Corringer, Durrien, Dague & Durieux, 1993; Dauge, Derrien, Durieux, Noble, Corringer et al, 1992; Rex, Barth, Voigt, Domeney & Fink, 1994a). In the African green monkey, the behavioral response to peripheral CCK-4 is immobility and/or freezing (Ervin, Palmour & Bradwejn,

1991). Interestingly, immobilization and freezing have also been observed in monkeys in response to social stress or threat and may provide behavioral equivalents of human fear or panic (Bolles & Fanselow, 1980; Friedman, Sunderland & Rosenblum, 1987).

In addition to these behavioral effects, alterations in ACTH, growth hormone and prolactin concentrations provoked by CCK agonists have been reported in rodents (Itoh, Hirota, Katsuura & Odaguchi, 1979; Reisine & Jensen, 1986; Katsuura, Ibibi & Matsushita, 1992; Kamilaris, Johnson, Calogero, Kalogeras, Bernardini et al, 1992; Vijayanin, Samson & McCann, 1979; Matsumura, Yamanoi, Yamamoto, Mori & Saito, 1984). Biro, Sarnyai, Penke, Szabo and Telegdy (1993) reported that intraventricular administration of CCK-8S induced an anxiogenic response and concomitant increases in cortisol in rats. Interestingly, Biro et al (1993) found that pretreatment with different dilutions of corticotropin-releasing factor (CRF) antiserum and different doses of a CRF receptor antagonist, alpha-helical, antagonized the anxiogenic and hormonal response to CCK-8S in a dose-dependent fashion. It was concluded that the anxiogenic and pituitary-adrenal-activating effects of CCK-8S are mediated, at least in part, by activation of the endogenous CRF system in the central nervous system.

It may be noted that the anxiogenic effects of CCK agonists have not been demonstrated by all investigators (Bayley & Dawson, 1993; Rupniak, Schaffer, Siegl & Iversen, 1993; Rex et al, 1994a), and dose efficacy and

behavioral patterns after CCK challenge appear to be influenced by organismic and experiential factors. Thus, 10-50 times lower doses of caerulein and pentagastrin were anxiogenic in rats and mice housed under overcrowded conditions (Harro, Pold, Vasar & Allikmets, 1989) compared to animals housed under noncrowded conditions (Vasar, Peuranen, Ööpik, Harro & Männistö, 1993a), whereas social isolation decreased the animal's sensitivity to the caerulein challenge (Vasar, Peuranen, Harro, Lang, Oreland & Männistö, 1993b). In African green monkey, behavioral activation engendered by CCK-4 challenge was found to be largely influenced by the baseline behavioral profile of the individual animal in its social environment (Ervin et al, 1991). For example, high CCK-4 challenge doses produced immobilization and freezing in ordinarily "anxious and fidgety" monkeys but promoted mild restlessness and activation in naturally "calm" monkeys.

The use of CCK agonists in clinical studies have been paralleled by the development of CCK antagonists with demonstrated ease of central penetration and selectivity for central (CCK_b) and peripheral (CCK_A) receptor subtypes (Silverman, Greenberg & Bank, 1987; Freidinger, 1989; Horwell, 1991; Woodruff & Hughes, 1991). In this respect, a number of investigations have revealed that CCK antagonists may possess anxiolytic properties. For example, systemic treatment with the CCK_b-receptor antagonist, L-365,260, increased exploratory behavior in the elevated plus-maze (Ratnaud, Darche, Piot, Stutzmann, Pohem et al, 1991; Singh, Lewis, Field, Hughes & Woodruff,

1991b). In contrast, the CCK_A receptor antagonist devazepide failed to induce modification of the plus-maze behavior, suggesting that CCK_B rather than CCK_A antagonists have anxiolytic properties. The CCK_B antagonist, CI-988, also has anxiolytic effects in rodent models of anxiety (Hughes, Boden, Costall, Domeney, Kelly et al, 1990; Costall, Domeney, Hughes, Kelly, Naylor et al, 1991; Singh et al, 1991a; Singh et al, 1991b), with behavioral effects comparable to those of diazepam (Costall et al, 1991) and chlordiazepoxide (Singh et al, 1991a). CI-988 has also been found to be as effective as diazepam in blocking aversive behavior in a marmoset "human threat" model (Costall et al, 1991). Powell and Barrett (1991) reported that CI-988 increased punished responding in squirrel monkeys, although the magnitude of this effect was smaller than that obtained with chloridiazepoxide. Nonetheless, in contrast to the effects of chloridiazepoxide, CI-988 did not affect unpunished responding.

Several other investigations have determined whether CCK antagonists can attenuate the anxiogenic effects of CCK agonists. Pretreatment with proglumide, a nonselective CCK_A/CCK_B antagonist, blocked the anxiogenic effects of caerulein on exploratory behavior in the mouse but acute treatment with low doses of diazepam had no effect (Harro et al, 1990a). Harro and Vasar (1991b) subsequently reported that systemic treatment with lorglumide and devazepide (CCK_A receptor antagonists), proglumide and L-365,260 antagonized the action of CCK-4 on exploratory behavior in the rat. Proglumide

and lorglumide were active at relatively low doses whereas devazepide blocked the behavioral effects of CCK-4 only at the highest dose. By comparison L-365,260 reversed the behavioral effects of CCK-4 completely at the lowest dose used. Singh and coworkers (1991a; 1991b) reported that the central CCK antagonist CI-988 produced a dose-dependent attenuation of the anxiogenic effects of intraventricular administration of pentagastrin in the rat elevated plus-maze. In contrast, devazepide was found to be inactive in this respect (Singh et al, 1991b). CI-988 did not ordinarily antagonize the anxiogenic effects of pentylenetetrazol (Singh et al, 1991b), unless the pharmacological challenge was considerable (a ten-fold increase). These observations would detract from a consideration that CI-988 might produce its effects via benzodiazepine receptors (Singh et al, 1991a). Palmour, Ervin, Bradwejn and Howbert (1991) also recently reported that the anxiogenic and cardiovascular effects of CCK-4 were antagonized by pretreatment with the CCK_B receptor antagonist, LY262,691, in African green monkeys. In a recent study of panic patients, pretreatment with L-365,260, a CCK_B receptor antagonist, significantly reduced the behavioral and cardiovascular effects of exogenous CCK-4 (Bradwejn, Koszycki, Couteux du Tertre, van Megen, den Boer et al, 1994a). In particular, the panic attack rate following the challenge was 0% for patients treated with a 50 mg dose of L-365,260 and 87% for those treated with placebo. It has also been demonstrated that single oral doses of CI-988 and L-365,260 attenuate the anxiogenic effects of CCK_B agonists in healthy volunteers (Lines,

Traub & Ambrose, 1993; Bradwejn, Koszycki, Paradis, Reece & Sedman, 1994b). These clinical data indicate that CCK_B receptors are a primary site of action of exogenous CCK-4, and suggest that CCK_B antagonists may have therapeutic benefits in the treatment of panic disorder.

Although there is ample evidence that CCK receptor ligands influence emotions in human and infrahuman subjects, it is unclear whether alterations in endogenous CCK mechanisms are associated with anxiety states. Harro et al (1990a) reported that CCK concentrations differed in rats following a brief episode of forced exploration in the elevated plus maze. In particular, rats with low exploratory behavior ("anxious" rats) had a significantly lower density of CCK receptors in the frontal cortex and hippocampus relative to rats with high exploratory behavior ("nonanxious" rats). Moreover, alterations in CCK receptor binding in rat frontal cortex have been observed after stressor exposure (Harro et al, 1990; Vasar, Harro, Pold & Lang, 1992; Vasar et al, 1993a; Vasar, Harro, Lang, Soosaar, Ööpik, Koks et al, 1993c). Lydiard, Ballenger, Laraia, Fossey and Beinfeld (1992) reported that panic patients exhibit decreased basal CCK-8S concentrations relative to normal control subjects, suggesting that panic disorder may be associated with an abnormal CCK turnover. Similarly, Brambilla and colleagues (Brambilla, Bellodi, Perna, Garberi, Panerai et al, 1993) noted that lymphocyte CCK-8 concentrations were markedly lower in panic disorder relative to normal control subjects and did not change after alprazolam therapy. To be sure, the development of more sensitive analytic techniques,

permitting measurement of shorter CCK fragments, including CCK-4, will help determine whether CCK turnover rate is aberrant in panic disorder.

Cholecystokinin/Norepinephrine Colocalization and Behavior

It is relevant to note in retrospect that considerable attention has been directed towards the assessment of variations in central NE activity to panic disorder. Such investigations have largely focused on the potential role of the locus coeruleus to the induction of anxiety and to the interface of the dorsal NE bundle with major ascending and descending catecholamine and indoleamine systems in provoking behavioral change. The relatively recent demonstration that CCK can promote anxiogenic effects in human and infrahuman subjects is intriguing in light of the immunocytochemical identification of CCK colocalization in and about the regions of the locus coeruleus as well as the nucleus tractus solitarius (Sutin & Jacobowitz, 1983); brain nuclei implicated in the promotion of panic attacks (Gorman et al, 1989). Further to this point, Kendrick et al (1991) reported that intravenously administered CCK in rats enhanced NE release in the paraventricular nucleus and the dorsal supraoptic nucleus. These data are consistent with an earlier report by Kadar, Fekete, Lonovics and Telegdy (1981) which demonstrated that CCK antisera decreased NE content in the hypothalamus, mesencephalon, amygdala, septum, striatum and the cerebral cortex of rats. It should be underscored that site dependent variability in the influence of CCK on central NE turnover is likely. For example,

Kendrick et al (1991) observed that in contrast to the effect of the neuropeptide in other hypothalamic nuclei surveyed, CCK-4 decreased NE release in the ventral supraoptic nucleus and Beresford, Hall, Clark, Hill and Hughs (1988) observed that CCK-8 reduced NE turnover in whole hypothalamus. The functional significance of such region specific alterations of NE activity remains to be determined. It should also be noted that Harro, Jossan and Oreland (1992a) recently demonstrated that depletion of dorsal NE concentrations induced by intraperitoneal DSP₄ administration increased CCK receptor density in the frontal cortex and hippocampus, while desipramine treatment attenuated the DSP₄-induced NE neurotoxicity and prevented CCK receptor alteration. Interestingly, however, Yamamoto and Kato (1988) reported that neonatal NE depletion following 6-OHDA treatment did not influence CCK receptor density in the adult rat. Finally, there are some recent behavioral data concerning the interaction between NE and CCK-4 in the promotion of anxiety and panic. Palmour and Ervin (1993, personal communication) demonstrated that propranolol, but not atenolol, decreased motor activation, fidgeting, threat and vigilance induced by CCK-4 in African green monkeys. Moreover, Bradwejn and Koszycki (1994b) reported that the antipanic agent, imipramine, antagonized the panicogenic effects of CCK-4 in panic disorder patients. These data suggest that perhaps both α - and β -NE receptor activation may be instrumental in interacting with endogenous CCK in promoting specific symptom profiles associated with panic disorder.

Cholecystokinin/GABA Colocalization and Behavior

It has been suggested that CCK and endogenous benzodiazepine agents are competitive agonists at the benzodiazepine receptor site. Treatment with benzodiazepine agonists has also been reported to antagonize the effects of CCK-8 in the periphery (Sugaya, Matsuda, Kubota, 1985; Kubota, Sugaya, Sunagane, Matsuoka & Urono, 1985; Kubota, Sugaya, Matsuda, Matsuoka & Terawaki, 1985) and neuronal responsivity to CCK-8 in rats is decreased following long-term administration of benzodiazepine agonists (Bouthillier & de Montigny, 1988). Such neurochemical variations can be linked, in some cases, to specific behavioral change. For example, Harro, Kiivet, Lang & Vasar (1990b) demonstrated that rats with low exploratory behavior in the elevated plus maze ("anxious rats") had a markedly lower density of benzodiazepine receptors in the frontal cortex and reduced CCK-8 receptor availability in the hippocampus relative to rats with high exploratory activity ("nonanxious rats"). Alteration in CCK receptor binding in rat has also been observed following termination of chronic diazepam administration (Harro, Lang & Vasar, 1990c) and treatment with the anxiogenic β -carboline, FG 7142, or the noncompetitive GABA agonist, picrotoxin (Vasar et al, 1992).

Administration of the CCK_A receptor antagonist, lorglumide, has been reported to potentiate the behavioral effects of diazepam (Panerai, Rovati, Bianchi, Bareggi & Mantegazza (1987). Further, some investigators have demonstrated that benzodiazepine agonists attenuate the anxiogenic effects of

CCK agonists in rats (Csonka et al, 1988), monkeys (Ervin et al, 1991) and human subjects (de Montigny, 1989). It has also been reported that administration of the benzodiazepine receptor antagonist, flumazenil, significantly antagonized the anxiogenic activity of CCK-8 and the anxiolytic effects of the CCK_A receptor antagonist, devazepide, and the CCK_B receptor antagonist, L-365,260, in rats (Chopin & Briley, 1993). However, administration of flumazenil to healthy subjects failed to antagonize the panicogenic effects of CCK-4 (Bradwejn, Koszycki Couteux du Tertre, Paradis & Bourin, 1994c), suggesting that CCK-4-induced panic symptoms may not be mediated by benzodiazepine receptors. Bradwejn et al (1994b) have suggested that the net effect of the benzodiazepine-CCK interaction may involve alteration of central GABA activity. It is notable in this respect that CCK is colocalized with GABA-synthesizing neurons in the cerebral cortex, hippocampus and basolateral amygdala (Hendry, Jones, De Felipe, Schmechel, Brandon et al, 1984; Jones & Hendry, 1986), and CCK-8S has been shown to enhance GABA release in the CNS (Sheenan & De Belleruche, 1984). However, Nakamura et al (1990) reported that caerulein decreased GABA concentrations and turnover rates and Bradwejn and de Montigny (1985b) reported that the influence of benzodiazepine agonists on CCK-8S-induced excitation of rat hippocampal neurons was not associated with a potentiation of GABA activity.

Cholecystokinin/Serotonin Colocalization and Behavior

Rybarczyk, Orosco, Rouch, Jacquot and Cohen (1990) reported that

intraperitoneal administration of CCK-8S decreased 5-HT concentrations and 5-HIAA levels in rat hippocampus whereas diazepam promoted 5-HT accumulation. The coadministration of CCK and diazepam precluded detection of 5-HT variations. It will be recalled that Br: dwejn and de Montigny (1984) previously reported that benzodiazepine administration antagonized the effects of CCK induced activation of hippocampal neurons. As such it should be considered that such electrophysiological effects may be influenced, at least in part, by alterations of central 5-HT activity. Indeed, it would be of considerable advantage to determine whether such alterations of indoleamine activity can be attributed to variations of neurochemical activity in the raphe system; a proposal consistent with the demonstration of CCK colocalization in this brain region. In addition, Fekete, Varszegi, Kadar, Penke, Kovacs and Telegdy (1981) reported that intraventricular administration of CCK-8 produced a significant decrease in 5-HT concentrations in the hypothalamus, the mesencephalon and the striatum. Kadar et al (1981) also reported that the antisera for CCK-8 decreased the content of 5-HT in the mesencephalon, the amygdala and the septum, but increased 5-HT content in the striatum. Itoh, Takashima and Katsuura (1988) observed that intraventricular administration of CCK-4 decreased 5-HT content and increased 5-HIAA in various regions of the brain of rat including the cortex, septum, hippocampus, mid-brain and hypothalamus. These changes were most pronounced with low doses of CCK-4. Serotonin turnover increased in most brain regions except the striatum and olfactory

tubercle. A recent microdialysis study revealed that CCK-4 potentiated the rise of 5-HT on exposure to the elevated plus maze in guinea-pigs (Rex, Fink & Marsden, 1994b). Further, the CCK_B antagonist, L-365,260, antagonized both the anxious behavior and the rise in 5-HT induced by CCK-4 (Rex et al, 1994). Taken together, these results strongly indicate that CCK-4 administered in small doses can increase central 5-HT activity in the rat.

The locus of the CCK/5-HT interaction has been proposed by some investigators to occur in the dorsal raphe nucleus, an area of conspicuous 5-HT activity. For example, Boden, Woodruff and Pinnock (1991) noted that CCK-8S dose-dependently activated a subpopulation of raphe neurons in rat brain. Such an effect appeared to be associated with activation of the CCK_A receptor type. Paudice and Raiteri (1991) also recently reported that 5-HT promoted release of CCK in the frontal cortex and nucleus accumbens of the rat. In particular, these investigators demonstrated that 5-HT induced release of CCK was attributable to an antagonism of the 5-HT₃ receptor (i.e., MDL 72222, ICS 205-930, ondansetron), but not to 5-HT₁ and 5-HT₂ antagonism (i.e., methiopen). The mixed nature of the latter pharmacological challenge would suggest that closer examination of 5-HT₁ and 5-HT₂ receptors may be in order. Indeed, Agnati, Fuxe, Benfenati, Celani, Battistini et al (1983) provided evidence for a 5-HT₂/CCK receptor interaction in the dorsal aspects of the cerebral cortex in the rat. Finally, Vasar et al (1993a) speculated on the potential for a CCK- 5HT₃ interaction in the mediation of anxiety. Consistent with the investigation of

Paudice and Raiteri (1991), the anxiogenic effect (e.g., anti-exploratory) of caerulein was also prevented by prior ondansetron treatment. Taken together, preliminary data suggest that the interaction of 5-HT and CCK activity may contribute to the expression of anxiety and tentatively implicate the 5-HT₂ and 5-HT₃ receptors in this respect.

Experimental Prospectus

The observation that pharmacological stimulation of CCK_B receptors with CCK-4 can provoke panic attacks in humans has generated considerable interest in the role of endogenous CCK in the pathophysiology of panic disorder. However, it should be apparent from the aforementioned discussion that the neurochemical (and ultimately the behavioral) basis of panic disorder cannot be attributed to the influence of an isolated transmitter or putative transmitter system. In order to assess the influence of extra-CCK mechanisms in the modulation of CCK function in human anxiety, the present study examined the involvement of 5-HT in the panicogenic activity of CCK-4. It would be presumptuous at this point to consider that the interaction between CCK and 5-HT in panic disorder is more deserving of investigation than an assessment of the interaction between CCK and other neuronal systems hypothesized to contribute to the induction of panic. Nevertheless, there are experimental protocols which permit relatively unobtrusive manipulations of central 5-HT neurochemistry and the induction of predictable behavioral change.

One technique which has been routinely employed to vary central 5-HT concentrations involves a relatively simple dietary manipulation which alters availability of the amino acid precursor tryptophan. In this respect, Biggio, Fadda, Fanni, Tagliomonte and Gessa (1974) initially reported that provision of a tryptophan deficient amino acid mixture in rats promoted a marked decline of

plasma and brain tryptophan levels as well as central 5-HT and 5-HIAA levels. Decreasing dietary tryptophan intake has also been shown to lower 5-HT and 5-HIAA concentrations in CSF and brain in nonhuman primates (Young, Ervin, Pihl & Finn, 1989). Five hours after consumption of a tryptophan deficient diet in human volunteers, plasma tryptophan concentrations are reduced to approximately 20% of control values. If a low protein diet is introduced on the day immediately preceding the tryptophan deficient diet, the fall in plasma tryptophan can be exacerbated (Delgado, Charney, Price, Aghajanian, Landis et al, 1990; Young, 1993, personal communication). Acute dietary depletion of tryptophan has been used to evaluate central 5-HT function in depression and other psychiatric disorders (Delgado et al, 1990; Delgado, Price, Miller, Salomon, Licinio et al, 1991; McDougle, Naylor, Goodman, Volkmar, Cohen et al, 1993; Weltzin, Fernstrom, McConaha & Kaye, 1994). Delgado et al (1990) reported that transient depletion of plasma tryptophan was associated with transient depressive relapse in antidepressant-remitted depressed patients, although tryptophan depletion did not worsen a depressed state in drug-free depressed patients (Delgado et al, 1991). A transient mood lowering has also been reported in healthy volunteers following acute tryptophan depletion (Young, Smith, Pihl & Ervin, 1985; Smith, Pihl, Young & Ervin, 1987), but such an effect may well be associated with prior mood (Abbott, Etienne, Franklin, Morgan, Sewitch et al, 1992) or genetic vulnerability to affective disorder (Benkelfat, Ellenbogen, Palmour & Young, unpublished manuscript).

To some extent, the neurochemical effects of tryptophan depletion and loading have been studied in nonhuman primates (Chamberlain, Ervin, Pihl & Young, 1987; Young and Ervin, 1984). There is a dose-dependent effect with respect to the lowering of plasma tryptophan, which plateaus with amino acid mixtures of about 50 grams. Cerebrospinal fluid tryptophan and 5HIAA fall in parallel with the decline in plasma tryptophan whereas the level of tyrosine and the catecholamine metabolites HVA and MHPG are unaffected (Young et al, 1989). A previous study has demonstrated a number of similarities between man and vervet monkey with regard to CSF amine metabolites following administration of a tryptophan-deficient amino acid diet (Young & Ervin, 1984; Young et al, 1985). These results are consistent with the idea that the behavioral effects of tryptophan deficient mixtures are mediated by changes in brain 5-HT unaccompanied by changes in brain catecholamines. In conclusion, the results of this study provide further support for the idea that the behavioral effects of tryptophan deficient mixtures are due to decreased brain 5-HT function (Young & Ervin, 1984). These data also suggest that significant depletion of 5-HT in humans can be achieved with a low protein diet followed by a tryptophan deficient mixture.

The present study was designed to assess whether human subjects who ingest a tryptophan-free amino acid diet would exhibit a differential response to CCK-4 challenge relative to subjects who consumed a control diet. In view of the hypothesized role of 5-HT in CCK-4-induced anxiety, it was expected

that subjects who consumed the tryptophan-free diet would (a) experience a greater number of panic symptoms and more frequent panic attacks following CCK-4 challenge, (b) show greater increases in physiological arousal following CCK-4 challenge and (c) display greater release of stress responsive hormones than subjects who consumed a control diet.

METHOD

Subjects

Forty males between 18 and 35 years of age, in good physical health as determined by medical history, physical examination, electrocardiogram and clinical laboratory tests, and within $\pm 15\%$ of their ideal weights (Table of "Desirable Weights of Adults", Metropolitan Life Insurance Company, 1983) participated in the study. Subject variables which excluded participation in the study included (a) a history of AXIS I DSM-III-R diagnosis, suicidal tendencies, convulsive disorder and infrequent panic attacks, (b) abnormal scores on any of the subscales of the Hopkins Symptom Checklist, (c) a history of panic or affective disorder in a first-degree relative, (d) physical illness in the three months immediately preceding the study, (e) a daily caffeine intake exceeding three cups of coffee (or equivalent), (f) a daily tobacco consumption of greater than five cigarettes (or equivalent) and (g) participation in a drug trial within one month prior to the start of the study.

Assessment

Psychological Measures

1. The Hopkins Symptom Checklist (SCL-90), a 90 item self-report psychiatric symptom inventory (Derogatis, Lipman & Covi, 1973) was used as a screening device (see Appendix 1). The scale was designed to assess current-point psychiatric symptom status. Symptoms were rated on a 5-point ordinal scale ranging from 0 "not at all" to 4 "extremely severe." These scores were interpreted with respect to nine symptom dimensions believed to represent the symptomatic behavior of psychiatric patients, including somatization, obsessive-compulsive tendency, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation and psychoticism. The score for each dimension was computed as the mean of its constituent items. Research on the psychometric properties of the SCL-90 has demonstrated its reliability (>0.80) and validity.

2. The Structured Clinical Interview DSM-III-R: Non-Patient Edition (NP-SCID) was used to screen for the presence of any current or lifetime AXIS-I psychiatric disorder (Spitzer, Williams, Gibbon & First, 1990). The interview focused on current and lifetime prevalence of mood disorders (major depression, dysthymia, bipolar disorder), substance abuse (alcohol, sedative-hypnotics, stimulant, opioids, hallucinogens, polydrug use), anxiety disorders (panic disorder with or without agoraphobia, agoraphobia without a history of panic attacks, social phobia, simple phobia, obsessive-compulsive disorder,

generalized anxiety disorder), somatoform disorders (somatization disorder, somatoform pain disorder, undifferentiated somatoform disorder, hypochondriasis), eating disorders (anorexia nervosa, bulimia nervosa) and adjustment disorder.

3. Panic Symptom Scale (PSS). The behavioral response of subjects to CCK-4 challenge was measured with an 18-item DSM-III-R derived panic inventory that has been administered in previous CCK-4 challenge studies (see Appendix 2). Subjects were directed to rate the severity of each item as absent (0), mild (1), moderate (2), severe (3) or very severe (4). Two separate scores were obtained from this panic inventory, (a) a score reflecting the total number of symptoms reported (i.e., number of symptoms with scores > 1) and (b) a sum intensity score (i.e., the sum of the intensity ratings). The occurrence of a panic attack was based on (1) DSM-III-R criteria of a panic attack (see Appendix 3) and (2) a score of 2 or more on the fear/anxiety/apprehension rating of the panic inventory. The latency to effect panic symptoms with CCK-4 and the duration of symptoms were recorded on the panic inventory. In addition to the 18 items appearing on the PSS, five items describing sensations rarely associated with clinical or experimentally-induced panic attacks were included to evaluate response bias towards item endorsement. These items were earache, itchy nose, stuffy nose, low back pain and itchy feet. The response items were rated on a 0 to 4 scale with response items being tallied and analyzed separately.

4. Visual Analog Scale of Mood and Symptom Variables (VAS). Thirteen

symptom and mood variables were measured (happy, sad, drowsy, anxious, nervous, energetic, calm, fearful, high, mellow, talkative, depressed and angry) before administration of the study diets and prior to and following CCK-4 challenge (see Appendix 4). Individual subjects rated mood state on a 100 mm visual analog scale from "not at all" (lefthand end of line) to "most ever" (righthand end of line). The subjects made a mark perpendicular to the line corresponding to mood state at that time.

Cardiovascular Measures

Blood pressure and heart rate were monitored for each subject with an automatic vital signs monitor (Dinamap®, Critikon, Canada, Model No. 1846 SX) that was attached to the subject's left arm. This apparatus provided continuous readings of vital signs at approximately 20 second intervals.

Tryptophan and Hormonal Measures

Plasma tryptophan was measured as an index of the extent of tryptophan depletion. Blood samples were collected in polypropylene tubes containing EDTA as an anticoagulant. Plasma was separated by centrifugation and stored in labelled vials at -20°C. Total plasma tryptophan was assayed by high-performance liquid chromatography (HPLC) with fluorometric detection in the laboratory of Dr. Simon Young (McGill University). The technical staff involved in the HPLC analysis was blind to the dietary treatment administered.

Hormones were measured by radioimmunoassay (RIA) and assayed blind to dietary treatment by the Endocrinology laboratory at St. Mary's Hospital.

Blood samples for ACTH determination were collected in chilled polypropylene tubes employing EDTA as an anticoagulant. The blood samples were immediately centrifuged and stored in labelled vials at -70°C. Plasma ACTH levels were measured employing a Diagnostic Systems Laboratories Kit. The limit of detection for ACTH was 6 pg/ml and the inter- and intra-assay coefficient of variation were 10% and 3.8%, respectively. Blood samples for serum cortisol, prolactin and growth hormone were collected, centrifuged, and stored in labelled vials at -20°C. Serum cortisol concentrations were determined using a kit from Kallestad Diagnostics (Quanticoat™); inter- and intra-assay variability were 4.7 and 3.7%, respectively. The lowest detectable dose that could be distinguished from the zero calibration standard was 0.5 µg/dL. Serum prolactin levels were measured using a kit supplied by Serono Diagnostics (MAIACLONE™); inter-assay variability was 7.3% and intra-assay variability 4.9%. The lower limit of detection of prolactin in serum was 0.3 ng/ml. Serum human growth hormone concentrations were measured using a kit from Diagnostic Products Corporation; inter- and intra-assay variability were 11.7% and 1.8%, respectively. The growth hormone assay has a detection limit of approximately 0.9 ng/mL. Plasma ACTH and serum cortisol, prolactin and growth hormone concentrations below the limit of quantitation were assigned a value of zero for mean calculations.

Cholecystokinin Challenge

CCK-4 was purchased from Peninsula (California, U.S.A.) and prepared

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PM-1 3½"x4" PHOTOGRAPHIC MICROCOPY TARGET
NBS 1010a ANSI/ISO #2 EQUIVALENT

PRECISIONSM RESOLUTION TARGETS

by GIS Medicament (Nantes, France). Ampoules containing 50 μ g/2.5 ml were prepared and contained sufficient solution to recover the injection volume (2.5 ml). Ampoules were refrigerated until use. The 50 μ g challenge dose of CCK-4 was used because previous studies have demonstrated that this dose of CCK-4 reliably elicits a panic attack in 50% of healthy subjects tested (Bradwejn et al, 1991; Bradwejn et al, 1994b; 1994c). Thus, this challenge dose would permit elicitation of measurable symptoms while permitting symptom exacerbation by subjects consuming the tryptophan-free amino acid diet.

Amino Acid Dietary Composition

The amino acid diet was composed of 15 amino acids according to the following formula: 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, 8.0 g; L-leucine, 13.5 g, L-lysine monohydrochloride, 11.0 g; L-methionine, 3.0 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. The control diet included the 15 amino acids with 2.3 g of L-tryptophan whereas the tryptophan-free diet excluded L-tryptophan. The amino acid mixture was prepared in the proportions available in human milk, which approximates the optimum amino acid composition for humans. Both glutamate and aspartate were excluded owing to toxicity concerns. This omission would not have affected the ability of the tryptophan-free mixture to lower central tryptophan availability because glutamate and aspartate are not considered to be essential amino acids. For the tryptophan-free amino acid mixture, 100 g

was ingested. This intake approximates the amino acid availability in a 500 g steak. Pre-prepared mixtures were stored in containers labelled in a double-blind fashion. The ingestion of the amino-acid mixture was made more palatable by blending it with 150 ml of water, 2 packets of low calorie sweetener and 100 ml of chocolate syrup. The chocolate syrup contained artificial chocolate flavour and did contain caffeine, phenylethylamine, or tyramine typically available in chocolate. Owing to the unpleasant taste of methionine, cysteine and arginine, however, these amino acids were encapsulated and administered immediately after the subject consumed the remaining amino acids in drink form.

Procedure

Screen

Subjects were recruited via an advertisement in local newspapers (see Appendix 5). Potential subjects were briefly screened over the telephone with respect to medical status, personal and family psychiatric history, smoking habits, alcohol and drug use, and previous participation in studies. They were informed of the nature of the study and the experimental treatments involved. In addition, subjects were informed that a urine sample would be collected to analyze for drug use. After returning the screening questionnaire (SCL-90), subjects who scored below the established cutoff were assessed in a one-hour structured interview based on the NP-SCID. Family history of psychiatric disorders was obtained from direct interview with the subject. Following the structured interview, subjects were interviewed by a psychiatrist who described

the experimental procedure, the effects of CCK-4 challenge, and the effects of the tryptophan-free amino acid diet. Upon successful completion of screening and acquisition of an informed consent form, eligible subjects underwent a physical examination including electrocardiogram and routine laboratory tests. At the end of the evaluation, a convenient experimental day was scheduled within the next two weeks, with the provision that positive findings from the urine analysis or abnormal laboratory results would lead to cancellation.

Experimental Intervention

Each subject was required to follow a low protein diet of approximately 2,212 calories the day before the acute tryptophan depletion and CCK-4 challenge (see Appendix 6 for diet composition). Although the low protein diet has not been studied systematically, experience suggest that it results in a somewhat greater tryptophan depletion in subjects receiving the tryptophan deficient amino acid diet (Simon Young, personal communication). The low protein diets were prepared by the investigator and were either delivered to the subject's home or picked up by the subject at St. Mary's Hospital. Although records of the exact amount of the diet consumed were not kept, subjects ate the majority of food and there was very good compliance with the diet as revealed by verbal report.

Subjects, who had fasted since midnight, were required to report to the Psychopharmacology Unit of St. Mary's Hospital at 8:30 am. On arrival, subjects completed the visual analog mood scale. Vital signs and blood samples

for tryptophan, ACTH, cortisol, prolactin and growth hormone determination were also obtained. At the end of prediet testing, subjects were randomly assigned to either the tryptophan-free amino acid diet or the balanced control amino acid diet condition under double-blind conditions. Administration of the amino acid diets generally took between 10-15 minutes. The diets were ingested in the presence of the investigator. The CCK-4 challenge was timed to occur five hours after administration of the amino acid diets. This period was used because previous studies have shown that maximal blood tryptophan depletion begins approximately 5 hours after ingestion of the tryptophan-free amino acid diet (Young et al, 1987). Subjects remained in the Psychopharmacology Unit during the period between administration of the amino acid diets and the start of the CCK-4 challenge. Supplementary eating or smoking was not be permitted during this waiting period, but subjects were given ad libitum access to water.

Thirty minutes prior to the CCK-4 challenge subjects were seated in a reclining chair in the laboratory. An intravenous catheter was inserted into the right antecubital vein through which physiological saline was slowly dripped. Blood was drawn through a three-way stopcock in the intravenous line. A blood pressure cuff with a pulse monitor attached to the Dinamap sphygmomanometer was applied to the left arm. Blood samples were collected 5 minutes prior to administration of CCK-4 for tryptophan determination and for baseline (pre-CCK) ACTH, cortisol, prolactin and growth hormone

concentrations. Baseline mood ratings and heart rate and blood pressure were also obtained.

After baseline (pre-CCK) measurements were obtained, subjects were given an intravenous single bolus injection of CCK-4 (50 μ g). They were asked to describe the symptoms experienced following the injection and to report when the effects of CCK-4 had dissipated. These symptoms, as well as their onset and duration, were recorded. Once the effects of the CCK-4 had abated, subjects were instructed to rate the intensity of each symptom listed on the PSS that occurred immediately following CCK-4 administration. Symptoms not appearing on the PSS but spontaneously reported by subjects were also recorded. The visual analog scale was administered to quantify mood changes that occurred during the period associated with peak panic symptoms. In addition, visual analog ratings of mood were made 5, 10 and 15 minutes after administration of the peptide. Samples for neuroendocrine determination were collected at 2, 5, 10 and 15 minutes after CCK-4 administration. A total of 150 ml of blood was drawn from each subject. Vital signs were obtained at 20 second intervals following the injection for a two minutes period and at 5, 10 and 15 minutes after CCK-4 administration.

Concurrent treatment with any medication including over-the counter products was prohibited throughout the study. Consumption of alcohol or xanthine-containing beverages and foods was not permitted for 12 hours prior to the study and during sample collection. Subjects were also required to refrain

from smoking on the day of the study. After completion of the final blood sampling, subjects were given a balanced meal. The amino acid diet codes were broken by a research nurse, and those who received the tryptophan-free amino acid diet were given 2 g of L-tryptophan in capsules. Subjects were allowed to leave after a minimum of one hour observation and were instructed to contact the Psychopharmacology Unit if they experienced any adverse events.

The study did not include a placebo injection, since negative results from normal subjects injected with placebo have been reported in several previous investigations on the panicogenic effects of CCK-4 (Bradwejn et al, 1991; Koszycki et al, 1991; Bradwejn et al, 1994b). Nevertheless, in order to exclude the possibility that the influence of acute tryptophan depletion on CCK-4-induced anxiety might be due to a placebo effect, a pilot study employing placebo injections was conducted in ten subjects. The results of the pilot study are given in Appendix 7. Briefly, placebo injection resulted in a very low incidence of symptoms and none of the subjects experienced a panic attack. Further, there was no difference between the tryptophan-free and control amino acid diet groups in behavioral response to placebo. Based on these results, it was reasonable to assume that the effects of acute tryptophan depletion on behavioral response to CCK-4 would not be due to a placebo-like effect.

Ethical Considerations

The prospectus was reviewed by the ethical review board of St. Mary's Hospital Centre, Montreal, Quebec, the Department of Psychology, Carleton

University, Ottawa, Ontario, and the Health Protection Branch of Health and Welfare Canada (HPB), Ottawa, Ontario. Written informed consent (see Appendix 8) was obtained from each subject after full explanation of the purpose of the study, risks and discomforts involved. Subjects were under close medical supervision. CCK-4 was administered by Dr. Bradwejn or a medical colleague. Subjects were paid \$140.00 for their participation in the study.

RESULTS

Subject Characteristics

Fifty-five subjects were screened for the study with the structured clinical interview (SCID), of whom 13 were excluded because of a positive psychiatric history ($n = 3$), positive family history of psychiatric disorders ($n = 3$), positive drug screen ($n = 3$), abnormal laboratory results ($n = 1$), abnormal medical examination ($n = 1$), and brief syncope episode during blood taking for routine laboratory tests ($n = 2$). Two subjects who were randomized to the control diet group withdrew from the experiment; one subject vomited shortly after ingesting the amino acid mixture and the second subject experienced a drop in blood pressure after the intravenous line was installed which prohibited further participation.

Forty subjects completed the study. The mean age ($\pm$ SEM) of the 20 subjects who were randomized to the control amino acid diet was 25.4 ± 1.0 years (range: 18 to 35 years) and the mean age of the 20 subjects who were randomized to the tryptophan-free amino acid diet was 23.8 ± 0.8 years (range: 18 to 31 years). The difference in age between the two amino acid diet groups was not statistically significant ($t(38) = 1.24$, $p = 0.222$). To examine the adequacy of the randomization scheme multivariate analyses of variance (MANOVA) were carried out separately on prediet hormonal (ACTH, cortisol, growth hormone and prolactin), cardiovascular (heart rate, systolic blood pressure and diastolic blood pressure), and visual analog scale mood variables.

These analyses failed to reveal significant differences between the amino acid diets for prediet hormonal ($F(4,35)=0.69$, $p=0.605$), cardiovascular ($F(3,36)=0.45$, $p=0.719$) and mood ($F(13,26)=1.61$, $p=0.144$) variables. Further, unpaired t-test comparing prediet total plasma tryptophan levels failed to detect significant differences between the amino acid diets ($t(38)=1.25$, $p=0.20$).

Changes in Plasma Tryptophan Levels

In order to validate the biochemical effects of the amino acid diets, paired t tests were computed to compare prediet values with values obtained five hours after ingestion of the diets. The tryptophan-free amino acid diet dramatically decreased total plasma tryptophan concentrations. Total plasma tryptophan levels decreased from a prediet value of 11.0 ± 0.3 ng/ml to 1.4 ± 0.4 ng/ml within five hours after administration of the tryptophan-free diet ($t(19)=31.00$, $p<0.001$). This represents a 91.6% decrease from prediet values. Subjects who ingested the control diet displayed an increase in total plasma tryptophan concentrations, as would be expected with normal food intake. Total plasma tryptophan level was 10.3 ± 0.4 ng/ml before the diet and increased to 18.0 ± 1.0 ng/ml five hours after the control diet, an increase of 76.8% from prediet values ($t(19)=8.91$, $p<0.001$). As expected, plasma tryptophan was markedly lower five hours after with the tryptophan-free versus the control amino acid diet ($t(38)=17.8$, $p<0.001$).

Baseline Findings

To test whether the dietary manipulation differentially affected baseline measures obtained prior to administration of CCK-4, analyses of variance (ANOVA) were performed on baseline scores using prediet scores as covariate. For cardiovascular measures, the average of the two baseline measures (obtained at time -5 and 0) were used in the analyses. The mean($\pm$ SEM) prediet and baseline values for physiological measures and visual analog scale mood measures are presented in Tables 1 and 2, respectively.

Insert Tables 1 and 2 about here

The analysis failed to detect significant differences between the amino diet groups for baseline ACTH ($F(1,39)=0.46$, $p=0.503$), cortisol ($F(1,39)=0.33$, $p=0.571$), prolactin ($F(1,39)=0.03=0.852$) and growth hormone ($F(1,39)=1.13$, $p=0.294$) concentrations. Moreover, no significant effect of diet occurred for baseline heart rate ($F(1,39)=0.18$, $p=0.673$), systolic blood pressure ($F(1,39)=1.38$, $p=0.247$) and diastolic blood pressure ($F(1,39)=0.10$, $p=0.757$). For mood variables, baseline ratings of depressed and fearful mood were significantly higher in the control than the tryptophan-free amino acid diet group ($F(1,39)=4.49$ and 4.22 , p 's <0.05), although the magnitude of the difference was small and cannot be considered clinically meaningful. No significant differences between the amino acid diets emerged

TABLE 1

Effect of the Amino Acid Diets on Baseline Physiological Measures

Measure	Diet	Prediet	Baseline
ACTH (pmol/L)	Control	14.8 ± 1.6	13.1 ± 1.8
	TRP-	12.8 ± 1.1	10.5 ± 1.0
Cortisol (nmol/L)	Control	470.6 ± 29.5	330.0 ± 23.5
	TRP-	468.9 ± 28.8	310.1 ± 25.0
Growth Hormone (µg/L)	Control	0.1 ± 0.1	3.1 ± 1.2
	TRP-	0.3 ± 0.2	1.4 ± 0.5
Prolactin (µg/L)	Control	12.0 ± 0.9	8.7 ± 0.8
	TRP-	12.7 ± 1.0	9.1 ± 1.0
Heart rate (bpm)	Control	77.1 ± 1.8	76.2 ± 2.9
	TRP-	73.9 ± 2.6	72.7 ± 2.2
Systolic Blood Pressure (mm Hg)	Control	118.5 ± 2.4	116.0 ± 2.9
	TRP-	119.8 ± 3.3	121.3 ± 3.3
Diastolic Blood Pressure (mm Hg)	Control	79.8 ± 2.7	64.1 ± 2.0
	TRP-	81.2 ± 2.3	65.1 ± 1.8

TRP- indicates tryptophan-free amino acid diet. Values are the mean (± SEM). Baseline hormonal levels were measured 5 minutes prior to CCK-4 administration. Baseline heart rate and blood pressure measures represent the average of two baseline measures (Time -5 and 0).

TABLE 2

Effect of the Amino Acid Diets on Baseline Mood Ratings

Variable	Diet	Prediet	Baseline
Nervous	Control	21.5 ± 4.0	34.3 ± 4.9
	TRP-	13.9 ± 3.6	20.5 ± 4.9
Anxious	Control	10.6 ± 2.5	23.5 ± 4.5
	TRP-	13.4 ± 4.6	19.4 ± 5.5
Fearful	Control	5.6 ± 1.8	14.5 ± 3.6*
	TRP-	1.0 ± 0.9	5.3 ± 2.4
Calm	Control	65.1 ± 5.0	53.0 ± 4.3
	TRP-	55.4 ± 5.3	48.1 ± 5.1
Mellow	Control	52.6 ± 5.1	48.0 ± 4.2
	TRP-	42.9 ± 5.9	38.8 ± 5.4
Drowsy	Control	25.9 ± 6.0	26.2 ± 5.2
	TRP-	22.3 ± 5.5	16.0 ± 4.8
Depressed	Control	3.4 ± 1.8	4.5 ± 1.4*
	TRP-	1.5 ± 1.0	0.8 ± 0.6
Sad	Control	4.6 ± 1.8	5.7 ± 2.7
	TRP-	1.4 ± 0.8	1.0 ± 0.5
Happy	Control	52.7 ± 2.6	41.8 ± 3.9
	TRP-	50.5 ± 2.5	45.2 ± 2.9
Talkative	Control	43.9 ± 4.6	38.7 ± 3.7
	TRP-	38.9 ± 3.6	36.7 ± 4.1
Energetic	Control	38.2 ± 4.5	31.6 ± 3.2
	TRP-	31.3 ± 3.6	27.5 ± 3.8
High	Control	8.8 ± 3.3	10.7 ± 3.6
	TRP-	9.3 ± 3.5	4.4 ± 2.1
Angry	Control	2.9 ± 1.6	1.5 ± 0.8
	TRP-	0.1 ± 0.1	0.5 ± 0.4

TRP- indicates tryptophan-free amino acid diet. Values represent the mean(±SEM). Baseline ratings were obtained 5 minutes prior to CCK-4 injection. * $p < 0.05$, control vs TRP- diet.

for baseline ratings of nervous ($F(1,39)=2.16$, $p=0.15$), anxious ($F(1,39)=1.81$, $p=0.186$), calm ($F(1,39)=0.08$, $p=0.782$), mellow ($F(1,39)=1.79$, $p=0.189$), talkative ($F(1,39)=0.005$, $p=0.944$), happy ($F(1,39)=0.533$, $p=0.47$), energetic ($F(1,39)=0.30$, $p=0.586$), drowsy ($F(1,39)=1.83$, $p=0.184$), sad ($F(1,39)=1.62$, $p=0.21$) and high ($F(1,39)=2.85$, $p=0.10$) mood.

CCK-4 CHALLENGE

Effect of Acute Tryptophan Depletion on Behavioral Responses to CCK-4 Injection

Occurrence of Panic Attacks. Eleven of 20 subjects (55%) who received the tryptophan-free amino acid diet had a panic attack after administration of CCK-4 compared with 13 of 20 subjects (65%) who received the control amino acid diet. The difference in panic rate was not statistically significant ($\chi^2 = 0.10$, $p=0.75$). Because all subjects had a rapid onset of symptoms and most reported at least 4 panic symptoms after administration of CCK-4, the principal determinant of a panic attack was an anxiety/fear/apprehension score of ≥ 2 on the Panic Symptom Scale.

There was little evidence that the propensity to panic following CCK-4 administration was attributed to higher levels of baseline anxiety. A Panic Status x Diet ANOVA, with the prediet value serving as the covariate, failed to yield a significant Panic effect or Diet x Panic interaction for visual analog ratings of anxious ($F(1,39)=0.04$, $p=0.851$ and $F(1,39)=0.99$, $p=0.327$),

fearful ($F(1,39) = 0.853$, $p = 0.362$ and $F(1,39) = 0.04$, $p = 0.834$) and nervous ($F(1,39) = 0.02$, $p = 0.892$ and $F(1,39) = 0.77$, $p = 0.386$) mood. For baseline physiological measures, no significant Panic or Diet x Panic effects occurred for ACTH ($F(1,39) = 0.07$, $p = 0.786$ and $F(1,39) = 0.37$, $p = 0.546$), cortisol ($F(1,39) = 0.12$, $p = 0.728$ and $F(1,39) = 0.07$, $p = 0.793$), prolactin ($F(1,39) = 0.69$, $p = 0.411$ and $F(1,39) = 0.27$, $p = 0.608$), growth hormone ($F(1,39) = 0.18$, $p = 0.683$ and $F(1,39) = 0.36$, $p = 0.55$), heart rate ($F(1,39) = 0.66$, $p = 0.423$ and $F(1,39) = 0.29$, $p = 0.590$) and systolic blood pressure ($F(1,39) = 2.49$, $p = 0.123$ and $F(1,39) = 0.72$, $p = 0.402$). The Diet x Panic interaction was significant for diastolic blood pressure ($F(1,39) = 4.68$, $p < 0.05$), although the Panic main effect was not significant ($F(1,39) = 0.59$, $p = 0.446$). The Panic x Diet interaction is accounted for by the fact that, within the control amino acid diet group, baseline diastolic blood pressure was lower in panickers than in nonpanickers (mean($\pm$ SEM): 61.5 ± 2.4 versus 69.0 ± 3.1 ; $F(1,19) = 6.56$, $p < 0.05$). There was also a tendency for diastolic blood pressure to be lower for panickers in the control amino acid diet than for panickers in the tryptophan-free amino acid diet (61.5 ± 2.4 versus 66.4 ± 2.0 , $F(1,19) = 3.65$, $p < 0.10$). Prediet and baseline measures for panickers and nonpanickers are presented in Table 3.

Insert Table 3 about here

TABLE 3

Baseline Mood and Physiological Measures for Panickers and Nonpanickers

Measure	Panickers	Nonpanickers
VAS Mood Ratings		
Anxious	21.7 ± 4.8	21.2 ± 5.4
Nervous	28.5 ± 4.8	25.7 ± 5.4
Fearful	11.8 ± 3.3	7.1 ± 2.7
Cardiovascular Measures		
Heart Rate (bpm)	75.4 ± 2.6	72.9 ± 2.6
Systolic BP (mm Hg)	115.5 ± 2.6	123.4 ± 3.7
Diastolic BP (mm Hg)	63.7 ± 1.6	66.0 ± 2.4
Hormonal Measures		
ACTH (pmol/L)	12.1 ± 1.5	11.4 ± 1.3
Cortisol (nmol/L)	326.0 ± 24.1	311.1 ± 23.1
Prolactin (µg/L)	8.7 ± 0.9	9.4 ± 0.8
Growth Hormone (µg/L)	2.4 ± 1.0	2.0 ± 0.8

Values are the mean ± SEM. Mood and hormonal variables were measured 5 minutes prior to CCK-4 administration. Heart rate and blood pressure measures are the average of two baseline measures (Time -5 and 0).

Panic Symptom Scale Scores. The data comparing the tryptophan-free and control amino acid diet groups on the number, sum intensity, time to onset, and duration of symptoms induced with CCK-4 are summarized in Table 4. All subjects reported at least one symptom after CCK-4 injection that ranged in intensity from mild to extremely severe. The time to onset of symptoms was rapid and the duration of symptoms was relatively short.

Insert Table 4 about here

A 2 (tryptophan-free versus control diet) x 2 (panic versus no panic) MANOVA was performed on the combined dependent variables. As shown in Table 4, the effect of CCK-4 itself, independent of the occurrence of a panic attack, was not affected by the dietary manipulation (Diet effect: $F(4,33) = 0.57$, $p = 0.687$). The MANOVA also failed to yield a significant Diet x Panic interaction ($F(4,33) = 1.07$, $p = 0.386$), although the main effect for Panic was statistically significant ($F(4,33) = 4.41$, $p < 0.01$). To investigate the Panic main effect more closely, univariate ANOVAs were computed for each dependent variable. These analyses revealed significant differences between panickers ($n = 24$) and nonpanickers ($n = 16$) (tryptophan-free and control diet groups combined) with respect to the mean ($\pm$ SEM) number of reported symptoms (12.7 ± 0.6 versus 8.9 ± 0.7 ; $F(1,36) = 13.63$, $p < 0.001$), sum intensity of symptoms (31.4 ± 2.1 versus 18.6 ± 2.2 ; $F(1,36) = 16.82$,

TABLE 4**Effect of the Amino Acid Diets on Behavioral Responses to CCK-4 Injection**

Variable	Control Diet	Tryptophan-free Diet
Number of reported symptoms	11.7 ± 0.9	10.7 ± 0.7
Sum intensity of symptoms	26.7 ± 2.9	25.8 ± 2.2
Time to onset of symptoms (seconds)	18.2 ± 1.0	19.1 ± 1.5
Duration of symptoms (seconds)	131.0 ± 12.9	132.9 ± 8.7

Values represent the mean($\pm$ SEM).

$p < 0.001$), and duration of symptoms (146.5 ± 7.9 versus 110.1 ± 13.7 seconds; $F(1,36) = 5.82$, $p < 0.05$). There was no evidence of a more rapid onset of action for CCK-4 in panickers versus nonpanickers (17.4 ± 0.7 versus 20.5 ± 2.0 seconds; $F(1,36) = 2.64$, $p = 0.113$).

Table 5 provides the mean ($\pm$ SEM) intensity rating for each DSM-III-R panic symptom induced with CCK-4 for the two dietary conditions. The

Insert Table 5 about here

MANOVA failed to reveal a significant effect for Diet ($F(18,19) = 0.68$, $p = 0.795$) for the 18 panic symptoms listed on the Panic Symptom Scale. The Diet x Panic interaction also failed to reach statistical significance ($F(18,19) = 0.71$, $p = 0.762$), but the Panic main effect was significant ($F(18,19) = 5.48$, $p < 0.001$). Subsequent univariate ANOVAs revealed that the mean($\pm$ SEM) ratings were higher in panickers than nonpanickers for anxiety/fear/apprehension (2.5 ± 0.1 versus 0.6 ± 0.1 ; $F(1,36) = 88.59$, $p < 0.001$), fear of losing control (1.0 ± 0.2 versus 0.06 ± 0.06 ; $F(1,36) = 15.74$, $p < 0.001$), dyspnea (2.9 ± 0.2 versus 2.1 ± 0.3 ; $F(1,36) = 6.19$, $p < 0.05$), unsteadiness (1.6 ± 0.3 versus 0.7 ± 0.02 ; $F(1,36) = 4.92$, $p < 0.05$), faintness (1.7 ± 0.3 versus 0.7 ± 0.2 ; $F(1,36) = 5.66$, $p < 0.05$), palpitations/rapid heart (3.0 ± 0.2 versus 1.5 ± 0.3 ; $F(1,36) = 18.76$, $p < 0.001$), trembling/shaking (1.0 ± 0.2 versus 0.4 ± 0.2 ; $F(1,36) = 4.34$, $p < 0.05$), nausea (2.6 ± 0.3 versus

TABLE 5
Effect of the Amino Acid Diets on Intensity Ratings of DSM-III-R Panic Symptoms Induced by CCK-4 Injection

Symptoms	Control Diet	Tryptophan-Free Diet
Dyspnea	2.4 ± 0.2	2.7 ± 0.3
Dizziness	1.2 ± 0.3	1.2 ± 0.3
Unsteadiness	1.5 ± 0.3	0.9 ± 0.2
Faintness	1.4 ± 0.3	1.1 ± 0.3
Palpitations/rapid heart	2.4 ± 0.2	2.4 ± 0.3
Trembling/shaking	0.9 ± 0.2	0.5 ± 0.2
Sweating	1.9 ± 0.3	2.5 ± 0.3
Choking	1.2 ± 0.2	1.3 ± 0.3
Nausea	1.9 ± 0.3	2.3 ± 0.4
Abdominal distress	1.8 ± 0.4	1.6 ± 0.3
Feeling detached/unreal	1.3 ± 0.3	0.8 ± 0.8
Paresthesia	1.8 ± 0.3	2.1 ± 0.3
Flushes/chills	2.0 ± 0.3	2.0 ± 0.2
Chest pain/discomfort	1.9 ± 0.3	1.7 ± 0.3
Anxiety/fear/apprehension	1.8 ± 0.2	1.5 ± 0.3
Fear of dying	0.1 ± 0.08	0.1 ± 0.07
Fear of losing control	0.6 ± 0.2	0.6 ± 0.2
Fear of going crazy	0.2 ± 0.1	0 ± 0

Symptoms are rated on a 0 (not present) to 4 (extremely severe) scale.
 Values are presented as the mean ± SEM

1.0 ± 0.4 ; $F(1,36) = 5.85$, $p < 0.05$) and chest pain/discomfort (2.2 ± 0.2 versus 1.3 ± 0.3 ; $F(1,36) = 4.61$, $p = < 0.05$). Ratings of dizziness also tended to be higher in panickers than nonpanickers (1.5 ± 0.3 versus 0.7 ± 0.3 , $F(1,36) = 3.56$, $p < 0.10$).

Symptoms not appearing on the PSS but spontaneously reported by subjects after CCK-4 administration were also recorded. These symptoms included headache ($n = 2$), lightheadedness ($n = 2$), dry mouth ($n = 2$), increased salivation ($n = 1$), lacrimation ($n = 1$), burning sensation ($n = 3$), tense/stiff muscles ($n = 3$), heat in throat ($n = 1$), feeling paralysed ($n = 1$), fatigue ($n = 1$), and heavy limbs ($n = 1$). The mean ($\pm$ SEM) number of spontaneously reported symptoms was 0.6 ± 0.2 for the tryptophan-free amino acid diet group and 0.4 ± 0.1 for the control amino acid diet group. In addition, the mean ($\pm$ SEM) number of spontaneously reported symptoms was 0.5 ± 0.1 for panickers and 0.4 ± 0.2 for nonpanickers. A two-way ANOVA (Diet, Panic status) failed to reveal a significant Diet ($F(1,39) = 0.67$, $p = 0.418$) or Panic ($F(1,39) = 0.12$, $p = 0.727$) effect, or interaction between Diet and Panic ($F(1,39) = 0.03$, $p = 0.873$) for the number of spontaneously reported symptoms.

Response Bias Items. Response bias towards item endorsement was analyzed separately by a two-way ANOVA (Diet, Panic Status). The number of reported response bias items for each dietary condition was minimal. Only four of the 40 subjects reported a response bias item. The items were itchy

feet ($n = 1$), earache ($n = 1$), low back pain ($n = 1$) and stuffy nose ($n = 1$). In all cases, the item was rated as being mild in intensity. The mean ($\pm$ SEM) number of response items was 0.15 ± 0.4 for the control amino acid diet group and 0.05 ± 0.2 for the tryptophan-free amino acid diet group. In addition, the mean ($\pm$ SEM) number of reported response items was 0.2 ± 0.4 and 0.0 ± 0.0 for panickers and nonpanickers, respectively. There were no statistically significant main effects for Diet ($F(1,39) = 0.78$, $p = 0.381$), Panic ($F(1,39) = 2.65$, $p = 0.112$) or the Diet x Panic interaction ($F(1,39) = 0.52$, $p = 0.476$) for the number of reported response bias items.

Visual Analog Scale Mood Ratings. A mixed-design ANOVA which incorporated Diet (tryptophan-free versus control) and Panic (panic versus no panic) as between subject factors, and Time of assessment (baseline, the peak of CCK-4-induced symptoms ("peak") and 5, 10 and 15 minutes postinjection) as the repeated measure was computed to evaluate whether changes in self-rated mood following intravenous injection of CCK-4 were differentially affected by the dietary manipulation. Logarithmic transformations were applied to ratings of nervous, talkative, happy, angry and energetic mood to improve the normality of their distribution. The main interest in this analysis was whether changes in mood states differed significantly in the tryptophan-free and control amino acid diet groups. In the mixed ANOVA this was manifested by a significant Diet x Time or Diet x Panic x Time interaction. The remaining Panic x Time interaction simply

indicated that ratings of VAS mood variables were significantly different in panickers and nonpanickers (tryptophan-depleted and control subjects combined). The source of significant ($p < 0.05$) or near significant ($P < 0.10$) interactions was evaluated by pre-planned contrasts on the time factor (baseline versus score at each time point).

The mean ($\pm$ SEM) change from baseline in the 13 mood variables for the amino acid diet groups is presented in Table 6. The visual analog scale used to quantify mood revealed that CCK-4 produced a wide variety of mood changes, as revealed by significant Time main effects for 12 of the 13 mood variable (see Appendix 9d). The most striking effect was found for items on the visual analog scale that represent anxious mood (i.e., anxious, nervous and fearful). These mood changes were transient and prominent at the peak of CCK-4 symptoms. The peak of CCK-4 symptoms was also accompanied by a marked decline in talkative, happy, calm and mellow mood. Although changes in sad, depressed, angry, energetic and high mood were statistically significant, they were not nearly as profound.

Insert Table 6 about here

Overall, the dietary manipulation had little effect on mood ratings after CCK-4 administration. The Diet x Time interaction was statistically significant for happy mood ($F(4, 144) = 6.57$, $p < 0.01$), which appeared to be the result of

TABLE 6

Effect of the Amino Acid Diets on Mood Ratings Following CCK-4 Injection

Mood State	Diet	Mean ($\pm$ SEM) Change From Baseline			
		Peak	+5 mins	+10 mins	+15 mins
Nervous	Control	28.6 $\pm$ 6.7	-11.6 $\pm$ 5.8	-22.5 $\pm$ 4.7	-25.7 $\pm$ 4.5
		TRP- 27.3 $\pm$ 6.4	-3.2 $\pm$ 3.5	-14.6 $\pm$ 3.9	-15.6 $\pm$ 4.0
Anxious	Control	44.6 $\pm$ 6.8	-1.9 $\pm$ 6.6	-11.4 $\pm$ 6.2	-12.9 $\pm$ 4.6
		TRP- 33.0 $\pm$ 7.0	-1.8 $\pm$ 4.9	-6.5 $\pm$ 4.4	-10.5 $\pm$ 5.1
Fearful	Control	37.2 $\pm$ 4.8	-7.3 $\pm$ 3.0 ^a	-8.5 $\pm$ 3.4	-9.1 $\pm$ 3.0
		TRP- 28.6 $\pm$ 5.7	0.3 $\pm$ 2.4	-3.5 $\pm$ 2.3	-4.6 $\pm$ 2.4
Calm	Control	-38.6 $\pm$ 6.3	-5.1 $\pm$ 5.5	2.0 $\pm$ 4.7	-0.2 $\pm$ 3.9
		TRP- -32.1 $\pm$ 8.0	-12.3 $\pm$ 4.6	-7.2 $\pm$ 5.3	7.4 $\pm$ 4.1
Mellow	Control	-38.2 $\pm$ 5.4	-7.0 $\pm$ 5.2	-1.6 $\pm$ 5.3	1.8 $\pm$ 5.7
		TRP- -29.5 $\pm$ 5.4	-2.2 $\pm$ 5.2	-1.6 $\pm$ 4.6	6.3 $\pm$ 4.0
Sad	Control	2.6 $\pm$ 3.6	-1.4 $\pm$ 2.2	1.4 $\pm$ 3.1	-1.7 $\pm$ 2.7
		TRP- 7.0 $\pm$ 3.9	0.8 $\pm$ 0.6	0.3 $\pm$ 0.6	0.0 $\pm$ 0.3
Depressed	Control	4.2 $\pm$ 3.1	-1.0 $\pm$ 1.6	1.9 $\pm$ 2.1	-0.4 $\pm$ 1.5
		TRP- 3.7 $\pm$ 2.2	1.4 $\pm$ 1.1	0.4 $\pm$ 0.3	-0.1 $\pm$ 0.3
Happy	Control	-29.6 $\pm$ 3.8 ^d	-9.4 $\pm$ 4.8 ^b	-0.6 $\pm$ 2.5 ^a	1.9 $\pm$ 1.8 ^a
		TRP- -39.7 $\pm$ 3.7	-1.4 $\pm$ 2.2	1.4 $\pm$ 3.1	-1.7 $\pm$ 2.7
Energetic	Control	-8.3 $\pm$ 5.6	-3.4 $\pm$ 5.3	2.7 $\pm$ 3.5	4.8 $\pm$ 3.1
		TRP- -4.1 $\pm$ 5.2	-4.3 $\pm$ 3.3	-1.7 $\pm$ 4.4	0.3 $\pm$ 3.9
Drowsy	Control	-12.3 $\pm$ 7.4	-1.9 $\pm$ 7.3	-10.3 $\pm$ 6.0	-11.9 $\pm$ 5.1
		TRP- 3.0 $\pm$ 9.5	-2.9 $\pm$ 6.7	-4.1 $\pm$ 4.7	-10.0 $\pm$ 3.8
Talkative	Control	-21.3 $\pm$ 4.7 ^b	-0.05 $\pm$ 2.7	12.0 $\pm$ 4.1	12.3 $\pm$ 4.7
		TRP- -25.8 $\pm$ 4.6	-8.2 $\pm$ 3.7	4.3 $\pm$ 2.5	6.3 $\pm$ 2.4
Angry	Control	4.7 $\pm$ 2.5	1.1 $\pm$ 1.2	-0.2 $\pm$ 0.4	-0.1 $\pm$ 0.4
		TRP- 1.9 $\pm$ 1.6	0.2 $\pm$ 0.3	0.2 $\pm$ 0.3	0.0 $\pm$ 0.1
High	Control	16.8 $\pm$ 6.5	3.1 $\pm$ 3.8	6.8 $\pm$ 4.3	4.5 $\pm$ 4.5
		TRP- 18.5 $\pm$ 6.0	13.2 $\pm$ 6.1	7.7 $\pm$ 4.2	2.5 $\pm$ 2.3

TRP- indicates tryptophan-free amino acid diet.

a: $p < 0.10$, control vs TRP- diet

b: $p < 0.05$, control vs TRP- diet

c: $p < 0.01$, control vs TRP- diet

d: $p < 0.001$, control vs TRP- diet

a greater decline from baseline in happy mood for the tryptophan-free amino acid diet group at the period associated with peak CCK-4 symptoms (Diet x Time: $F(1,36) = 16.75$, $p < 0.001$) and 5 minutes postinjection (Diet x Time: $F(1,36) = 6.24$, $p < 0.05$). Further, a trend towards a greater increase from baseline in happy mood was observed for the control amino acid diet group 10 and 15 minutes postinjection (Diet x Time: $F(1,36) = 3.23$ and 3.64 , p 's < 0.10). ANOVA on the fearful, calm and talkative ratings showed a marginally significant interaction between Diet and Time ($F(4,144) = 2.00$, 2.05 and 3.79 , p 's < 0.10). Planned contrasts on the time factor revealed that the tryptophan-free amino acid diet group experienced a significantly greater decrease from baseline in talkative mood at the period of peak CCK-4 symptoms (Diet x Time: $F(1,36) = 5.16$, $p < 0.05$). The control amino acid diet group tended to exhibit a greater decline from baseline in fearful mood 5 minutes postinjection (Diet x Time: $F(1,36) = 3.99$, $p < 0.10$). With respect to calm mood, none of the planned comparisons approached statistical significance.

The repeated measures ANOVA revealed significant Panic x Time interactions for several mood ratings, including fearful ($F(4,144) = 9.83$, $p < 0.01$), anxious ($F(4,144) = 2.74$, $p < 0.05$), happy ($F(4,144) = 4.25$, $p < 0.01$), calm ($F(4,144) = 4.89$, $p < 0.001$), mellow ($F(4,144) = 2.57$, $p < 0.05$) and angry ($F(4,144) = 3.15$, $p < 0.05$) mood. As shown in Table , panickers exhibited a greater increase from baseline in fearful (Panic x Time: $F(1,36) = 17.69$, $p < 0.001$) and anxious (Panic x Time: $F(1,36) = 6.49$,

$p < 0.05$) mood, and greater decrease from baseline in happy (Panic x Time: $F(1,36) = 10.87$, $p < 0.01$) and calm (Panic x Time: $F(1,36) = 10.0$, $p < 0.01$) mood at the period associated with peak CCK-4 symptoms. Further, compared to nonpanickers, panickers tended to exhibit a greater decline from baseline in mellow mood ($F(1,36) = 3.31$, $p < 0.10$) and a greater increase from baseline in angry mood ($F(1,36) = 3.85$, $p < 0.10$) at the period of peak CCK-4 symptoms. Planned comparisons also revealed that panickers tended to be more anxious than nonpanickers 5 minutes postinjection ($F(1,36) = 3.42$, $p < 0.10$).

Insert Table 7 about here

Finally, a significant Diet x Panic x Time interaction emerged for nervous mood ($F(4,144) = 3.28$, $p < 0.05$), which appeared to be the result of a significant Diet x Panic x Time interaction at the period associated with peak CCK-4 symptoms ($F(1,36) = 7.87$, $p < 0.01$). A more detailed analysis of this interaction revealed that within the tryptophan-free amino acid diet group, panickers exhibited a greater increase from baseline in nervous mood than did nonpanickers (mean ($\pm$ SEM) change: 37.5 ± 7.3 versus 14.9 ± 10.1 ; $F(1,18) = 7.18$, $p < 0.05$). No significant differences between panickers and nonpanickers were evident in the control amino acid diet group.

TABLE 7

Effect of CCK-4 on Mood Ratings: Comparison Between Panickers and Nonpanickers

Mood State	Panic Status	Mean ($\pm$ SEM) Change From Baseline			
		Peak	+ 5 mins	+ 10 mins	+ 15 mins
Nervous	Panic	33.3 $\pm$ 5.9 ¹	-6.6 $\pm$ 4.9	-18.8 $\pm$ 4.2	-21.7 $\pm$ 4.3
	No Panic	20.0 $\pm$ 7.1	-8.7 $\pm$ 4.4	-18.1 $\pm$ 4.6	-19.1 $\pm$ 4.4
Anxious	Panic	48.7 $\pm$ 5.9 ^b	4.2 $\pm$ 5.7 ^a	-6.7 $\pm$ 5.8	-9.5 $\pm$ 5.2
	No Panic	23.9 $\pm$ 7.2	-11.0 $\pm$ 4.7	-12.4 $\pm$ 3.5	-14.9 $\pm$ 3.4
Fearful	Panic	44.0 $\pm$ 4.2 ^d	-3.1 $\pm$ 3.1	-6.2 $\pm$ 2.9	-7.2 $\pm$ 2.7
	No Panic	16.4 $\pm$ 4.3	-4.1 $\pm$ 1.9	-5.6 $\pm$ 2.9	-6.4 $\pm$ 2.8
Calm	Panic	-47.5 $\pm$ 4.9 ^a	-12.1 $\pm$ 4.5	-4.4 $\pm$ 4.8	3.0 $\pm$ 3.4
	No Panic	-17.2 $\pm$ 8.6	-3.6 $\pm$ 5.8	0.1 $\pm$ 5.3	4.4 $\pm$ 5.2
Mellow	Panic	-39.8 $\pm$ 5.0 ^a	-8.7 $\pm$ 4.9	0.9 $\pm$ 4.5	2.5 $\pm$ 4.4
	No Panic	-25.0 $\pm$ 5.4	1.4 $\pm$ 5.3	-5.4 $\pm$ 5.5	6.3 $\pm$ 5.7
Sad	Panic	4.9 $\pm$ 4.1	-1.2 $\pm$ 1.8	-0.7 $\pm$ 2.2	-1.5 $\pm$ 2.2
	No Panic	4.6 $\pm$ 2.5	1.1 $\pm$ 0.7	3.1 $\pm$ 2.1	0.1 $\pm$ 0.3
Depressed	Panic	5.4 $\pm$ 2.9	-0.2 $\pm$ 1.1	1.6 $\pm$ 1.6	0.1 $\pm$ 1.1
	No Panic	1.9 $\pm$ 1.9	0.8 $\pm$ 1.8	0.6 $\pm$ 1.2	-0.9 $\pm$ 0.9
Happy	Panic	-40.6 $\pm$ 2.4 ^c	-8.6 $\pm$ 3.8	3.1 $\pm$ 2.9	4.3 $\pm$ 2.4
	No Panic	-25.8 $\pm$ 5.1	-4.4 $\pm$ 4.9	2.5 $\pm$ 4.0	6.7 $\pm$ 4.0
Energetic	Panic	-7.0 $\pm$ 5.3	-4.9 $\pm$ 3.1	0.5 $\pm$ 3.6	2.5 $\pm$ 3.9
	No Panic	-5.0 $\pm$ 5.4	-2.3 $\pm$ 6.3	0.4 $\pm$ 4.5	2.6 $\pm$ 2.4
Drowsy	Panic	-4.4 $\pm$ 8.8	-1.7 $\pm$ 7.2	-7.7 $\pm$ 5.0	-10.7 $\pm$ 4.2
	No Panic	-5.0 $\pm$ 7.8	-3.5 $\pm$ 6.1	-6.4 $\pm$ 6.0	-11.3 $\pm$ 4.9
Talkative	Panic	-27.4 $\pm$ 3.7	-4.7 $\pm$ 3.5	10.2 $\pm$ 2.9	10.4 $\pm$ 3.0
	No Panic	-17.9 $\pm$ 5.8	-3.2 $\pm$ 2.7	5.1 $\pm$ 4.3	7.6 $\pm$ 4.9
Angry	Panic	5.4 $\pm$ 2.4 ^a	1.0 $\pm$ 1.0	-0.2 $\pm$ 0.4	-0.1 $\pm$ 0.3
	No Panic	0.2 $\pm$ 0.2	0.2 $\pm$ 0.1	0.4 $\pm$ 0.4	0.1 $\pm$ 0.1
High	Panic	24.5 $\pm$ 5.6	13.4 $\pm$ 5.2	12.0 $\pm$ 4.2	8.0 $\pm$ 3.3
	No Panic	7.4 $\pm$ 5.7	0.3 $\pm$ 4.1	0.1 $\pm$ 3.1	-3.1 $\pm$ 3.2

a: $p < 0.10$; b: $p < 0.05$; c: $p < 0.01$; d: $p < 0.001$.

Effect of Acute Tryptophan Depletion on Cardiovascular Responses to CCK-4 Injection

A mixed-design ANOVA was conducted separately on heart rate and systolic and diastolic blood pressure with Diet (tryptophan-free versus control) and Panic status (panic versus no panic) as between subject factors and Time of assessment (0, 20, 40, 60, 80, 100, 120, 300, 600, 900 seconds) as the repeated measures factor. In the repeated measures ANOVA, the main focus was on whether the Diet effect was Time dependent (Diet x Time and Diet x Panic x Time interactions). The source of significant ($p < 0.05$) or near significant ($P < 0.10$) interactions was evaluated by pre-planned contrasts on the time factor (time 0 versus score at each time point).

Heart Rate. Heart rate data were lost due to equipment failure from one subject, so analysis was carried out on 39 subjects. Figure 1 illustrates the effect of the amino acid diets on the mean change from baseline in heart rate after CCK-4 injection.

Insert Figure 1 about here

The mean maximum increment in heart rate from baseline occurred 60 seconds after CCK-4 administration for both diets, with heart rate gradually declining over the remainder of the test period. There was no overall Diet ($F(1,35) = 0.00$, $p = 0.946$) or Panic ($F(1,35) = 0.21$, $p = 0.648$) effect or Diet

FIGURE 1

Effect of the amino acid diets on mean ($\pm$ SEM) change from baseline in heart rate following CCK-4 injection.

x Panic interaction ($F(1,35) = 2.61, p = 0.115$). The overall effect of Time was significant ($F(9,315) = 60.94, p < 0.001$), but the Diet x Time ($F(9,315) = 1.05, p = 0.397$) and Diet x Panic x Time ($F(9,315) = 0.55, p = 0.839$) interactions failed to approach statistical significance, indicating that CCK-4-induced changes in heart rate were not significantly affected by the amino acid diet manipulation. The ANOVA also failed to yield a significant Panic x Time interaction ($F(9,315) = 0.35, p = 0.956$), indicating that panickers and nonpanickers exhibited comparable changes over time in heart rate following CCK-4 injection. The mean change from baseline in heart rate for panickers and nonpanickers is illustrated in Figure 2.

Insert Figure 2 about here

Blood Pressure. Figure 3 illustrates the effect of diet on the mean change from baseline in systolic blood pressure after CCK-4 injection. The mean

Insert Figure 3 about here

maximal increase in systolic blood pressure occurred 60 seconds after administration of CCK-4, with values declining gradually over the remainder of the test period. Tests of between-subject effects revealed that the overall Panic effect was significant ($F(1,36) = 4.48, p < 0.05$). The Panic effect is accounted

FIGURE 2

Mean ($\pm$ SEM) change from baseline in heart rate following CCK-4 injection for panickers and nonpanickers.

FIGURE 3

Effect of the amino acid diets on mean($\pm$ SEM) change from baseline in systolic blood pressure following CCK-4 injection.

for by the fact that the overall (all assessment periods combined) systolic blood pressure scores tended to be higher for nonpanickers relative to panickers.

The overall Diet effect ($F(1,36) = 1.13$, $p = 0.294$) and Diet x Panic interaction ($F(1,36) = 0.05$, $p = 0.822$) were not significant. The overall Time effect was highly significant ($F(9,324) = 6.27$, $p < 0.001$), but there was no significant Diet x Time ($F(9,324) = 0.57$, $p = 0.820$) or Diet x Panic x Time interaction ($F(9,324) = 0.68$, $p = 0.728$), indicating that the dietary manipulation had no effect of CCK-4-induced changes in systolic blood pressure. Further, the Panic x Time interaction was not significant ($F(9,324) = 1.07$, $p = 0.381$), suggesting that panickers and nonpanickers showed comparable changes over time in systolic blood pressure. The mean change from baseline in systolic blood pressure for panickers and nonpanickers is shown in Figure 4.

Insert Figure 4 about here

Figure 5 illustrates the effect of the amino acid diets on the mean change from baseline in diastolic blood pressure after CCK-4 injection. The mean maximal increase in diastolic blood pressure occurred 40 seconds after administration of CCK-4 for both diets, with values gradually decreasing over the remainder of the testing period. The overall Diet ($F(1,36) = 0.32$, $p = 0.576$) and Panic ($F(1,36) = 1.14$, $p = 0.293$) effects and Diet x Panic interaction ($F(1,36) = 1.08$, $p = 0.306$) were not significant. The overall Time effect was

FIGURE 4

Mean($\pm$ SEM) change from baseline in systolic blood pressure following CCK-4 injection for panickers and nonpanickers.

significant ($F(9,324)=6.73$, $p<0.001$), but neither the Diet x Time ($F(9,323)=0.82$, $p=0.602$) nor the Diet x Panic x Time ($F(9,323)=0.92$, $p=0.507$) interaction approached statistical significance. Thus, changes from baseline in diastolic blood pressure were unaffected by the dietary manipulation.

Insert Figure 5 about here

The repeated measures ANOVA produced a significant Panic x Time interaction ($F(9,324)=2.10$, $p<0.05$). The mean change from baseline in diastolic blood pressure in panickers and nonpanickers is illustrated in Figure 6. Subsequent analysis of the Panic x Time interaction indicated that the mean increase from baseline was significantly greater in panickers than nonpanickers 40 seconds after CCK-4 injection (Panic x Time: $F(1,36)=4.99$, $p<0.05$). At the 600 second time point (i.e., 10 minutes postinjection), diastolic blood pressure was significantly lower than baseline values in nonpanickers relative to panickers (Panic x Time: $F(1,36)=4.51$, $p<0.05$).

Insert Figure 6 about here

FIGURE 5

Effect of the amino acid diets on mean($\pm$ SEM) change from baseline in diastolic blood pressure following CCK-4 injection.

FIGURE 6

Mean($\pm$ SEM) change from baseline in diastolic blood pressure following CCK-4 injection for panickers and nonpanickers.

** $p < 0.05$, panickers vs nonpanickers

Effect of Acute Tryptophan Depletion on Neuroendocrine Responses to CCK-4 Injection

The effect of the amino acid diets on hormonal responses to CCK-4 injection was analyzed by a mixed-design ANOVA, with Diet (tryptophan-free versus control) and Panic status (panic versus no panic) as between subject factors and Time of assessment (-5, 2, 5, 10 and 15 minutes) as the repeated measures factor. In these analyses, the main focus was on whether the Diet effect was Time dependent (Diet x Time and Diet x Panic x Time interaction). Only significant ($p < 0.05$) or marginally significant ($p < 0.10$) interactions were examined by pre-planned contrasts on the time factor (baseline value versus value at each time point).

ACTH. Figure 7 illustrates the effect of the dietary manipulation on the mean change from baseline in plasma ACTH. Plasma ACTH increased following

Insert Figure 7 about here

administration of CCK-4 for both dietary conditions. The time to peak for ACTH release was 5 minutes after administration of the peptide, with levels remaining clearly elevated at the end of the 15 minute sampling period. A repeated measures ANOVA performed on logarithmically transformed ACTH data indicated that the overall Diet ($F(1,36) = 0.11$, $p = 0.741$) and Panic

FIGURE 7

Effect of the amino acid diets on mean ($\pm$ SEM) change from baseline in plasma ACTH concentrations following CCK-4 injection.

* $p < 0.10$, tryptophan-free vs control amino acid diet.

** $p < 0.05$, tryptophan-free vs control amino acid diet.

($F(1,36) = 0.71$, $p = 0.404$) effect and Diet x Panic interaction ($F(1,36) = 0.02$, $p = 0.887$) were not significant. The overall Time effect was highly significant ($F(4,144) = 48.01$, $p < 0.001$), the Diet x Time interaction marginally significant ($F(4,144) = 2.33$, $p < 0.10$), and the Diet x Panic x Time interaction nonsignificant ($F(4,144) = 1.26$, $p = 0.290$). As shown in Figure 7, the Diet x Time interaction is accounted for by the fact that the mean increase from baseline in plasma ACTH was greater for the tryptophan-free than control amino acid diet group 2 and 15 minutes postinjection (Diet x Time: $F(1,36) = 4.47$ and 5.09 , p 's < 0.05), with a similar tendency noted 5 minutes postinjection (Diet x Time: $F(1,36) = 3.79$, $p < 0.10$).

The repeated measures ANOVA also yielded a significant Panic x Time interaction ($F(4,144) = 3.74$, $p < 0.05$). The Panic x Time interaction is illustrated in Figure 8. Although increases in ACTH occurred in both panickers and nonpanickers, the mean increase from baseline in ACTH was significantly greater in panickers than nonpanickers 5, 10 and 15 minutes postinjection (Panic x Time: $F(1,36) = 4.39$, 4.50 and 4.96 , p 's < 0.05).

Insert Figure 8 about here

Cortisol. Figure 9 displays the mean change from baseline in cortisol for the amino acid diets. Serum cortisol steadily rose for both dietary conditions, with the peak increase occurring 15 minutes after administration of L-CK-4. No

FIGURE 8

Mean($\pm$ SEM) change from baseline in plasma ACTH concentrations for panickers and nonpanickers following CCK-4 injection.

****p<0.05, panickers vs nonpanickers**

significant Diet ($F(1,36) = 0.04$, $p = 0.847$), Panic ($F(1,36) = 0.60$, $p = 0.445$) or Diet x Panic ($F(1,36) = 0.10$, $p = 0.745$) effects were found. The overall Time effect was highly significant ($F(4,144) = 122.59$, $p < 0.001$). More importantly, the Diet x Time interaction ($F(4,144) = 2.78$, $p < 0.05$) was significant, indicating that the dietary manipulation differentially affected cortisol response to CCK-4.

Insert Figure 9 about here

Post hoc analysis of the Diet x Time interaction revealed that the mean increase from baseline in cortisol tended to be higher in the tryptophan-free than the control amino acid diet group 15 minutes postinjection (Diet x Time: $F(1,36) = 3.21$, $p < 0.10$). Neither the Panic x Time ($F(4,144) = 0.58$, $p = 0.677$) nor the Diet x Panic x Time ($F(4,144) = 0.43$, $p = 0.785$) interactions were significant. Figure 10 displays the mean change from baseline in cortisol for panickers and nonpanickers.

Insert Figure 10 about here

Growth hormone. The effect of the dietary manipulation on mean change from baseline in growth hormone is displayed in Figure 11. As can be seen, serum growth hormone concentrations rose slightly after CCK-4 injection, with

FIGURE 9

Effect of the amino acid diets on mean($\pm$ SEM) change from baseline in serum cortisol concentrations following CCK-4 injection.

* $p < 0.10$, tryptophan-free vs control amino acid diet.

FIGURE 10

Mean($\pm$ SEM) change from baseline in serum cortisol concentrations for panickers and nonpanickers following CCK-4 injection.

the peak increase occurring 15 minutes postinjection for both amino acid diet groups. The overall Diet ($F(1,36)=0.77$, $p=0.385$) and Panic ($F(1,36)=0.97$, $p=0.331$) effect and the Diet x Panic interaction ($F(1,36)=0.64$, $p=0.430$) were not significant. The overall Time effect was significant ($F(4,144)=3.41$, $p<0.05$), but the Diet x Time ($F(4,144)=0.41$, $p=0.799$) and Diet x Panic x Time ($F(4,144)=0.94$, $p=0.443$) interactions failed to approach significance.

Insert Figure 11 about here

The repeated measures ANOVA revealed that the Panic X Time interaction was highly significant ($F(4,144)=6.65$, $p<0.001$). As can be seen in Figure 12, post hoc tests revealed that the mean change from baseline in serum growth hormone concentrations was significantly greater in panickers than in nonpanickers 2 (Panic x Time: $F(1,36)=11.42$, $p<0.01$), 5 (Panic x Time: $F(1,36)=10.31$, $p<0.01$), 10 (Panic x Time: $F(1,36)=5.88$, $p<0.05$) and 15 (Panic x Time: $F(1,36)=9.88$, $p<0.01$) minutes postinjection.

Insert Figure 12 about here

Prolactin. Figure 13 illustrates the effect of the dietary manipulation on mean changes from baseline in prolactin concentrations. Administration of CCK-4 produced robust increases in serum prolactin, with the peak increase

FIGURE 11

Effect of the amino acid diets on mean($\pm$ SEM) change from baseline in serum growth hormone concentrations following CCK-4 injection.

FIGURE 12

Mean($\pm$ SEM) change from baseline in serum growth hormone concentrations for panickers and nonpanickers following CCK-4 injection.

**** $p < 0.05$, panickers vs nonpanickers**

***** $p < 0.01$, panickers vs nonpanickers**

occurring 10 minutes after administration of the peptide for both amino acid diet groups. Tests of between-subject effects revealed the overall Diet ($F(1,36) = 1.21, p = 0.278$) and Panic ($F(1,36) = 0.66, p = 0.422$) effect and Diet x Panic interaction ($F(1,36) = 0.37, p = 0.547$) were not significant. The overall Time effect was significant ($F(4,144) = 37.89, p < 0.001$) and the Diet x Time interaction marginally significant ($F(4,144) = 2.11, p < 0.10$). The Diet x Panic x Time interaction was not significant ($F(4,144) = 0.52, p = 0.721$). Post hoc analysis revealed that the increase from baseline in prolactin release was greater for the tryptophan-free amino acid diet group 2 and 5 minutes postinjection (Diet x Time: $F(1,36) = 4.37$ and $5.04, p's < 0.05$). Although tryptophan-depleted subjects also displayed a greater increase from baseline in prolactin 10 and 15 minutes postinjection, differences between the dietary groups failed to approach statistical significance.

The repeated measures ANOVA also revealed a significant Panic x Time interaction ($F(4,144) = 10.17, p < 0.01$). The Panic x Time interaction is illustrated in Figure 14. Post hoc analysis revealed that panickers exhibited a greater increase from baseline in prolactin concentrations 5 minutes postinjection (Panic x Time: $F(1,36) = 7.42, p < 0.01$), with a similar tendency noted 10 and 15 minutes postinjection ($F(1,36) = 3.59$ and $3.32, p's < 0.10$).

Insert Figures 13 and 14 about here

FIGURE 13

Effect of the amino acid diets on mean($\pm$ SEM) change from baseline in serum prolactin concentrations following CCK-4 injection.

**** $p < 0.05$, tryptophan-free vs control amino acid diet.**

FIGURE 14

Mean($\pm$ SEM) change from baseline in serum prolactin concentrations for panickers and nonpanickers following CCK-4 injection.

*** $p < 0.10$, panickers vs nonpanickers**

**** $p < 0.01$, panickers vs nonpanickers**

Correlations Between Neuroendocrine, Cardiovascular and Behavioural Measures After CCK-4 Injection

In order to compare covariation patterns in behavioral, cardiovascular and neuroendocrine responses to CCK-4 injection, Spearman rank order correlations were computed separately for each amino acid diet group. In these analyses, the neuroendocrine variables were the maximum change score from baseline and the cardiovascular variables were the maximum change score from baseline that occurred within the first 120 seconds after CCK-4 injection. For behavioral ratings, only the principal outcome measures derived from the PSS were used (i.e., number of reported symptoms, sum intensity of symptoms, onset and duration of symptoms, and intensity of individual panic symptoms). A composite score of visual analog scale items reflecting anxiety-related mood (anxious, fearful and nervous) at the period of peak CCK-4 symptoms was also used in the analyses.

Correlations between Neuroendocrine and Cardiovascular Measures. An overview of the relationship between neuroendocrine and cardiovascular changes after CCK-4 injection is presented in Table 8.

Insert Table 8 about here

Within the control amino acid diet group, Spearman correlations showed a significant relationship between the maximal change in plasma ACTH

TABLE 8

Correlations between Neuroendocrine and Cardiovascular Responses to CCK-4 Injection

Variable	Diet	ACTH	Cortisol	Growth Hormone	Prolactin
Heart Rate	Control	-0.13	0.005	-0.15	0.03
	TRP-	0.15	-0.04	0.14	0.35
Systolic Blood Pressure	Control	0.24	0.06	-0.04	0.12
	TRP-	-0.04	-0.02	0.003	0.06
Diastolic Blood Pressure	Control	0.47*	0.26	0.36	0.03
	TRP-	0.31	-0.03	0.30	0.38

TRP- indicates tryptophan-free amino acid. Neuroendocrine and cardiovascular values are maximum change from baseline. * $p < 0.05$ (two-tailed tests).

concentrations and the maximal change in diastolic blood pressure ($r=0.47$, $p<0.05$). No other correlations were statistically significant.

Correlations between Neuroendocrine and Behavioral Measures. Table 9 reveals the intercorrelations between neuroendocrine changes and behavioral measures obtained after CCK-4 injection.

Insert Table 9 about here

As can be seen, CCK-4-induced neuroendocrine changes bore no significant association with behavioral responses to CCK-4 administration for subjects in the tryptophan-free amino acid diet group. The only notable exception was that prolactin changes correlated positively with the duration of symptoms induced with CCK-4 ($r=0.64$, $p<0.01$). By contrast, behavioral response to CCK-4 injection was highly correlated with neuroendocrine alterations within the control diet group. As shown in Table 9, there was a positive relationship between maximum prolactin changes and the number ($r=0.60$, $p<0.01$), sum intensity ($r=0.55$, $p<0.05$) and duration ($r=0.57$, $p<0.01$) of symptoms. Alterations in ACTH were found to be positively correlated with the sum intensity ($r=0.56$, $p<0.05$) and duration ($r=0.54$, $p<0.05$) of symptoms. Furthermore, growth hormone changes correlated positively with the number ($r=0.52$, $p<0.05$) and sum intensity ($r=0.57$, $p<0.01$) of symptoms, and the composite score for the visual analog scale

TABLE 9
Correlations Between Neuroendocrine and Behavioral Responses to CCK-4 Injection

Variable	Diet	ACTH	Cortisol	Growth Hormone	Prolactin
Number of symptoms	Control	0.41	-0.16	0.52 [*]	0.60 ^{**}
	TRP-	-0.01	0.08	-0.06	0.06
Sum intensity of symptoms	Control	0.56 [*]	-0.08	0.57 ^{**}	0.55 [*]
	TRP-	0.27	-0.07	0.10	0.31
Time to onset of symptoms	Control	0.07	-0.16	-0.04	0.03
	TRP-	-0.36	0.01	-0.18	0.01
Duration of symptoms	Control	0.54 [*]	-0.10	0.40	0.57 ^{**}
	TRP-	0.43	-0.003	0.27	0.64 ^{**}
VAS anxious/ nervous/fearful mood	Control	0.41	-0.21	0.60 ^{**}	0.44
	TRP-	-0.08	-0.03	-0.004	-0.11

TRP- indicates tryptophan-free amino acid diet.

* $p < 0.05$, ** $p < 0.01$ (two-tailed tests).

rating reflecting anxiety-related mood ($r=0.60$, $p<0.01$). There were no significant correlations between cortisol changes and any of the behavioral measures.

Correlative results for neuroendocrine changes and intensity ratings of individual panic symptoms are shown in Table 10. An inspection of the data

Insert Table 10 about here

reveals that among subjects in the control dietary condition, ACTH changes correlated positively with ratings of dyspnea ($r=0.46$, $p<0.05$), faintness ($r=0.48$, $p<0.05$), sweating ($r=0.68$, $p<0.01$), hot flushes/chills ($r=0.70$, $p<0.001$) and anxiety/fear/apprehension ($r=0.53$, $p<0.05$). Prolactin changes correlated positively with ratings of dyspnea ($r=0.59$, $p<0.01$), choking ($r=0.56$, $p<0.01$) and anxiety/fear/apprehension ($r=0.51$, $p<0.05$), and growth hormone changes correlated positively with ratings of dizziness ($r=0.48$, $p<0.05$), nausea ($r=0.52$, $p<0.05$), anxiety/fear/apprehension ($r=0.60$, $p<0.01$) and fear of going crazy ($r=0.55$, $p<0.05$). In the tryptophan-free amino acid diet group ratings of nausea positively correlated with changes in ACTH ($r=0.60$, $p<0.01$), prolactin ($r=0.51$, $p<0.05$) and growth hormone ($r=0.59$, $p<0.01$), whereas ratings of feeling detached/unreal correlated inversely with changes in ACTH ($r=-0.49$, $p<0.05$) and prolactin ($r=-0.48$, $p<0.05$). ACTH changes also correlated positively with ratings of

TABLE 10

Correlations between Neuroendocrine Responses and Panic Symptoms Induced by CCK-4

Symptom	Diet	ACTH	Cortisol	Growth Hormone	Prolactin
Dyspnea	Control TRP-	0.46* 0.41	-0.03 -0.23	0.36 0.18	0.59** 0.28
Dizziness	Control TRP-	0.35 0.47*	-0.003 -0.22	0.48* 0.35	0.38 0.36
Unsteady	Control TRP-	0.003 -0.08	-0.05 -0.31	0.10 -0.08	0.26 -0.09
Faintness	Control TRP-	0.48* 0.25	0.06 -0.44	0.44 0.05	0.38 0.17
Palpitation/ rapid heart	Control TRP-	0.19 0.17	0.02 -0.04	0.21 0.07	0.24 0.45
Trembling/ shaking	Control TRP-	0.09 -0.06	-0.12 0.27	0.28 -0.08	0.26 0.05
Sweating	Control TRP-	0.68** -0.15	-0.08 0.03	0.29 -0.22	0.23 -0.02
Choking	Control TRP-	0.11 -0.07	0.16 0.01	0.44 -0.20	0.56** 0.07
Nausea	Control TRP-	0.19 0.60**	-0.15 -0.09	0.52* 0.59**	0.45 0.51*
Abdominal Distress	Control TRP-	0.33 0.18	-0.23 0.004	0.20 0.08	0.37 0.24
Feeling unreal/detached	Control TRP-	0.39 -0.49*	-0.03 0.03	0.43 -0.28	0.22 -0.48*
Paresthesia	Control TRP-	0.35 0.23	-0.42 0.30	0.18 0.21	0.40 0.25
Hot flushes/ chills	Control TRP-	0.70*** 0.05	0.07 -0.10	0.12 -0.11	0.40 0.01
Chest pain/ discomfort	Control TRP-	0.08 -0.20	-0.24 0.11	-0.005 -0.28	0.36 -0.22
Fear/anxiety/ apprehension	Control TRP-	0.53* 0.11	0.22 0.02	0.60** 0.14	0.51* 0.41
Fear of dying	Control TRP-	0.05 0.27	0.34 -0.04	0.22 -0.12	-0.0003 0.16
Fear of losing control	Control TRP-	-0.16 -0.05	0.04 0.19	0.40 -0.03	0.42 0.10
Fear of going crazy	Control TRP-	0.21 -	-0.04 -	0.55* -	0.33 -

TRP- indicates tryptophan-free amino acid diet. *p<0.05, **p<0.01, ***p<0.001 (two-tailed tests).

dizziness ($r = 0.47$, $p < 0.05$).

Correlations between Cardiovascular and Behavioral Measures. Spearman correlations between cardiovascular and behavioral measures are illustrated in Table 11. There was a positive correlation between maximal changes in

Insert Table 11 about here

diastolic blood pressure and the sum intensity of symptoms in the control diet group ($r = 0.48$, $p < 0.05$). Within the tryptophan-free amino acid group, maximal changes in diastolic blood pressure were inversely correlated with the time to onset of symptoms ($r = -0.52$, $p < 0.05$). No other correlations reached statistical significance.

Table 12 shows the association between CCK-4-induced cardiovascular changes and individual panic symptoms. Heart rate changes did not correlate

Insert Table 12 about here

significantly with any of the individual panic symptoms in either the tryptophan-free or control amino acid diet group. Among subjects in the control diet group, diastolic blood pressure correlated positively with ratings of faintness ($r = 0.58$, $p < 0.01$), sweating ($r = 0.59$, $p < 0.01$) and hot flushes/chills ($r = 0.48$, $p < 0.05$), whereas systolic blood pressure correlated inversely with ratings of

TABLE 11

Correlations Between Behavioral and Cardiovascular Responses to CCK-4 Injection

Variable	Diet	Heart Rate	Systolic BP	Diastolic BP
Number of symptoms	Control	0.06	-0.09	0.37
	TRP-	0.25	-0.06	-0.11
Sum intensity of symptoms	Control	-0.04	-0.13	0.48*
	TRP-	0.27	-0.07	-0.10
Time to onset of symptoms	Control	-0.31	-0.21	-0.36
	TRP-	-0.45	-0.14	-0.52*
Duration of symptoms	Control	-0.05	0.33	0.42
	TRP-	0.10	-0.08	0.07
VAS anxious/ nervous/fearful mood	Control	0.08	0.07	0.36
	TRP-	0.37	0.27	0.22

TRP- indicates tryptophan-free amino acid diet.

* $p < 0.05$ (two-tailed tests).

TABLE 12

Correlations between Cardiovascular Responses and DSM-III-R Panic Symptoms Induced by CCK-4

Measure	Diet	Heart Rate	Systolic BP	Diastolic BP
Dyspnea	Control TRP-	-0.26 0.12	0.19 -0.10	0.26 0.06
Dizziness	Control TRP-	-0.04 -0.15	-0.20 -0.56*	0.44 -0.29
Unsteady	Control TRP-	-0.09 0.25	-0.56* -0.06	0.17 -0.08
Faintness	Control TRP-	0.06 0.16	-0.14 -0.24	0.58** -0.14
Palpitation/rapid heart	Control TRP-	-0.11 0.42	-0.18 0.42	0.17 0.54*
Trembling/shaking	Control TRP-	-0.01 0.34	-0.06 0.20	0.05 0.11
Sweating	Control TRP-	0.16 0.25	-0.01 -0.10	0.59** -0.32
Choking	Control TRP-	-0.22 -0.14	0.18 -0.06	0.08 -0.38
Nausea	Control TRP-	0.04 0.36	-0.29 -0.04	0.21 0.25
Abdominal Distress	Control TRP-	0.14 0.32	0.05 0.04	0.24 -0.19
Feeling unreal detached	Control TRP-	0.07 0.09	-0.25 -0.14	0.29 -0.27
Paresthesia	Control TRP-	0.17 0.44	0.11 -0.04	0.27 0.28
Hot flushes/chills	Control TRP-	0.04 0.22	0.16 -0.06	0.48* -0.34
Chest pain/discomfort	Control TRP-	-0.23 -0.30	-0.006 0.03	-0.23 -0.24
Fear/anxiety/apprehension	Control TRP-	0.02 0.02	-0.22 0.31	0.39 0.24
Fear of dying	Control TRP-	0.44 -0.04	0.19 -0.13	0.36 -0.29
Fear of losing control	Control TRP-	0.22 -0.11	-0.22 -0.08	-0.15 -0.19
Fear of going crazy	Control TRP-	0.13 -	-0.42 -	-0.04 -

TRP- indicates tryptophan-free amino acid diet. * $p < 0.05$, ** $p < 0.01$ (two-tailed tests).

unsteadiness ($r = -0.56$, $p < 0.05$). Within the tryptophan-free amino acid diet group, diastolic blood pressure correlated positively with ratings of palpitations/rapid heart ($r = 0.54$, $p < 0.05$), whereas systolic blood pressure correlated negatively with dizziness ($r = -0.56$, $p < 0.01$).

DISCUSSION

Consistent with the data of previous investigations (e.g., de Montigny, 1989; Bradwejn et al, 1991c; Koszycki, Cox & Bradwejn, 1993; Bradwejn et al, 1994b; 1994c), activation of CCK₈ receptors with the CCK agonist, CCK-4, elicited a marked anxiogenic response in healthy subjects, as demonstrated by significant increases in behavioral indices of anxiety, cardiovascular arousal, and release of stress hormones. The onset of the behavioral effects of CCK-4 was rapid, with symptoms appearing within 20 seconds after intravenous administration of the peptide, and the duration of symptoms was relatively short.

The neuroanatomical site of action of the anxiogenic effects of CCK-4 remains unclear. There is no conclusive evidence that peripheral CCK-4 crosses the blood-brain barrier and it cannot be assumed that CCK peptides are widely distributed in the brain after systemic administration. It has been proposed that the brainstem region that ordinarily functions to monitor sympathetic nerve discharge and vasomotor tone is a potential site of anxiogenic action of CCK-4 (Branchereau, Böhme, Champagnat, Morin-Surin, Durieux et al, 1992; Harro et al, 1993; Bradwejn & Koszycki, 1994a). Indeed, this region is not fully shielded by the blood-brain barrier and is accessible to peripherally administered CCK. In this respect, CCK pathways, binding sites and immunoreactivity have been identified in the nucleus of the solitary tract, the medullary nucleus and the parabrachial nucleus, among other central sites (Dampney, Goodchild,

Robertson & Montgomery, 1982). Considerable cross innervation is evident among these nuclei and ascending NE input to the locus coeruleus is detectable (Ross, Ruggiero, Park, Hoh, Sved et al, 1984). In addition, these brainstem sites innervate limbic and cortical regions and CCK is found among the numerous networks connecting all these regions. Conceivably, CCK-4 associated stimulation of CCK_b receptors in the brainstem contributes to the rapid onset of both somatic and affective symptoms of panic. Although the possibility exists that the behavioral effects of peripherally administered CCK-4 are mediated via the vagus nerve, the demonstration that subdiaphragmatic vagotomy (Vasar, Lang, Harro, Koks, Volke et al, 1994) and CCK_A receptor antagonists (Harro et al, 1993) were ineffective in preventing the anxiogenic effect of the mixed CCK_A/CCK_b agonist, caerulein, in rats, makes it likely that brainstem rather than peripheral CCK receptors contribute to the promotion of anxiety.

It should also be considered that systemic administration of CCK-4 produces prominent respiratory and cardiovascular alterations in humans through a direct action on the brainstem (Bradwejn et al, 1994a; Bradwejn & Koszycki, 1994a). There are numerous demonstrations that brainstem nuclei contribute to respiratory and cardiovascular regulation, among other vegetative functions (Jordan & Spyer, 1986). Increases in breathing amplitude, respiration, blood pressure and heart rate have been noted following electrical or pharmacological stimulation of the nucleus of the solitary tract, medullary nuclei

and parabrachial nucleus (Marovitch, Kumada & Reis, 1982; Jordan & Spyer, 1986). Further, there are experimental data that CCK interacts with these brainstem nuclei in modulating cardiorespiratory function. Microiontophoretic application of CCK-8S, a mixed CCK_A/CCK_B agonist, to the nucleus of the solitary tract has been reported to decrease both neuronal firing and respiratory frequency in cats; effects which are reversed by administration of CCK-4, a CCK_B agonist (Denavit-Saubie, Hurle, Morin-Surun, Foutz & Champagnat, 1985). Exogenous administration of CCK-4 to human subjects provokes increases in respiratory frequency, tidal volume and minute ventilation (Legrand, Bates & Bradwejn, unpublished data). Further, in panic patients, acute administration of the CCK_B receptor antagonist, L-365,260, attenuates respiratory and cardiovascular arousal following CCK-4 injection, presumably through blockade of CCK receptors located in the nucleus of the solitary tract (Bradwejn et al, 1994a).

While the data of the present investigation suggest that CCK-4 stimulates prolactin, ACTH/cortisol and growth hormone release in humans, it is not clear whether such effects occur through direct pituitary activation or via the hypothalamus. Anatomical studies have detected appreciable amounts of CCK in the hypothalamo-hypophyseal system, in neurons of the paraventricular and the supraoptic nuclei (Larsson & Rehfeld, 1981; Morley, Melmed, Briggs, Carlson, Hershman, 1979; Beinfeld, Meyer & Brownstein, 1980; Anhut, Mayer & Knepel, 1983; Tang & Man, 1991). It is generally assumed that CCK alters

hormonal release from the anterior pituitary by acting as a transmitter or neuromodulator (Vijayan et al, 1979). For instance, CCK is colocalized with corticotropin-releasing factor and vasopressin in parvocellular neurons (Mezey, Reisine, Skirboll, Beinfeld & Kiss, 1986). Both systemic and intracerebral administration of CCK-8 stimulate corticotropin-releasing hormone that results in ACTH and cortisol secretion in rats (Reisine & Jensen, 1986; Verbalis, Stricker, Robinson & Hoffman, 1991; Kamilaris, Johnson, Calogero, Kalogeras, Bernardini, et al, 1992). There is also evidence that peripherally administered CCK-peptides stimulate pituitary hormone release through the activation of the nucleus of the solitary tract and area postrema which modulate hypothalamic function (Crawley & Schwaber, 1984; Luckman, Hamamura, Antonijevic, Dye & Leng, 1993). However, peripherally administered CCK may also stimulate hormonal release via activation of the vagus nerve. The suppression of CCK-8- and caerulein-induced neuroendocrine effects in rats by subdiaphragmatic vagotomy (Itoh, Katsuura, Hirota, Odaguchi, 1980) and treatment with selective CCK_A receptor antagonists (Katsuura et al, 1992; Luckman et al, 1993) tends to support this hypothesis. However, while it appears that at least part of the neuroendocrine action of nonselective CCK agonists is mediated through activation of CCK_A receptors, it remains unclear whether peripheral administration of selective CCK_B receptor agonists, such as CCK-4, stimulate hormonal release by activating peripheral CCK receptors.

The potential neurochemical mechanisms associated with the anxiogenic

effects of CCK-4 remain to be elucidated. However, the capacity for CCK-4 to provoke symptoms of anxiety may be related to an interaction between the 5-HT and CCK systems (Paudice & Raiteri, 1991; Vasar et al, 1993a; Bradwejn & Koszycki, 1994b). In evaluating the role of 5-HT in the anxiogenic action of CCK-4, the present study examined the influence of acute depletion of the 5-HT precursor, tryptophan, on behavioral, cardiovascular and neuroendocrine sensitivity to systemic CCK-4 in healthy males. The tryptophan depletion procedure was effective in lowering plasma tryptophan. Five hours after ingesting a tryptophan-free amino acid diet, total plasma tryptophan decreased by a mean of 92%. Although no direct measurement of central 5-HT availability was obtained in this study, data available from infrahuman experimentation suggest that the dietary manipulation employed in this investigation produced a reduction in central 5-HT by inhibition of 5-HT synthesis. For example, in the rat, a diet lacking in tryptophan provoked brain and serum tryptophan reductions of 90% and 75%, respectively, and brain 5-HT and 5-HIAA concentration decreases of 58% and 76%, respectively (Biggio et al, 1974). In nonhuman primates, a tryptophan-free diet produced a 51% decline in CSF tryptophan and a 34% decline of CSF 5-HIAA, in the absence of central variations of HVA or MHPG (Young et al, 1989). Human CSF studies have revealed that increasing or decreasing dietary tryptophan consistently elevates or lowers brain 5-HT synthesis and that plasma amino acid availability reliably parallels 5-HT variations in CSF (Perez-Cruet, Chase & Murphy, 1974; Young

& Gauthier, 1981). Taken together, it seems likely that a reduction in plasma tryptophan of the magnitude observed in the present study produced a parallel decrease in brain 5-HT.

Surprisingly, despite pronounced reductions in plasma tryptophan and presumably central 5-HT availability, significant behavioral and cardiovascular alterations in response to CCK-4 injection were not detected. To be sure, acute tryptophan depletion had no demonstrable influence on the number, intensity, latency or duration of symptoms induced by CCK-4. Changes in heart rate and blood pressure following CCK-4 injection were also unaffected by the dietary manipulation. Panic attack frequency, as well as the symptom profile of panickers, were comparable among tryptophan-depleted and control subjects. Visual analog ratings of mood revealed only a greater decrease in happy and talkative mood after CCK administration in the tryptophan-depleted group, in the absence of any intensification of self-rated anxiety, fearfulness or nervousness. Parenthetically, the lack of effect of acute tryptophan depletion on baseline mood indicates that the rigorous screening procedure employed in the study was successful in generating a sample of subjects who were not vulnerable to the mood lowering effects of tryptophan depletion (cf. Abbott et al, 1992; Benkelfat et al, unpublished manuscript).

Acute tryptophan depletion did not affect hormonal secretion, although it potentiated the stimulatory action of CCK-4 on prolactin and ACTH/cortisol secretion. The demonstration that variations of 5-HT activity contribute to

hypothalamic control of anterior pituitary hormone secretion in humans is well documented (Yatham & Steiner, 1993). Although it is not clear which 5-HT receptor subtype mediates prolactin and ACTH/cortisol secretion, there are data to suggest that 5-HT₁ and 5-HT₂ receptors contribute to such an effect (Yatham & Steiner, 1993). While the data of the present investigation suggest that CCK-4-induced excitation of prolactin- and ACTH-secreting neurons may be mediated by serotonergic mechanisms, it should be emphasized that the contribution of transmitters other than 5-HT to CCK-4-induced activation of prolactin and ACTH/cortisol secretion cannot be excluded. Indeed, it is conceivable that tryptophan depletion may have altered the neurochemical balance between 5-HT and other neurotransmitter systems, including NE and DA (McDougale et al, 1993). Likewise, acute tryptophan depletion may have affected neuropeptide availability, second messenger systems and receptor sensitivity and/or synthesis (McDougale et al, 1993). Accordingly, one must be cautious in interpreting these data as indicative of a direct effect of 5-HT on CCK-evoked hormonal release.

While the present results demonstrate that acute tryptophan depletion did not influence CCK-4-induced anxiety and cardiovascular arousal, it would be premature to conclude that 5-HT and CCK do not interact in the mediation of anxiety. Various 5-HT receptors have been identified in the brain and there is evidence that at least two of these 5-HT receptors are functionally associated with CCK activity. In particular, an apparent relationship between CCK and the

5-HT₃ receptor was demonstrated by Vasar et al (1993a) who revealed that the anxiogenic action of caerulein was antagonized by the 5-HT₃ receptor antagonist, ondansetron, in mice. The locus of the CCK-5-HT₃ interaction was proposed to occur at the nucleus of the solitary tract where 5HT₃ and CCK₈ receptors are colocalized. Electrophysiological studies have also pointed to a possible interaction between CCK and 5-HT₂ receptors in the mediation of anxiety (Agnati et al, 1983) and an association between CCK and 5-HT₂ receptors in the mediation of defensive aggression in rats has been noted (Vasar, 1992). It should be pointed out that there are no studies of how 5-HT receptor subtypes (e.g., 5HT₁, 5HT₂, 5-HT₃, 5-HT₄) respond to acute tryptophan depletion. It is conceivable that the tryptophan depletion paradigm produced a general lowering of 5-HT availability without directly or appreciably altering the sensitivity of the 5-HT receptor(s) that mediates the anxiogenic action of CCK-4 (e.g., 5-HT₃). Such an explanation is not improbable considering that 5-HT receptor subtypes show different affinities to 5-HT (Peroutka, Schmidt, Sleight & Harrington, 1990). Clearly, it will be important to establish the contribution of specific 5-HT receptor subtypes to the behavioral and cardiovascular aspects of CCK-4-induced anxiety in humans.

There is reason to believe that the neurochemical and behavioral concomitants of the interaction between 5-HT and CCK may be mediated by the interactive influence of environmental stimuli. Consistent with the aforementioned proposition, Rex et al (1994b) observed that whereas CCK-4

was ineffective in altering basal levels of 5-HT in the cerebral cortex of guinea pigs, it potentiated the release of 5-HT in animals exposed to aversive stimulation (i.e., the elevated plus maze). It appears, therefore, that CCK may modulate 5-HT neurotransmission only if 5-HT is altered after stressor exposure. These data raise the intriguing possibility that the anxiety and cardiovascular arousal engendered by a combined tryptophan depletion/CCK-4 challenge would have been markedly altered if subjects were exposed to a stressor prior to administration of CCK-4.

Another possible reason why there was no robust effect of acute tryptophan depletion on behavioral or cardiovascular sensitivity to CCK-4 challenge in human subjects is that reductions in 5-HT in brain regions where CCK_B receptors largely predominant, and where CCK-4 produces its anxiogenic effects, may not have been sufficient. Moreover, several neurochemical changes may have occurred as an adaptive response to acute lowering of brain tryptophan and 5-HT availability, and such adaptive changes may have obscured or prevented the effects of acute tryptophan depletion on CCK-4-induced anxiety and cardiovascular arousal. Interestingly, self-rated nervousness was increased following a combined acute tryptophan depletion and yohimbine challenge in healthy subjects (Goddard, 1994), suggesting that the NE system is more sensitive than the CCK system to alterations in 5-HT availability. Nevertheless, the effect of a combination of tryptophan depletion and yohimbine on self-rated nervousness was modest, and other anxiety-related

mood states such as fearfulness and anxiety were not augmented by the combined treatments (Goddard, personal communication, 1994). It should be underscored that the subject pool employed in this study was comprised of healthy subjects with no personal or family history of mental disorders. Accordingly, it would not be unreasonable to assume that acute tryptophan depletion would enhance CCK-4-induced anxiety in patients with panic disorder whose CCK and/or 5-HT system is in some way compromised. In such individuals, adaptive changes to transient alterations in 5-HT availability may be minimized.

The capacity for acute tryptophan depletion to potentiate CCK-4-induced neuroendocrine alterations without affecting behavioral or cardiovascular concomitants of CCK-4-induced anxiety demonstrates that the neuroendocrine system is exquisitely sensitive to alterations in brain neurochemistry. This sensitivity might simply reflect a lower threshold for change in endocrine as compared to behavioral or cardiovascular indices of anxiety. A similar conclusion was reached by Abelson Curtis, Nesse, Fantone, Pyke et al (1994b) who reported that administration of the CCK₈ receptor antagonist, CI-988, at a low dose attenuated the endocrine (i.e., ACTH, cortisol and prolactin secretion) but not the behavioral (i.e., anxiety) sequelae of benzodiazepine withdrawal. It was concluded that larger doses of CI-988 would be necessary to attenuate the behavioral consequences of benzodiazepine withdrawal. Although the present study failed to reveal an effect of tryptophan depletion on

the expression of fear and anxiety induced by CCK-4, it is notable that tryptophan-depleted subjects rated themselves as being less happy and talkative than control subjects at the peak of CCK-4-induced panic symptoms. Hence, it may be tentatively concluded that while acute depletion of the 5-HT precursor, tryptophan, is sufficient to produce subtle changes in behavioral indices of CCK-4-induced anxiety, prolonged exposure to a tryptophan deficient diet may be necessary to obtain more profound behavioral effects.

Systemic administration of CCK-4 elicits panic attacks in normal subjects which are comparable to those experienced naturally and those induced by CCK-4 in patients with panic disorder (Bradwejn et al, 1991b). In the present study, 60% of subjects experienced a panic attack. The propensity to panic following CCK-4 administration was unrelated to baseline levels of arousal. Panickers were clearly more sensitive to the behavioral effects of CCK-4 than were nonpanickers. They reported a greater number of symptoms, more intense symptoms and symptoms of a longer duration. Panickers also displayed greater changes in visual analog ratings of anxious, fearful, calm, mellow, happy and talkative mood following CCK-4 challenge than nonpanickers. Although there was no differential heart rate or systolic blood pressure response to CCK-4 between panickers and nonpanickers, panickers did show a greater elevation in diastolic blood pressure during the period associated with peak panic symptoms. Perhaps the most interesting difference between panickers and nonpanickers was the exaggerated ACTH, prolactin and growth hormone

response to CCK-4. The cortisol response to CCK was also greater in panickers but differences between panickers and nonpanickers failed to reach statistical significance. Hormonal changes associated with clinical panic attacks have been observed, including increases in prolactin, cortisol and growth hormone (Ko et al, 1983; Cameron et al, 1987; Woods et al, 1987). There is ample evidence that panic patients are hypersensitive to the behavioral and hormonal effects of a variety of panicogenic agents (Nutt, 1990). Thus, it appears that the healthy subjects who panicked after CCK-4 administration in the present study displayed characteristics which are similar to those which have been observed in patients with panic disorder.

The finding that subjects who panicked with CCK-4 were more sensitive to the behavioral and neuroendocrine effects of this peptide than those who did not panic highlights some interesting findings from animal and human research on the role of the CCK system in anxiety. Two CCK receptor subtypes have been identified: peripheral (CCK_A) and central (CCK_B) (Dourish & Hill, 1987). These receptors, which were originally characterized pharmacologically, have recently been cloned (Wank, Pisegna & De Weerth, 1992; Kopin, Lee, McBride, Miller, Lu et al, 1992). The intravenous injection of CCK-4, the agonist at the CCK_B receptor, reliably and dose-dependently provokes panic attacks and concomitant increases in heart rate and blood pressure in patients with panic disorder (Bradwejn et al, 1991a; Bradwejn et al, 1992b; Bradwejn et al, 1992c). Blockade of CCK_B receptors with single oral doses of selective CCK_B

antagonists decrease the panicogenic action of CCK-4 in panic disorder (Bradwejn et al, 1994a) and normal subjects (Bradwejn et al, 1994b), suggesting that CCK_B receptors mediate the panicogenic effects of this peptide. There is also considerable evidence from studies with laboratory animals that CCK_B receptors mediate anxiety. For instance, anxious rats have been reported to exhibit increased CCK receptor densities in brain regions where CCK_B receptors largely predominate (Harro et al, 1990b). Exposing rats to the smell of a predator resulted in marked elevations of CCK-4 in many brain regions, including the nucleus solitary tract, central amygdala, frontal and central cortices, the olfactory bulb and the dorsal and ventral striatum, whereas elevations in CCK-8 levels occurred only in the ventral striatum (Pavlasovic, Bednar, Qureshi & Sodersten, 1993). Further, studies of selective CCK agonists and antagonists suggest a clear role for CCK_B receptors in anxiogenic behavior in animals (e.g., Singh et al, 1991a; 1991b; Rex et al, 1994a). Taken together, human and animal data provide compelling evidence that subjects prone to panic attacks or anxiety might exhibit a hyperactivity of the CCK_B system.

Recent data have suggested that panic anxiety might not be exclusively the result of an enhanced CCK_B system. In fact, there is growing speculation that pathological anxiety might stem from a disruption of the interaction between CCK_A and CCK_B receptors favoring an overactive CCK_B system (Harro et al, 1993; Bradwejn & Koszycki, 1994a). Both CCK_A and CCK_B receptors are present in the brainstem and Branchereau et al (1992) have revealed that CCK_A

and CCK_B receptors mediate opposite neuronal activity in brainstem regions. In particular, Branchereau et al (1992) observed that CCK_B receptor stimulation increased the spontaneous activity of rat nucleus tractus solitarius neurons whereas CCK_A receptor stimulation diminished neural activity in this brainstem region. The opposite effects of CCK_A and CCK_B receptor subtypes have also been observed in behavioral paradigms of anxiety. In this regard, Männistö, Lang, Harro, Peuranen, Bradwejn et al (1993) reported that pretreatment of rats with devazepide, a selective CCK_A receptor antagonist, potentiated the anxiogenic action of the mixed CCK_A/CCK_B receptor agonist caerulein in the plus-maze, whereas the selective CCK_B antagonists LY28853 and L-365,260 abolished the anxiogenic action of caerulein. It appears therefore, that endogenous CCK acting at CCK_B receptors is anxiogenic whereas endogenous CCK acting at CCK_A receptors is anxiolytic. Presumably, the enhanced sensitivity of CCK_B receptors in individuals who are predisposed to anxiety might result from an imbalance between anxiogenic and anxiolytic CCK receptors. The concept of a functional antagonism between CCK_A and CCK_B receptors is further substantiated by neuroendocrine studies. Männistö et al (1993) showed that administration of L-365,260 produced an inhibition of caerulein-stimulated growth hormone secretion in rats, whereas devazepide potentiated caerulein-induced rise in growth hormone. Interestingly, when administered alone, caerulein, L-365-260, and devazepide had no demonstrable effect on growth hormone secretion.

The data derived from infrahuman studies suggest that individuals who are predisposed to anxiety syndromes, such as panic disorder, might exhibit an enhanced CCK_B (or $\text{CCK}_B > \text{CCK}_A$) receptor activity associated with an enhanced growth hormone secretion to CCK-4. In contrast, individuals who are not predisposed to pathological anxiety might exhibit a balanced level of CCK_A and CCK_B (or $\text{CCK}_A =$ or $> \text{CCK}_B$) activity associated with a blunted growth hormone secretion to CCK-4. The different pattern in growth hormone secretion after CCK-4 injection in panickers and nonpanickers of the present investigation tends to support such an hypothesis. Indeed, whereas panickers showed an exaggerated growth hormone response to CCK-4 injection, nonpanickers showed either no change or a decrease in growth hormone secretion. It would be interesting to determine if a similar pattern of growth hormone secretion is evident following nonpharmacologically-induced anxiety or panic. Since significant baseline differences in growth hormone levels were not found between panickers and nonpanickers, it could be argued that CCK-4 injection unmasked the imbalance between CCK_A and CCK_B receptor activity or a predisposition to anxiety in subjects who panicked. Presumably, this imbalance is more conspicuous in patients who suffer from clinical panic attacks. In accordance with this view, panic patients, relative to control subjects, have been reported to exhibit decreased CSF and lymphocyte concentrations of CCK-8S, a mixed $\text{CCK}_A/\text{CCK}_B$ agonist (Lydiard et al, 1992; Brambilla et al, 1993), and an enhanced anxiogenic response to selective CCK_B receptor agonists

(Abelson & Nesse, 1990; Bradwejn et al, 1991b; Van Megen, Westenberg, den Boer, Haigh & Traub, 1994).

It has also been reported that variation in endogenous CCK levels correlate with self-reported anxiety. For instance, Feldman, Walker, Goldschmiedt and Cannon (1992) found a positive correlation between serum concentrations of gastrin, a CCK_B agonist, and self-reported tension, conflict and anxiety in normal men. Likewise, Uvnäs-Moberg, Arn, Jonsson, Ek and Milsonne (1993) reported that gastrin levels correlated with high levels of anxiety in normal women. A potential role for endogenous CCK as an anticipatory stress factor has also been suggested. In this respect, Philipp, Wilckens, Friess, Platte and Pirke (1992) reported that plasma CCK-8S concentrations were markedly elevated in marathon runners prior to competition as compared to control conditions. In addition, plasma ACTH, cortisol and NE were elevated prior to and increased substantially after the run. However, CCK-8S levels remained at prerun values. Harro et al (1993) have postulated that CCK is a normal endogenous anticipatory stress modulator that may contribute to the genesis of anxiety disorders due to some alteration in the normal regulation of CCK. Conceivably such an alteration is expressed, at least in part, as an imbalance between CCK_A and CCK_B receptors in the brainstem.

The hypothesized imbalance between CCK_A and CCK_B receptor activity might also be evident at higher brain sites. For example, microinjection of CCK into the central amygdaloid nucleus of rats provoked behaviors thought to

reflect fear and anxiety (Fekete, Lengyel, Hegedus, Penke, Zarandy et al, 1984; Csonka et al, 1988). Likewise, exposure to footshock produced substantial increases in CCK concentrations in limbic regions and in the prefrontal cortex in rats (Siegal, Dücker, Fuchs, Pahnke & Wuttke, 1985). Moreover, forebrain CCK receptor binding correlates with anxiety states. In particular, social isolation, benzodiazepine withdrawal and administration of FG 7142, an anxiogenic β -carboline, or picrotoxin, the non-competitive GABA antagonist, provoke an upregulation of [3 H]-CCK₈ binding in frontal cortex in the rat (Harro & Vasar, 1991a; Harro et al, 1990b; Vasar et al, 1992; Vasar et al, 1993b). This upregulation may be of physiological importance to the role of CCK in stress and anxiety since considerable CCK receptor binding is also observed in anxious rats selected according to low levels of exploratory behavior in the elevated plus-maze (Harro et al, 1990b). Conceivably, conspicuous cortical CCK receptor density is a correlate of either anticipatory anxiety and/or deficits in behavioral adaptation to stress (Harro et al, 1993). Such an interpretation is supported by data available from postmortem examination of brain tissue of suicide victims which revealed upregulation of CCK receptor binding in frontal and the cingulate cortex relative to control subjects. Although the diagnostic status of these subject was unknown, it can be assumed that they experienced considerable anticipatory anxiety prior to death (Harro, Marcusson & Oreland, 1992).

In view of the suggestion that CCK_A and CCK_B receptor activity are

involved in the promotion of fear and anxiety, it is tempting to speculate that factors which influence the density/sensitivity of these receptors contribute to the vulnerability of some individuals to anxiety and panic attacks. For instance, 5-HT activity may influence the balance between CCK_A and CCK_B receptors and an intact 5-HT system might be necessary, among other factors, to maintain this balance. In the present study, it was interesting to note that plasma ACTH and serum prolactin and growth hormone concentrations correlated with behavioral response to CCK-4 injection in control subjects but not in tryptophan-depleted subjects. This latter finding suggests that an adaptive behavioral and/or hormonal response to stressful stimuli may be dependent upon the integrity of the 5-HT system. Possibly, a disrupted or underactive 5-HT system contributes to a dysregulation of neurotransmitter systems involved in stress or anxiety regulation. It is conceivable that the clinical effectiveness of antipanic agents which increase 5-HT availability (e.g., imipramine, clomipramine) stem, at least in part, from their ability to restore the balance between anxiolytic (CCK_A) and anxiogenic (CCK_B) CCK receptors. Consistent with this proposal is the finding that chronic treatment with imipramine markedly decreased behavioral and cardiovascular sensitivity to systemic CCK-4 in patients with panic disorder (Bradwejn & Koszycki, 1994b). In a similar vein, a potential mechanism mediating the action of 5-HT₃ antagonists, which are anxiolytic and which block the anxiogenic effects of CCK in experimental animals (Costa, 1993; Vasar et al, 1993a), is the inhibition of endogenous CCK

release (Paudice & Raiteri, 1991) or alteration of CCK_A and CCK_B receptor sensitivity.

Summary

The data of this investigation revealed that the prolactin and ACTH/cortisol response to CCK-4 was influenced by acute tryptophan depletion, suggesting that 5-HT mechanisms within the hypothalamic-pituitary axis are involved, at least in part, in CCK-4 induced activation of stress hormones. Although the effects of acute tryptophan depletion on response to CCK-4 were not robust enough to be evident at a behavioral or cardiovascular level, it would be premature to conclude that 5-HT mechanisms are not involved in the anxiogenic effects of CCK-4, since the tryptophan depletion paradigm may not have altered the sensitivity of the 5-HT receptor subtype(s) that specifically interacts with CCK-4 or sufficiently reduce 5-HT in brain regions where CCK_B receptors are present. It should also be emphasized that the results of this study apply only to healthy males. Additional studies with patients with panic disorder are necessary in further evaluate the functional significance of acute tryptophan depletion on response to exogenous CCK-4. Finally, the data of this dissertation revealed that healthy subjects who panicked after CCK-4 administration were more sensitive to the behavioral, cardiovascular and hormonal effects of this peptide. The enhanced sensitivity to CCK-4 evident in panickers may stem from a disruption of the interaction between CCK_A and CCK_B receptors, favoring an overactive CCK_B system.

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Appendix 1 SCL-90

Name: _____

Date: _____

Below is a list of problems and complaints that people sometime have. Please read each one carefully. After you have done so, please fill in one of the number spaces to the right that best describes how much that problem has bothered or distressed you during the past week including today.

HOW MUCH WERE YOU BOTHERED BY:	Not at all	A little bit	Moderately	Quite a bit	Extremely
1. Headaches	0	1	2	3	4
2. Nervousness or shakiness inside	0	1	2	3	4
3. Unwanted thoughts, words, or ideas that won't leave your mind	0	1	2	3	4
4. Faintness or dizziness	0	1	2	3	4
5. Loss of sexual interest or pleasure	0	1	2	3	4
6. Feeling critical of others	0	1	2	3	4
7. The idea that someone else can control your thoughts	0	1	2	3	4
8. Feeling others are to blame for most of your troubles	0	1	2	3	4
9. Trouble remembering things	0	1	2	3	4
10. Worried about sloppiness or carelessness	0	1	2	3	4
11. Feeling easily annoyed or irritated	0	1	2	3	4
12. Pains in heart or chest	0	1	2	3	4
13. Feeling afraid in open spaces or on the streets	0	1	2	3	4
14. Feeling low in energy or slowed down	0	1	2	3	4
15. Thoughts of ending your life	0	1	2	3	4
16. Hearing voices that other people do not hear	0	1	2	3	4
17. Trembling	0	1	2	3	4
18. Feeling that most people cannot be trusted	0	1	2	3	4
19. Poor appetite	0	1	2	3	4
20. Crying easily	0	1	2	3	4
21. Feeling shy or uneasy with the opposite sex	0	1	2	3	4
22. Feeling of being trapped or caught	0	1	2	3	4

HOW MUCH WERE YOU BOTHERED BY:	Not at all	A little bit	Moderately	Quite a bit	Extremely
23. Suddenly scared for no reason	0	1	2	3	4
24. Temper outbursts that you could not control	0	1	2	3	4
25. Feeling afraid to go out of your house alone	0	1	2	3	4
26. Blaming yourself for things	0	1	2	3	4
27. Pains in lower back	0	1	2	3	4
28. Feeling blocked in getting things done	0	1	2	3	4
29. Feeling lonely	0	1	2	3	4
30. Feeling blue	0	1	2	3	4
31. Worrying too much about things	0	1	2	3	4
32. Feeling no interest in things	0	1	2	3	4
33. Feeling fearful	0	1	2	3	4
34. Your feelings being easily hurt	0	1	2	3	4
35. Other people being aware of your private thoughts	0	1	2	3	4
36. Feeling others do not understand you or are unsympathetic	0	1	2	3	4
37. Feeling that people are unfriendly or dislike you	0	1	2	3	4
38. Having to do things very slowly to insure correctness	0	1	2	3	4
39. Heart pounding or racing	0	1	2	3	4
40. Nausea or upset stomach	0	1	2	3	4
41. Feeling inferior to others	0	1	2	3	4
42. Soreness of your muscles	0	1	2	3	4
43. Feeling that you are watched or talked about by others	0	1	2	3	4
44. Trouble falling asleep	0	1	2	3	4
45. Having to check and double-check what you do	0	1	2	3	4
46. Difficulties in making decisions	0	1	2	3	4
47. Feeling afraid to travel on buses, subways or trains	0	1	2	3	4
48. Trouble getting your breath	0	1	2	3	4
49. Hot or cold spells	0	1	2	3	4

HOW MUCH WERE YOU BOTHERED BY:	Not at all	A little bit	Moderately	Quite a bit	Extremely
50. Having to avoid certain things, places, or activities because they frighten you	0	1	2	3	4
51. Your mind going blank	0	1	2	3	4
52. Numbness or tingling in parts of your body	0	1	2	3	4
53. A lump in your throat	0	1	2	3	4
54. Feeling hopeless about the future	0	1	2	3	4
55. Trouble concentrating	0	1	2	3	4
56. Feeling weak in parts of your body	0	1	2	3	4
57. Feeling tense or keyed up	0	1	2	3	4
58. Heavy feelings in your arms or legs	0	1	2	3	4
59. Thoughts of death or dying	0	1	2	3	4
60. Overeating	0	1	2	3	4
61. Feeling uneasy when people are watching or talking about you	0	1	2	3	4
62. Having thoughts that are not your own	0	1	2	3	4
63. Having urges to beat, injure or harm someone	0	1	2	3	4
64. Awakening in the early morning	0	1	2	3	4
65. Having to repeat the same actions such as touching, counting, washing	0	1	2	3	4
66. Sleep that is restless or disturbed	0	1	2	3	4
67. Having urges to break or smash things	0	1	2	3	4
68. Having ideas or beliefs that others do not share	0	1	2	3	4
69. Feeling very self-conscious with others	0	1	2	3	4
70. Feeling uneasy in crowds, such as shopping or at a movie	0	1	2	3	4
71. Feeling everything is an effort	0	1	2	3	4
72. Spells of terror or panic	0	1	2	3	4
73. Feeling uncomfortable about eating or drinking in public	0	1	2	3	4
74. Getting into frequent arguments	0	1	2	3	4
75. Feeling nervous when you are left alone	0	1	2	3	4

HAVE YOU BEEN BOTHERED BY:	Not at all	A bit	Quite a bit	Moderately	Extremely
76. Others not giving you proper credit for your achievements	0	1	2	3	4
77. Feeling lonely even when you are with people	0	1	2	3	4
78. Feeling so restless you couldn't sit still	0	1	2	3	4
79. Feelings of worthlessness	0	1	2	3	4
80. Feeling that familiar things are strange or unreal	0	1	2	3	4
81. Shouting or throwing things	0	1	2	3	4
82. Feeling afraid you will faint in public	0	1	2	3	4
83. Feeling that people will take advantage of you if you let them	0	1	2	3	4
84. Having thoughts about sex that bothered you a lot	0	1	2	3	4
85. The idea that you should be punished for you sins	0	1	2	3	4
86. Feeling pushed to get things done	0	1	2	3	4
87. The idea that something is seriously wrong with your body	0	1	2	3	4
88. Never feeling close to another person	0	1	2	3	4
89. Feelings of guilt	0	1	2	3	4
90. The idea that something is wrong with your mind	0	1	2	3	4

Appendix 2. Panic Symptom Scale

Name: _____ ID: _____ Date: _____

Below is a list of symptoms you might have experienced as a result of receiving the CCK-4 injection. Please indicate on a scale from 0 to 4 how severe/intense each symptom you experienced was.

Severity rating:

- 0 not present
- 1 mildly severe/intense
- 2 moderately severe/intense
- 3 very severe/intense
- 4 extremely severe/intense

Feeling short of breath and/or
smothering sensation 0 1 2 3 4

Dizziness 0 1 2 3 4

Unsteady feeling 0 1 2 3 4

Faintness 0 1 2 3 4

Palpitations and/or
accelerated heart 0 1 2 3 4

Earache 0 1 2 3 4

Nose itching 0 1 2 3 4

Trembling and/or shaking 0 1 2 3 4

Sweating 0 1 2 3 4

Choking feeling 0 1 2 3 4

Panic Symptom Scale

Stuffy nose*	0 1 2 3 4
Nausea	0 1 2 3 4
Abdominal distress	0 1 2 3 4
Low back pain*	0 1 2 3 4
Feeling unreal and/or detached from your body	0 1 2 3 4
Numbness and/or tingling in parts of your body	0 1 2 3 4
Hot flashes and/or cold chills	0 1 2 3 4
Itchy feet*	0 1 2 3 4
Chest pain and/or discomfort	0 1 2 3 4
Anxiety, fear and/or apprehension	0 1 2 3 4
Fear of dying	0 1 2 3 4
Fear of losing control	0 1 2 3 4
Fear of going crazy	0 1 2 3 4

Other symptoms:

0 1 2 3 4
0 1 2 3 4
0 1 2 3 4

a) Number of PSS symptoms reported: ____
(number of items scored 1 or higher)

b) Sum intensity of PSS symptoms: ____
(sum of intensity scores)

c) Onset of symptoms: ____

d) Duration of symptoms: ____

e) Number of response bias items: ____

OBSERVATIONS/COMMENTS:

* Response bias items

Appendix 3. DSM-III-R Criteria for a Panic Attack

A panic attack is a discrete episode of intense anxiety or discomfort which reaches a peak within ten minutes of the beginning of the first symptom noticed in the attack. The attack involves at least four of the following sensations.

1. dyspnea or smothering sensations
2. dizziness, unsteady feeling or faintness
3. palpitations or tachycardia
4. trembling or shaking
5. sweating
6. choking
7. nausea or abdominal distress
8. depersonalization or derealization
9. paresthesia
10. flushes or chills
11. chest pain or discomfort
12. fear of dying
13. fear of going crazy or losing control.

Appendix 4.

Visual Analog Scale

Name: _____

ID _____

Date: _____

Time of Evaluation: _____

	Not at all	Moderately	Extremely (most ever)	Score (mm)
talkative				_____
happy				_____
drowsy				_____
nervous				_____
sad				_____
calm				_____
depressed				_____
anxious				_____
energetic				_____
fearful				_____
mellow				_____
high				_____
angry				_____

Appendix 5. Advertisement for Subject Recruitment

VOLUNTEERS NEEDED FOR RESEARCH ON ANXIETY

The division of Psychopharmacology of St. Mary's Hospital Centre is currently seeking healthy male volunteers to participate in a research protocol on anxiety. All subjects will be required to undergo evaluation (medical and psychological) to determine whether they are eligible for the study. Eligible subjects who agree to participate will be required to spend about seven hours at our clinic. In the morning, they will receive a milkshake containing several amino acids and in the afternoon they will receive an injection of a peptide (protein) which may or may not induce symptoms of anxiety. You will be paid \$140.00 for your total participation.

Volunteers must be:

- *Males, 18-40 years old
- *Non- or light smoker (<5 cigarettes/day)
- *Non- or light drinker
- *No history of psychological/emotional problems
- *No family history (parents, brothers, sisters) of psychiatric illness

If you meet these requirements and think you might be interested in participating please leave your name and phone number at the following number 345-3077 (specify that you are a volunteer). You will be contacted by a research nurse (Kim or Janice) as soon as possible.

Appendix 6. LOW PROTEIN DIET

	Weight (g)	Protein (g)	Fat (g)	Carbohy- drates (g)	kcal
<u>BREAKFAST</u>					
Banana 2	228	2.4	2	54	210
Orange juice	129	0.8	0	13	52
White toast 2 slices	42	4.0	2	24	128
Margarine	10	0	8	0	68
Jelly	42	0	0	30	116
Decaf coffee/tea		0	0	0	
Coffee whitner (1 tsp)		0.1	1	1.8	16
Sugar 2 packages	8	0	0	8	32
<u>LUNCH</u>					
Shredded lettuce	80	0.7	0	2	10
Raw carrots	55	0.6	0	5	23
Raw celery (1 stalk)	40	0.3	0	2	6
Tomatoe (1)	123	1.3	0	6	27
Cucumber (1 cup)	52	0.3	0	2	7
Salad oil (1 tbsp)		0.9	5.7	1.1	55
Raisins (1 package)	45	1.5	0	36	136
Apple (1)	140	0	0	21	82
Peach (1)	90	0.6	0	10	38
Cookies (2)	45	0.9	2.8	12.5	77
Decaf coffee/tea					
Coffee whitner (1 tsp)		0.1	1	1.8	16
Sugar 2 packages	8	0	0	8	32
<u>DINNER</u>					
Stir fried vegetable					
Onions(3 tbsp)	40	0	0	3	12
Carrots	55	0.5	0	4	17
Celery(1 stalk)	40	0.3	0	1	6
Broccoli(1/2 cup)	44	1.4	0	2	11
Cauliflower(1/2 cup)	50	1.2	0	2	11
Mushrooms(1/2 cup)	35	0.9	0	1	39
Green pepper(1/2 cup)	50	0	0	3	13
Oil (3 tbsp)	45	0	44	0	386
Applesauce (1/2 cup)	128	0.2	0	25	97
Peach (1)	90	0.6	0	10	38
<u>SNACK</u>					
Raisins (1 package)	45	1.5	0	36	136
Cookies (2)	45	0.9	2.8	12.5	77

Appendix 7

Results of Pilot Study with Placebo Injection

TABLE 1

Effects of the Amino Acid Diets on Behavioral Response to Placebo Injection

Variable	Control Diet (n = 5)	Tryptophan Free Diet (n = 5)	W	Exact P
Number of PSS symptoms	0.80 ± 0.6	0.60 ± 0.4	28.0	1.00
Sum intensity of PSS symptoms	0.80 ± 0.6	0.60 ± 0.4	28.0	1.00
Number of response bias items	0.0 ± 0.0	0.0 ± 0.0	27.5	1.00

Values are the mean ± SEM. The Wilcoxon test was employed because of the small sample size.

TABLE 2.
Effect of the Amino Acid Diets on the Intensity of DSM-III-R Panic Symptoms Induced by Placebo Injection

Symptoms	Control Diet	Tryptophan Free Diet	W	P
Dyspnea	0.0 ± 0.0	0.2 ± 0.2	25.0	.6905
Dizziness	0.0 ± 0.0	0.0 ± 0.0	27.5	1.00
Unsteadiness	0.0 ± 0.0	0.0 ± 0.0	27.5	1.00
Faintness	0.0 ± 0.0	0.0 ± 0.0	27.5	1.00
Palpitations/rapid heart	0.4 ± 0.4	0.4 ± 0.4	30.0	.6905
Trembling/shaking	0.2 ± 0.2	0.0 ± 0.0	27.5	1.00
Sweating	0.0 ± 0.0	0.0 ± 0.0	27.5	1.00
Choking	0.0 ± 0.0	0.0 ± 0.0	27.5	1.00
Nausea	0.0 ± 0.0	0.0 ± 0.0	27.5	1.00
Abdominal distress	0.0 ± 0.0	0.0 ± 0.0	27.5	1.00
Feeling detached/unreal	0.0 ± 0.0	0.0 ± 0.0	27.5	1.00
Paresthesia	0.0 ± 0.0	0.0 ± 0.0	27.5	1.00
Hot flushes/chills	0.0 ± 0.0	0.0 ± 0.0	27.5	1.00
Chest pain/discomfort	0.0 ± 0.0	0.0 ± 0.0	27.5	1.00
Anxiety/fear/apprehension	0.2 ± 0.0	0.0 ± 0.0	30.0	.3171
Fear of dying	0.0 ± 0.0	0.0 ± 0.0	27.5	1.00
Fear of losing control	0.0 ± 0.0	0.0 ± 0.0	27.5	1.00
Fear of going crazy	0.0 ± 0.0	0.0 ± 0.0	27.5	1.00

Values are the mean ± SEM. The Wilcoxon test was employed because of the small sample size.

TABLE 3

Effect of the Amino Acid Diets on Visual Analog Ratings of Anxious, Fearful and Nervous Mood Following Placebo Injection

Time of Assessment	Diet	Anxious Mood	Fearful Mood	Nervous Mood
Pre-placebo	Control	9.8 ± 4.7	3.2 ± 2.5	8.8 ± 5.4
	TRP -	11.0 ± 5.0	4.4 ± 4.4	11.0 ± 8.9
Peak	Control	15.8 ± 10.7	6.4 ± 6.4	9.0 ± 9.0
	TRP-	12.0 ± 5.7	3.0 ± 3.0	10.8 ± 6.6
+ 5 mins.	Control	6.0 ± 4.3	0.0 ± 0.0	1.0 ± 1.0
	TRP-	9.4 ± 4.3	1.4 ± 1.4	6.4 ± 4.7
+ 10 mins.	Control	6.0 ± 4.1	0.0 ± 0.0	0.0 ± 0.0
	TRP-	9.6 ± 5.0	3.4 ± 3.4	3.6 ± 2.2
+ 15 mins.	Control	7.8 ± 7.8	0.0 ± 0.0	2.4 ± 1.6
	TRP-	4.2 ± 1.9	2.4 ± 2.4	1.6 ± 1.0

Values are the mean ± SEM. Control indicates control amino acid diet and TRP- indicates tryptophan-free amino acid diet.

Friedman Analysis of Variance revealed no significant changes over time for the control or tryptophan-free amino acid diet groups, respectively, for ratings of anxious mood ($F=2.04$, $df=4$, $p=.7280$ and $F=6.04$, $df=4$, $p=.1962$), fearful mood ($F=1.44$, $df=4$, $p=.8372$ and $F=0.80$, $df=4$, $p=.9384$) and nervous mood ($F=1.80$, $df=4$, $p=.7725$ and $F=2.92$, $df=4$, $p=.5713$).

Appendix 8. Informed Consent Form

THE ROLE OF SEROTONIN IN CCK-4-INDUCED PANIC SYMPTOMS

I, _____ am invited to participate as a healthy volunteer in a study on the effect of tryptophan depletion by dietary means on cholecystokinin tetrapeptide (CCK-4)-induced panic attacks. The study will be conducted by Dr. Bradwejn and colleagues. The details of their project having been explained to me and I understand that:

The goal of the project is to study the effect of tryptophan depletion on CCK-4-induced panic attacks. CCK is a peptide (protein) that occurs naturally in the human brain. This natural peptide had been postulated by Dr. Bradwejn and his colleagues to play a role in anxiety and panic attacks. Tryptophan is an amino acid (building block of proteins) which is present in protein meals.

Subjects who are interested in participating in this study will be questioned about their general physical health and about whether they or any family member, including parents and siblings, have a history of emotional problems (e.g. depression or anxiety). This evaluation will determine whether the subject is eligible for participation in the study. Subjects will be required to undergo a physical examination and medical tests (blood tests, urine test, electrocardiogram) to assure that they are fit for the study. These tests will be performed at St. Mary's Hospital.

Subjects who are selected for the study will be asked to come to St Mary's Hospital on one day. Subjects will be asked follow a low protein diet which will be provided by the researchers the day before the test day and to arrive at St. Mary's Hospital at 8:30 AM on the test day without having eaten since midnight. On the test day, subjects will be given either a milk shake containing all the amino acids normally found in protein or a milk shake containing every amino acids except tryptophan. Five hours later the subject will be given one injection of CCK-4. The experiment will finish around 5:00 P.M.

On the test day, subjects will be asked to answer questionnaires and to fill out pencil and paper tests. Blood pressure and pulse will be measured on several occasions before and after the injection of CCK-4. Blood will be taken before the ingestion of the milk-shakes and before and after the injection of CCK-4. The total amount of blood drawn will be 150 ml.

It is possible that injections of CCK₄ might produce symptoms associated with anxiety and/or panic. These symptoms include: dizziness, unsteady feeling or faintness, palpitations or accelerated heart beat, trembling or shaking,

sweating, choking, nausea or abdominal distress, feeling detached from your body, feeling unreal, numbness or tingling, flushes or chills, chest pain or discomfort, fear of going crazy, losing control, or a fear of dying. Subjects may or may not experience any of the above symptoms and it is unlikely that they will experience all of them. The duration of symptoms usually last from 2 to 5 minutes.

Subjects may also experience momentary and/or minor discomfort associated with the various procedures that are required. For instance, there may be some pain or bruising associated with the injections or the blood drawing.

Participation in this project could contribute to the scientific knowledge of psychiatric disorders and their treatment. Participation is voluntary. Withdrawal from the project is permitted at any time.

All documents pertaining to your participation in the project will remain strictly confidential.

All questions concerning this project will be answered appropriately by the investigators.

I understand that there is no specific advantage to me of participating in this study, but that information from this type of study may eventually be useful in diagnosis and treatment of anxiety and panic disorders.

I understand that any expenses for transportation or lost work time will be reimbursed to me up to a maximum of \$140 for the completed experiment.

I understand that any questions I may have concerning this research will be answered by Dr. Jacques Bradwejn, Diana Koszycki (psychologist) or one of the research assistants (Kim or Janice) who can be reached at 345-3584 or 345-3077.

Signed on the _____ day of _____, 19_____
 _____ Signature of the Subject
 _____ Signature of the Witness

I, _____ certify that I have explained to the person above, the nature of the project, the possible risks, and the liberty of accepting or refusing participation, as well as of withdrawing from the project at any point in time during the study.

Signature of Physician

APPENDIX 9**Analysis of Variance Tables**

APPENDIX 9a

ANOVA for Data in Table 1

Analysis of variance of baseline heart rate in subjects assigned to a tryptophan-free or control amino acid diet. Prediet heart rate score was used as a covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	1316.136	1316.136	18.200	.000
Main Effects					
Diet	1	33.029	33.029	0.457	.505
Explained	2	1349.165	674.582	9.329	.000
Residual	37	2675.610	72.314		
Total	39	4024.775	103.199		

Analysis of variance of baseline systolic blood pressure in subjects assigned to a tryptophan-free or control amino acid diet. Prediet systolic blood pressure score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	1979.110	1979.110	15.102	.000
Main Effects					
Diet	1	107.644	107.644	0.821	.371
Explained	2	2086.755	1043.377	7.962	.001
Residual	37	4848.745	131.047		
Total	39	6935.500	177.833		

Analysis of variance of baseline diastolic blood pressure in subjects assigned to a typtophan-free or control amino acid diet. Prediet diastolic blood pressure score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	231.011	231.011	3.173	.083
Main Effects					
Diet	1	3.042	3.042	0.042	.839
Explained	2	234.053	117.027	1.607	.214
Residual	37	2693.722	72.803		
Total	39	2927.775	75.071		

Analysis of variance of baseline ACTH concentrations in subjects assigned to a tryptophan-free or control amino acid diet. Prediet ACTH level was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	1094.319	1094.319	69.541	.000
Main Effects					
Diet	1	7.208	7.208	0.458	.503
Explained	2	1101.527	550.764	34.999	.000
Residual	37	582.248	15.736		
Total	39	1683.775	43.174		

Analysis of variance of baseline cortisol concentrations in subjects assigned to a tryptophan-free or control amino acid diet. Prediet cortisol level was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	156.670	156.670	0.013	.910
Main Effects					
Diet	1	3949.446	3949.446	0.327	.571
Explained	2	4106.116	2053.058	0.170	.845
Residual	37	447495.784	12094.481		
Total	39	451601.900	11579.536		

Analysis of variance of baseline growth hormone concentrations in subjects assigned to a tryptophan-free or control amino acid diet. Prediet growth hormone level was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	18.578	18.578	1.010	.321
Main Effects					
Diet	1	20.815	20.815	1.132	.294
Explained	2	39.392	19.696	1.071	.353
Residual	37	680.379	18.389		
Total	39	719.771	18.456		

APPENDIX 9b**ANOVA for the Data in Table 2**

Analysis of variance of baseline VAS nervous mood in subjects assigned to a tryptophan-free or control amino acid diet. Prediet nervous score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	4845.725	4825.725	12.526	.000
Main Effects					
Diet	1	834.175	834.175	2.156	.150
Explained	2	5679.900	2839.950	7.341	.002
Residual	37	14313.700	386.857		
Total	39	19993.600	512.656		

Analysis of variance of baseline VAS anxious mood in subjects assigned to a tryptophan-free or control amino acid diet. Prediet anxious score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	9577.426	9577.426	37.761	.000
Main Effects					
Diet	1	460.103	460.103	1.814	.186
Explained	2	10037.528	5018.764	19.787	.000
Residual	37	9384.472	253.634		
Total	39	19422.000	498.000		

Analysis of variance of baseline VAS fearful mood in subjects assigned to a tryptophan-free or control amino acid diet. Prediet fearful score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	53.624	53.624	0.279	.600
Main Effects					
Diet	1	810.163	810.163	4.219	.047
Explained	2	863.787	431.893	2.249	.120
Residual	37	7104.988	192.027		
Total	39	7968.775	204.328		

Analysis of variance of baseline VAS talkative mood in subjects assigned to a tryptophan-free or control amino acid diet. Prediet talkative score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	2869.076	2869.076	12.029	.001
Main Effects					
Diet	1	1.173	1.173	0.005	.944
Explained	2	2870.249	1435.124	6.017	.005
Residual	37	8825.251	238.520		
Total	39	11695.500	299.885		

Analysis of variance of baseline VAS happy mood in subjects assigned to a tryptophan-free or control amino acid diet. Prediet happy score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	40.832	40.832	0.171	.682
Main Effects					
Diet	1	127.438	127.438	0.533	.470
Explained	2	168.270	84.135	0.352	.706
Residual	37	8847.705	239.127		
Total	39	9015.975			

Analysis of variance of baseline VAS sad mood in subjects assigned to a tryptophan-free or control amino acid diet. Prediet sad score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	271.705	271.705	3.685	.063
Main Effects					
Diet	1	119.823	119.823	1.625	.210
Explained	2	391.528	195.764	2.655	
Residual	37	2727.847	73.726		
Total	39	3119.375	79.984		

Analysis of variance of baseline VAS depressed mood in subjects assigned to a tryptophan-free or control amino acid diet. Prediet depressed score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	212.282	212.282	10.185	.003
Main Effects					
Diet	1	93.624	93.624	4.492	.041
Explained	2	305.907	152.953	7.338	.002
Residual	37	771.193	20.843		
Total	39	1077.100	27.618		

Analysis of variance of baseline VAS calm mood in subjects assigned to a tryptophan-free or control amino acid diet. Prediet calm score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	2335.010	2335.010	5.822	.021
Main Effects					
Diet	1	31.052	31.052	0.077	.782
Explained	2	2366.062	1183.031	2.950	.065
Residual	37	14839.713	401.073		
Total	39	17205.775	441.174		

Analysis of variance of baseline VAS mellow mood in subjects assigned to a tryptophan-free or control amino acid diet. Prediet mellow score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	4115.171	4115.171	10.517	.003
Main Effects					
Diet	1	701.657	701.657	1.793	.189
Explained	2	4816.828	2408.414	6.155	.005
Residual	37	14477.072	391.272		
Total	39	19293.900	494.715		

Analysis of variance of baseline VAS high mood in subjects assigned to a tryptophan-free or control amino acid diet. Prediet high score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	1217.729	1217.728	8.288	.007
Main Effects					
Diet	1	418.191	418.191	2.846	.100
Explained	2	1635.920	817.960	5.567	.008
Residual	37	5435.980	146.918		
Total	39	7071.00	181.331		

Analysis of variance of baseline VAS angry mood in subjects assigned to a tryptophan-free or control amino acid diet. Prediet angry score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	157.576	157.576	30.637	.000
Main Effects					
Diet	1	0.019	0.019	0.004	.952
Explained	2	157.595	78.797	15.320	.000
Residual	37	190.305	5.143		
Total	39	347.900	8.921		

Analysis of variance of baseline VAS drowsy mood in subjects assigned to a tryptophan-free or control amino acid diet. Prediet score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	2315.144	2315.144	5.018	.031
Main Effects					
Diet	1	845.731	845.731	1.833	.184
Explained	2	3160.875	1580.438	3.425	.043
Residual	37	17017.500	461.392		
Total	39	20232.375	518.779		

Analysis of variance of baseline VAS energetic mood in subjects assigned to a tryptophan-free or control amino acid diet. Prediet energetic score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	523.316	523.316	2.124	.153
Main Effects					
Diet	1	74.268	74.268	0.301	.586
Explained	4	597.585	298.792	1.213	.309
Residual	37	9116.190	246.384		
Total	39	9713.775	249.071		

APPENDIX 9c**ANOVA for the Data in Table 3**

Analysis of variance of baseline fearful mood in panickers and nonpanickers.
 Prediet fearful score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	53.624	53.624	0.271	.606
Main Effects					
Diet	1	815.200	815.200	4.119	.050
Panic	1	168.890	168.890	0.853	.362
Interaction	1	8.869	8.869	0.045	.834
Explained	4	1041.546	260.386	1.316	.283
Residual	35	6927.229	197.921		
Total	39	7968.775	204.328		

Analysis of variance of baseline anxious mood in panickers and nonpanickers.
 Prediet anxious score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	9577.426	9577.426	36.766	.000
Main Effects					
Diet	1	468.755	468.755	1.799	.188
Panic	1	9.281	9.281	0.036	.851
Interaction	1	257.782	257.782	0.990	.327
Explained	4	10304.591	2576.148	9.889	.000
Residual	35	9117.409	260.497		
Total	39	19422.00	19422.00		

Analysis of variance of baseline nervous mood in panickers and nonpanickers.
Prediet nervous score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	4845.725	4845.725	12.116	.001
Main Effects					
Diet	1	811.789	811.789	2.030	.163
Panic	1	7.540	7.540	0.019	.892
Interaction	1	307.968	307.968	0.770	.386
Explained	4	5995.408	1498.852	3.748	.012
Residual	35	13998.192	399.948		
Total	39	19993.600	512.656		

Analysis of variance of baseline heart rate in panickers and nonpanickers.
 Prediet heart rate score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	1316.136	1316.126	17.478	.000
Main Effects					
Diet	1	25.990	25.990	0.345	.561
Panic	1	34.467	34.467	0.458	.503
Interaction	1	5.522	5.522	0.073	.788
Explained	4	1389.154	347.289	4.612	.004
Residual	35	2635.621	75.303		
Total	39	4024.775	103.199		

Analysis of variance of baseline systolic blood pressure in panickers and nonpanickers. Prediet systolic blood pressure score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	1979.110	1979.110	15.956	.000
Main Effects					
Diet	1	76.418	76.418	0.616	.438
Panic	1	262.580	262.580	2.117	.155
Interaction	1	244.833	244.833	1.974	.169
Explained	4	2594.167	648.542	5.229	.002
Residuals	35	4341.333	124.038		
Total	39	6935.500	177.833		

Analysis of variance of baseline diastolic blood pressure in panickers and nonpanickers. Prediet diastolic blood pressure score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	231.011	231.001	3.269	.079
Main Effects					
Diet	1	3.908	3.908	0.055	.815
Panic	1	5.878	5.878	0.083	.775
Interaction	1	214.257	214.257	3.032	.090
Explained	4	454.188	113.547	1.607	.194
Residuals	35	2473.587	70.674		
Total	39	2927.775	75.071		

Analysis of variance of baseline ACTH concentrations in panickers and nonpanickers. Prediet ACTH score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	1094.319	1094.319	66.620	.000
Main Effects					
Diet	1	8.438	8.438	0.257	.775
Panic	1	6.553	6.553	0.399	.532
Interactions	1	6.098	6.098	0.371	.546
Explained	4	1108.856	277.214	16.876	.000
Residuals	35	574.919	16.426		
Total	39	1683.775	43.174		

Analysis of variance of baseline cortisol concentrations in panickers and nonpanickers. Prediet cortisol score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	156.670	156.670	0.012	.912
Main Effects					
Diet	1	3422.076	3422.076	0.269	.607
Panic	1	1557.965	1557.965	0.123	.728
Interaction					
Explained	4	6549.484	1637.371	0.129	.971
Residuals	35	445052.416	12715.783		
Total	39	451601.900	11579.536		

4 of/de 4

PM-1 3½"x4" PHOTOGRAPHIC MICROCOPY TARGET
NBS 1010a ANSI/ISO #2 EQUIVALENT

PRECISIONSM RESOLUTION TARGETS

Analysis of variance of baseline growth hormone concentrations in panickers and nonpanickers. Prediet growth hormone score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	18.578	18.578	0.970	.331
Main Effects					
Diet	1	17.787	17.787	0.929	.342
Panic	1	3.242	3.242	0.169	.683
Interaction	1	6.961	6.961	0.364	.550
Explained	4	49.596	12.399	0.648	.632
Residual	35	670.175	19.148		
Total	39	719.771	18.456		

Analysis of variance of baseline prolactin concentrations in panickers and nonpanickers. Prediet prolactin score was used as the covariate.

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Covariate	1	86.603	86.603	5.867	.021
Main Effects					
Diet	1	0.127	0.127	0.009	.927
Panic	1	10.227	10.227	0.693	.411
Interaction					
Explained	4	101.293	25.323	1.716	.169
Residual	35	516.607	14.760		
Total	39	617.900	15.844		

APPENDIX 9d

ANOVA for Data in Tables 6 and 8

Analysis of variance with repeated measures for VAS fearful mood following administration of CCK-4 in subjects assigned to the tryptophan-free or control amino acid diet and in panickers and nonpanickers

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Tests of Between-Subject Effects	1,36				
Diet		1763.53	1763.53	3.66	.064
Panic		4294.41	4294.41	8.90	.005
Diet x Panic		296.02	296.02	0.61	.439
Tests Within-Subjects Effects	4,144				
Time		37067.14	9266.79	63.82	.000
Diet x Time		1162.19	290.55	2.00	.097
Panic x Time		5382.86	1345.71	9.27	.000
Diet x Panic x Time		117.62	29.40	0.20	.937

Analysis of variance with repeated measures for VAS anxious mood following administration of CCK-4 in subjects assigned to the tryptophan-free or control amino acid diet and in panickers and nonpanickers

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Tests of Between-Subject Effects	1,36				
Diet		449.94	449.94	0.42	.523
Panic		5123.31	5123.31	4.75	.036
Diet x Panic		739.92	739.92	0.69	.413
Tests Within-Subjects Effects	4,144				
Time		58174.21	14543.55	46.70	.000
Diet x Time		1009.34	252.33	0.81	.521
Panic x Time		3408.95	3408.95	2.74	.031
Diet x Panic x Time		314.64	78.66	0.25	.908

Analysis of variance with repeated measures for VAS nervous mood following administration of CCK-4 in subjects assigned to the tryptophan-free or control amino acid diet and in panickers and nonpanickers

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Tests of Between-Subject Effects	1,36				
Diet		13.19	13.19	2.74	.106
Panic		3.07	3.07	0.64	.430
Diet x Panic		15.51	15.51	2.81	.102
Tests Within-Subjects Effects	4,144				
Time		172.74	43.06	5.87	.000
Diet x Time		4.26	1.06	1.26	.290
Panic x Time		1.29	0.32	0.38	.822
Diet x Panic x Time		11.12	2.78	3.28	.013

* Data analysis was performed of logarithmically transformed data

Analysis of variance with repeated measures for VAS calm mood following administration of CCK-4 in subjects assigned to the tryptophan-free or control amino acid diet and in panickers and nonpanickers

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Tests of Between-Subject Effects	1,36				
Diet		1562.37	1562.37	1.91	.175
Panic		570.98	570.98	0.70	.409
Diet x Panic		0.85	0.85	0.00	.974
Tests Within-Subjects Effects	4,144				
Time		32148.41	8037.10	28.13	.000
Diet x Time		2343.85	585.96	2.05	.090
Panic x Time		5592.97	1398.24	4.89	.001
Diet x Panic x Time		1042.29	260.57	0.91	.459

Analysis of variance with repeated measures for VAS mellow mood following administration of CCK-4 in subjects assigned to the tryptophan-free or control amino acid diet and in panickers and nonpanickers

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Tests of Between-Subject Effects	1,36				
Diet		3179.72	3179.72	2.26	.142
Panic		380.60	380.60	0.27	.606
Diet x Panic		13.74	13.74	0.01	.922
Tests Within-Subjects Effects	4,144				
Time		32884.81	8221.20	34.14	.000
Diet x Time		384.45	96.11	0.40	.809
Panic x Time		2478.14	619.53	2.57	.040
Diet x Panic x Time		1064.98	266.25	1.11	.356

Analysis of variance with repeated measures for VAS drowsy mood following administration of CCK-4 in subjects assigned to the tryptophan-free or control amino acid diet and in panickers and nonpanickers

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Tests of Between-Subject Effects	1,36				
Diet		779.63	779.63	0.71	.405
Panic		1680.68	1680.68	1.53	.224
Diet x Panic		1512.87	1512.87	1.38	.248
Tests Within-Subjects Effects	4,144				
Time		2946.80	736.70	2.18	.074
Diet x Time		1428.28	357.12	1.06	.380
Panic x Time		49.12	239.41	0.71	.587
Diet x Panic x Time		957.63	239.41	0.71	.587

Analysis of variance with repeated measures for VAS energetic mood following administration of CCK-4 in subjects assigned to the tryptophan-free or control amino acid diet and in panickers and nonpanickers

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Tests of Between-Subject Effects	1,36				
Diet		17.43	17.43	4.43	.042
Panic		6.82	6.82	1.73	.197
Diet x Panic		6.24	6.24	1.58	.216
Tests Within-Subjects Effects	4,144				
Time		24.25	6.06	7.25	.000
Diet x Time		0.47	0.12	0.14	.967
Panic x Time		0.91	0.23	0.27	.896
Diet x Panic x Time		2.64	0.66	0.79	.535

* Data analysis was performed of logarithmically transformed data

Analysis of variance with repeated measures for VAS sad mood following administration of CCK-4 in subjects assigned to the tryptophan-free or control amino acid diet and in panickers and nonpanickers

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Tests of Between-Subject Effects	1,36				
Diet		244.21	244.21	1.40	.245
Panic		281.89	281.89	1.61	.212
Diet x Panic		542.89	542.89	3.10	.087
Tests Within-Subjects Effects	4,144				
Time		771.15	192.79	2.78	.029
Diet x Time		177.67	44.42	0.64	.635
Panic x Time		127.83	31.96	0.46	.765
Diet x Panic x Time		88.25	22.06	0.32	.866

Analysis of variance with repeated measures for VAS depressed mood following administration of CCK-4 in subjects assigned to the tryptophan-free or control amino acid diet and in panickers and nonpanickers

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Tests of Between-Subject Effects	1,36				
Diet		344.94	344.94	1.75	.194
Panic		165.56	165.56	0.84	.365
Diet x Panic		541.61	541.61	2.75	.106
Tests Within-Subjects Effects	4,144				
Time		374.42	93.61	2.70	.033
Diet x Time		71.89	17.97	0.52	.722
Panic x Time		101.38	25.34	0.73	.572
Diet x Panic x Time		36.34	9.09	0.26	.902

Analysis of variance with repeated measures for VAS happy mood following administration of CCK-4 in subjects assigned to the tryptophan-free or control amino acid diet and in panickers and nonpanickers

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Tests of Between-Subject Effects	1,36				
Diet		4.69	4.69	3.79	.059
Panic		0.33	0.33	0.26	.611
Diet x Panic		0.27	0.27	0.22	.645
Tests Within-Subjects Effects	4,144				
Time		158.63	39.66	76.41	.000
Diet x Time		13.63	3.41	6.57	.000
Panic x Time		8.82	2.21	4.25	.003
Diet x Panic x Time		1.76	0.44	0.85	.497

* Data analysis was performed of logarithmically transformed data

Analysis of variance with repeated measures for VAS high mood following administration of CCK-4 in subjects assigned to the tryptophan-free or control amino acid diet and in panickers and nonpanickers

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Tests of Between-Subject Effects	1,36				
Diet		748.61	748.61	0.63	.433
Panic		1374.37	1374.37	1.15	.290
Diet x Panic		138.50	138.50	0.12	.735
Tests Within-Subjects Effects	4,144				
Time		5772.45	1443.11	5.82	.000
Diet x Time		729.54	182.38	0.74	.569
Panic x Time		1631.91	407.98	1.65	.166
Diet x Panic x Time		615.33	153.83	0.62	.649

Analysis of variance with repeated measures for VAS angry mood following administration of CCK-4 in subjects assigned to the tryptophan-free or control amino acid diet and in panickers and nonpanickers

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Tests of Between-Subject Effects	1,36				
Diet		21.17	21.17	8.66	.006
Panic		6.44	6.44	2.63	.113
Diet x Panic		7.96	7.96	3.26	.079
Tests Within-Subjects Effects	4,144				
Time		3.56	0.89	3.30	.013
Diet x Time		0.45	0.11	0.42	.796
Panic x Time		3.40	0.85	3.15	.016
Diet x Panic x Time		0.65	0.16	0.60	.663

* Data analysis was performed of logarithmically transformed data

Analysis of variance with repeated measures for VAS talkative mood following administration of CCK-4 in subjects assigned to the tryptophan-free or control amino acid diet and in panickers and nonpanickers

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Tests of Between-Subject Effects	1,36				
Diet		9.43	9.43	4.44	.042
Panic		0.31	0.31	0.15	.703
Diet x Panic		3.79	3.79	1.79	.190
Tests Within-Subjects Effects	4,144				
Time		115.85	28.96	41.69	.000
Diet x Time		6.53	1.63	2.35	.057
Panic x Time		2.10	0.52	0.75	.557
Diet x Panic x Time		4.00	1.00	1.44	.223

* Data analysis was performed of logarithmically transformed data

APPENDIX 9e (Figures 1 and 2)

Analysis of variance with repeated measures for changes in heart rate following administration of CCK-4 in subjects assigned to the tryptophan-free or control amino acid diet and in panickers and nonpanickers

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Tests of Between-Subject Effects	1,35				
Diet		4.59	4.59	0.00	.962
Panic		399.86	399.86	0.20	.659
Diet x Panic		5219.23	5219.23	2.58	.117
Tests of Within-Subject Effects	9,315				
Time		70399.59	7822.18	1.10	.363
Diet x Time		1188.13	132.01	0.34	.961
Panic x Time		367.68	40.85	0.34	.961
Diet x Panic x Time		604.17	67.13	0.56	.830

APPENDIX 9f (Figures 3 and 4)

Analysis of variance with repeated measures for changes in systolic blood pressure following administration of CCK-4 in subjects assigned to the tryptophan-free or control amino acid diet and in panickers and nonpanickers

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Tests of Between-Subject Effects	1,36				
Diet		2236.70	2236.70	1.12	.296
Panic		8958.67	8958.67	4.50	.041
Diet x Panic		143.46	143.46	0.07	.790
Tests of Within-Subject Effects	9,324				
Time		6682.53	742.50	6.68	.000
Diet x Time		560.69	62.30	0.56	.829
Panic x Time		1121.39	124.60	1.12	.347
Diet x Panic x Time		773.22	85.91	0.77	.641

APPENDIX 9g (Figures 5 and 6)

Analysis of variance with repeated measures for changes in diastolic blood pressure following administration of CCK-4 in subjects assigned to the tryptophan-free or control amino acid diet and in panickers and nonpanickers

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Tests of Between-Subject Effects	1,36				
Diet		270.09	270.09	0.31	.582
Panic		844.29	844.29	0.96	.333
Diet x Panic		872.82	872.82	1.00	.325
Tests of Within-Subject Effects	9,324				
Time		3490.79	387.87	6.85	.000
Diet x Time		436.25	48.47	0.86	.565
Panic x Time		1062.11	118.01	2.08	.031
Diet x Panic x Time		404.93	44.99	0.79	.622

APPENDIX 9h (Figures 7 and 8)

Analysis of variance with repeated measures for changes in ACTH concentrations following administration of CCK-4 in subjects assigned to the tryptophan-free or control amino acid diet and in panickers and nonpanickers

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Tests Between-Subjects Effects	1,36				
Diet		0.11	0.11	0.11	.741
Panic		0.72	0.11	0.71	.404
Diet x Panic		0.02	0.11	0.02	.887
Tests Within-Subject Effects	4,144				
Time		6.23	1.56	48.01	.000
Diet x Time		0.30	0.08	2.33	.059
Panic x Time		0.49	0.12	3.74	.006
Panic x Diet x Time		0.16	0.04	1.26	.290

* Data analysis was performed of logarithmically transformed data

APPENDIX 9i (Figures 9 and 10)

Analysis of variance with repeated measures for changes in cortisol concentrations following administration of CCK-4 in subjects assigned to the tryptophan-free or control amino acid diet and in panickers and nonpanickers

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Tests Between-Subjects Effects	1,36				
Diet		1924.37	1924.37	0.04	.847
Panic		30476.34	30476.34	0.60	.445
Diet x Panic		5099.57	5099.57	0.10	.754
Tests Within-Subject Effects	4,144				
Time		639586.50	159896.63	122.59	.000
Diet x Time		14528.44	3632.11	2.78	.029
Panic x Time		3030.30	757.57	0.58	.677
Diet x Panic x Time		2254.93	563.73	0.43	.785

APPENDIX 9j (Figures 11 and 12)

Analysis of variance with repeated measures for changes in growth hormone concentrations following administration of CCK-4 in subjects assigned to the tryptophan-free or control amino acid diet and in panickers and nonpanickers

<u>Source of Variation</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Tests Between-Subject Effects	1,36				
Diet		31.43	81.43	0.77	.385
Panic		102.27	102.27	0.97	.331
Diet x Panic		66.95	66.95	0.64	.430
Tests Within-Subject Effects	4,144				
Time		18.87	4.72	3.41	.011
Diet x Time		2.28	0.57	0.41	.799
Panic x Time		36.76	9.19	6.65	.000
Diet x Panic x Time		5.19	1.30	0.94	.443

APPENDIX 9j (Figures 13 and 14)

Analysis of variance with repeated measures for changes in prolactin concentrations following administration of CCK-4 in subjects assigned to the tryptophan-free or control amino acid diet and in panickers and nonpanickers

<u>Source of Variation</u>	<u>df</u>	<u>MM</u>	<u>SS</u>	<u>F</u>	<u>P</u>
Tests Between-Subject Effects	1,36				
Diet		321.57	321.57	1.21	.278
Panic		174.88	174.88	0.66	.422
Diet x Panic		98.20	98.20	0.37	.547
Tests Within-Subject Effects	4,144				
Time		2314.87	578.72	37.89	.000
Diet x Time		128.87	32.22	2.11	.083
Panic x Time		218.11	54.53	3.57	.008
Diet x Panic x Time		31.81	7.95	0.52	.721

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