# ORIGINAL ARTICLES

# Pentagastrin Infusions in Patients with Panic Disorder I. Symptoms and Cardiovascular Responses

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*Cholecystokinin (CCK) may mediate human anxiety and animal data suggest that cholecystokinin antagonists could provide an important advance in the treatment of anxiety disorders. The study of CCK receptor systems in psychiatric patients has, however, been severely limited by the lack of available probes. We utilized intravenous infusions of pentagastrin, a selective CCK-B receptor agonist, and studied behavioral and cardiovascular responses in 10 patients with panic disorder and 10 normal controls. Pentagastrin produced substantial symptomatology, including anxiety, and increases in heart rate and blood pressure, in both patients and controls. Patients were more sensitive to the panicogenic effects of the pentagastrin. Panic attacks occurred in 70% of patients and 0% of controls. Patients' symptom responses were very similar to their "typical" panic attacks and to symptoms produced by $CCK_4$. Pentagastrin provides a readily available alternative to $CCK_4$ for studying the CCK receptor system and exploring its involvement in human anxiety.*

**Key Words:** Pentagastrin, cholecystokinin, panic disorder, symptoms, heart rate, blood pressure

## Introduction

A growing body of basic research suggests that cholecystokinin (CCK) may mediate anxiety. CCK agonists, such as $CCK_4$ and pentagastrin, are anxiogenic in both animals and humans (Abelson and Nesse 1990; Bradwejn et al 1991; Singh et al 1991) and CCK receptor antagonists can block anxiety (Bradwejn et al 1993; Singh et al 1991). Preclinical studies using recently developed, highly selective CCK-B receptor antagonists suggest that these drugs have potential utility in the treatment of anxiety disorders and drug abuse (Costall et al 1991; Hughes et al 1991) and may provide anxiolytic activity without significant sedation, tolerance, or withdrawal on abrupt discontinuation (Costall et al 1991). These drugs may also provide critical new tools for studying CCK receptor systems in humans.

Despite advances in our basic understanding of CCK systems and the therapeutic potential of new antagonists, there have been few detailed human studies of the behavioral, clinical, physiological, and neuroendocrine effects of available CCK agonists. The largest body of work to date, by Bradwejn and colleagues, demonstrates that $CCK_4$ can induce panic attacks and that panic patients are more sensitive to this panicogenic effect than normal subjects (Brad-

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wejn et al 1991). This Montreal group has provided extensive data to support the use of $CCK_4$ as a laboratory model of panic (Bradwejn et al 1990, 1991, 1992a, 1992b, 1993). To date, however, no replications from other laboratories have been published, and few neuroendocrine studies have been done. Wider study may be inhibited by limited access to $CCK_4$ for human use in many countries, including the United States.

Pentagastrin is a 5-amino acid synthetic analogue of gastrin developed for diagnostic evaluation of gastric acid secretory function (Baron 1972). It contains $CCK_4$ as its carboxyl terminal sequence. Gastrin and CCK are closely related peptide families and both pharmacological (Lotti and Chang 1989) and cloning data (Pisegna et al 1992) suggest that the gastrin and CCK-B receptors are identical. Two CCK receptors have been identified (Saito et al 1980) and were initially dichotomized as central (CCK B or brain) and peripheral (CCK-A or alimentary) types. Though recent work suggests that both receptors are found in the brain (Woodruff and Hughes 1991), the CCK-B subtype predominates and is widely distributed centrally in all mammals studied (Woodruff and Hughes 1991). Gastrin-like immunoreactivity was first demonstrated in the brain in 1975 (Vanderhaeghen et al 1975) and is now known to be comprised of CCK/gastrins of varying length molecular forms (Morley et al 1984; Sauter and Frick 1983). True gastrins (Rehfeld 1978a, 1978b), the CCK octapeptide ($CCK_8$), and smaller CCK fragments (Morley et al 1984; Sauter and Frick 1983) have all been isolated from the mammalian brain and have demonstrated differing affinities for CCK-A and B receptors. Both receptors have high affinity for sulfated $CCK_8$, whereas desulfated forms (including gastrin, pentagastrin, desulfated $CCK_8$, and $CCK_4$) are selective for the gastrin/CCK-B receptor (Hughes et al 1990). The tetrapeptide (Phe-Asp-Met-Trp) carboxyl terminal sequence shared by naturally occurring gastrins, pentagastrin, and $CCK_4$ appears to be the minimal structure necessary for activity at gastrin/CCK-B receptors. $CCK_4$ is the most selective CCK-B receptor agonist available for use in humans. Pentagastrin has slightly greater affinity and lower selectivity for the CCK-B receptor than $CCK_4$, but is still highly selective (700-fold selectivity for the CCK-B over CCK-A receptor) (Hughes et al 1990). It is the most selective CCK-B receptor agonist available in the United States.

We began using pentagastrin infusions in 1988 to stimulate vasoactive peptide release in patients with panic disorder. Pentagastrin's ability to release such peptides (Ahlman et al 1985; Oberg et al 1989; Vinik et al 1990) and the similarity of its "side effect" profile to the symptoms of a panic attack suggested it could contribute to the search for mechanisms of panic symptom production. Bradwejn's work has now confirmed that CCK-B agonists induce anxiety and panic (Bradwejn et al 1991), though data relevant to mechanisms remain scant. Confirmation of Bradwejn's findings and the search for mechanisms would be greatly facilitated if the more readily available agonist, pentagastrin, could be shown to produce effects similar to $CCK_4$. This report presents behavioral and cardiovascular data from the first two phases of our pentagastrin studies of panic disorder patients and normal subjects. The data support the viability of pentagastrin as an alternative to $CCK_4$ for the laboratory study of panic. Because of our interest in mechanisms, neuroendocrine data was also collected and will be reported separately. Preliminary reports of some neuroendocrine (Abelson et al 1991) and behavioral data (Abelson and Nesse 1990) have already appeared, but included data from only 10 subjects who did not receive placebo control infusions.

## Methods and Materials

### *Subjects*

All 20 subjects gave informed consent and were free of psychotropic medication for at least 2 weeks prior to study. They were medically healthy as determined by history, physical examination, and screening laboratory tests. All subjects, including controls, were evaluated using a Structured Clinical Interview for DSM-III-R (Spitzer and Williams 1986). The patients were recruited through newspaper advertisements and from routine referrals to our anxiety disorders program and were paid $100 for their participation in the protocol. They all met DSM-III-R criteria for panic disorder or panic disorder with agoraphobia. They did not meet criteria for current major depression or substance abuse (within the past 6 months) and did not have any history of primary depression or psychosis. Control subjects were age-matched and gender-matched to the patients and did not meet criteria for any Axis I disorder. All women had normal menstrual cycles and were studied within 10 days of onset of menses. Total medication exposure in the months prior to study was minimal. One control subject had a few doses of a nonsteroidal antiinflamatory drug during her period 10 days prior to study and another had a few puffs from an albuterol inhaler for seasonal allergies over a week prior to study. Only two patients had been on daily pharmacological treatment for panic disorder (one was taking buspirone and another alprazolam), but both discontinued their medication over a month prior to study. A third patient had used occasional doses of lorazepam (2-3 times/week) up until 3 weeks prior to study.

### *Design*

The study design was partially shaped by constraints imposed by the neuroendocrine component. It was conducted in 2 phases in a clinical research center (CRC). In the first

phase five patients and five controls received a single infusion of pentagastrin in conjunction with frequent blood sampling to provide detailed data on hormonal response patterns. In phase two sampling frequency was reduced to allow addition of a placebo infusion session without exceeding blood volume limits. An additional five patients and five controls were recruited and were admitted on two occasions a week apart, receiving a saline placebo infusion on visit one and pentagastrin on visit two (administered in a single-blind fashion). We administered the active substance second because panic patients are reactive to novel situations and therefore stress-reactive endocrine measures were likely to be closer to baseline levels during the second visit. Phase two was identical to phase one except for the addition of the placebo infusion and the reduced blood sampling frequency.

Subjects were told that the study focused on stress hormones, whose release was stimulated by pentagastrin. The side effect profile of pentagastrin was fully described orally and in the written consent form and subjects were explicitly informed that some of the side effects were similar to symptoms commonly experienced in panic attacks. The consent form mentioned the possibility that a panic attack could occur during the study. When patients expressed apprehension about having a panic attack in response to the infusion they were told that our intent was not to induce an attack but rather to examine hormonal responses to the drug. They were assured that any intense symptomatic responses to the drug would last only a few minutes and that the study psychiatrist would be at their side to supportively assist them through whatever symptoms and anxiety they might experience. Our goal was to provide adequate reassurance and support to minimize undue distress since our primary interests were pharmacological, neuroendocrine, and mechanistic.

Control subjects were given verbal descriptions of DSM-III-R defined panic attacks prior to the study, with an attempt to connect these descriptions to their own reports of real fear experiences. Sufficient details and discussion were provided to insure that control subjects could appropriately report whether they experienced a panic attack during the procedures.

### *Procedures and Measures*

Subjects were admitted to the CRC the night prior to study and had no food from 10 PM until completion of the protocol. They were awakened at 7:30 AM and at 8 AM an indwelling heparin lock catheter was inserted into an antecubital vein. Baseline blood samples were drawn between 8:30 and 8:59 AM. At 9 AM pentagastrin (commercially available Peptavlon,™ Wyeth-Ayerst Laboratories, Philadelphia, PA) was infused into the heparin lock, in view of the patient, in less than 1 min, at a dosage of 0.6 μg/kg, in a saline vehicle of less than 1 ml. Anxiety symptoms were monitored using a version of the Acute Panic Inventory (API) (Dillon et al 1987), modified to include panic attack symptoms as listed in DSM-III-R, with some of the compound items listed separately (e.g., "nausea or abdominal distress" listed as 2 separate items). We also added six additional potential side effects of pentagastrin (unusual taste or smell, drowsiness, blurred vision, headache, sadness, irritability). The instrument has a total of 25 items. Subjects rated their own symptoms on a 4-point scale from 0 (none at all) to 3 (severe). Subjects also gave a yes or no response to the question, "Did you, or are you now having a panic attack?" Symptom ratings obtained 30 min and 10 min before infusion were averaged to provide baseline levels. Additional ratings were obtained at 5, 10, 20, 30, and 45 min after infusion. The +5 min symptom ratings provided a measure of peak symptoms as all subjects experienced an acute symptom peak within the first few minutes after pentagastrin infusion and were told to rate these symptoms at the +5 min rating. When both of the subjects' arms were occupied (due to blood pressure monitoring and blood drawing) symptom ratings were reported verbally to the study physician for recording. The study physician was not blind to diagnosis. Patient responses to pentagastrin were such that a physician blind would have been difficult to maintain had it been attempted.

Heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured using an Air-Shields automated monitor. Recordings were obtained 30, 10, and 2 min prior to infusion, approximately 2, 5, 7, and 10 min after infusion, and every 5 min thereafter for 60 min.

### *Data Analysis*

We first analyzed responses from the pentagastrin infusion visit for all subjects using three-way, repeated measures analyses of variance (ANOVA) with phase and diagnosis (patient versus control) as between group variables and time as a within group variable. The effect of phase was examined to determine if prior experience with the infusion procedure (placebo session) altered responses to pentagastrin for phase two subjects. The time-by-diagnosis interaction reflected patient/control group differences in responsivity to pentagastrin. A second set of ANOVAs examined responses to pentagastrin and placebo in phase two subjects only. In these analyses diagnosis was the between group variable; drug (placebo/pentagastrin) and time were within group variables. The drug-by-time-by-diagnosis interaction in these analyses reflects the degree to which patients and controls responded differently to the two conditions (placebo versus pentagastrin) and thus provides the most stringent test of whether patients were more sensitive to specific pharmacological effects of the drug.

To analyze overall symptom responses to pentagastrin,

we applied the just-described ANOVAs to three dependent measures derived from the symptom rating scales: (1) number of symptoms present (those rated at least mild); (2) total symptom intensity (sum of individual symptom ratings); and (3) the score on the anxiety item ("worried, nervous, anxious") of the modified API. The time factor in the ANOVAs for symptom measures involved ratings obtained prior to infusion (mean baseline) and 5 min after infusion (peak symptoms).

To analyze cardiovascular responses to pentagastrin, we applied the above ANOVAs separately to SBP, DBP, and HR. Because of some missed samples and variability in individual measures, cardiovascular data was averaged to obtain four time points for analysis: mean baseline, immediate postinfusion (a single measurement obtained 1 to 3 min after infusion), 5 to 10 min mean, 10 to 30 min mean, and 45 to 60 min mean.

Additional analyses (described in Results section) were used to assess the duration of the symptom responses to pentagastrin, to compare patients and controls in frequency of pentagastrin-induced panic attacks, to compare pentagastrin-induced attacks to "typical" panic attacks, and to compare pentagastrin-induced attacks to $CCK_4$-induced attacks.

## Results

One control subject in phase one had a resting norepinephrine level of over 1000 pg/ml, suggesting a neuroendocrine abnormality, and he was dropped from all analyses. The final sample consisted of 9 control subjects (mean age = 26.2 ± 4.8 years; one man) and 10 panic patients (mean age = 28.3 ± 8.3 years; 2 men). The patient and control groups did not differ significantly in age or body weight. The patients had an average age of panic disorder onset of 25.9 (±7.7) years. Patients in phase two averaged 13.4 (±5.5) full-symptom panic attacks in the month prior to study screening (comparable data not available for phase one patients). Subjects in phase one and phase two did not differ in mean body weight ($t = 1.0, p = 0.32$); but subjects in phase one tended to be slightly older than those in phase two (30.3 ± 8.4 years versus 24.6 ± 3.4 years, $t = 2.0$, $p = 0.06$). Patient groups in the two phases did not differ in age of onset or duration of illness.

### *Panic Attacks*

Seven of 10 panic patients subjectively reported having a panic attack within the first 5 min after pentagastrin infusion, compared to only one of nine control subjects. None of the five patients and five controls who received a placebo infusion reported a panic attack following the placebo. Applying the criteria for a pharmacologically induced panic attack originally proposed by Klein's group (Dillon et al 1987), quantified by requiring that the symptom profile meet DSM-III-R's four symptom criterion and that there be at least a two point increase from baseline in the subject's rating of anxiety, pentagastrin induced panic attacks in 7 of 10 patients and zero of nine controls (Fisher's exact test, $p = 0.003$).

### *Overall Symptom Responses*

The initial three-way ANOVA (phase-by-diagnosis-by-time) showed no significant main effects or interactions involving phase for any of the three symptom measures, indicating that whether patients were naive to the infusion situation when they received pentagastrin (phase 1) or had prior experience with it (phase 2) had no effect on symptom responses. Combined data for the two phases are summarized in Figure 1. The main effect of diagnosis was significant in all three ANOVAs. Patients, relative to controls, endorsed a greater number of symptoms [$F(1,15) = 12.2, p = 0.003$], experienced greater symptom intensity [$F(1,15) = 14.9, p = 0.002$], and rated their anxiety higher [$F(1,15) = 10.4, p = 0.006$] throughout the pentagastrin infusion procedure. The main effect of time was also significant in all three ANOVAs, due to the substantial rise in number of symptoms [$F(1,15) = 104.5, p = 0.0001$], total symptom intensity [$F(1,15) = 98.4$, $p = 0.0001$], and anxiety [$F(1,15) = 36.7$, $p = 0.0001$] produced in both groups by pentagastrin infusion. The time-by-diagnosis interaction was significant for total symptom intensity [$F(1,15) = 7.3$, $p = 0.016$] and anxiety ratings [$F(1,15) = 5.6, p = 0.032$] but not for number of symptoms [$F(1,15) = 1.9, p = 0.187$]. These analyses and Figure 1 reveal that: (1) panic patients endorsed greater symptomatology on all measures at all time points; (2) patients also responded to pentagastrin with greater rises in anxiety and symptom intensity than did controls (reflected in the graphs in differences in slope); but (3) the rise in *number* of symptoms in response to the infusion did not differ significantly between groups.

A second set of ANOVAs was necessary because the act of being infused (infusions were given in view of the subjects) could elicit "placebo" responses that should be removed to see true pharmacological effects of active drug (placebo response data is included in Figure 1). Differential responsivity to pentagastrin versus placebo is reflected in interaction effects involving drug in the three-way (drug-by-time-by-diagnosis) ANOVAs based on the placebo-controlled phase two data. Drug-by-time interactions were significant for number of symptoms [$F(1,8) = 30.9$, $p = 0.0005$] and total symptom intensity [$F(1,8) = 36.0$, $p = 0.0003$], but not for anxiety rating [$F(1,8) = 0.8, p = 0.41$], indicating that pentagastrin produced substantially greater rises in symptom number and intensity than did placebo; but the anxiety response to pentagastrin was not significantly greater than the anxiety response to the act of being infused.

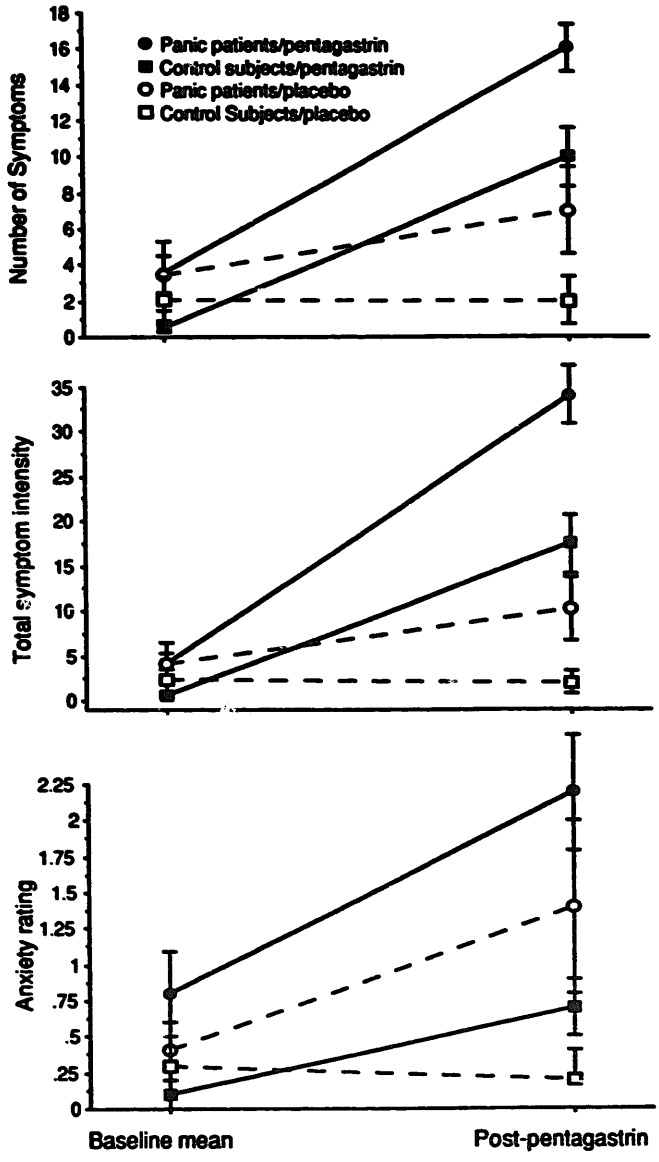


Figure 1. Mean number of symptoms, total symptom intensity, and anxiety ratings at baseline and following infusion (error bars show SEMs). The solid lines (closed symbols) show responses to pentagastrin in the total subject groups (10 panic patients and 9 controls). The dotted lines (open symbols) show responses to placebo infusions in the subgroups studied in phase 2 (5 patients and 5 controls).

None of the six interaction effects involving both drug and diagnosis reached significance (drug-by-diagnosis; $p > 0.50$ for number of symptoms and $p > 0.13$ for total symptom intensity and anxiety rating; drug-by-time-by diagnosis: $p > 0.80$ for number of symptoms and anxiety rating, $p > 0.30$ for total symptom intensity). These results suggest that patient versus control differences in symptom and anxiety responses (seen in the initial analyses) reflect differential responsivity to receiving an infusion and not differential responsivity to the pharmacological content of the infusion.

Two additional statistical tests support this conclusion. First, patients showed significantly greater symptom responses to the placebo infusion than did controls. Analysis of placebo day change from baseline scores (postinfusion symptom ratings minus baseline ratings) demonstrated that patients had greater increases in number of symptoms [$t(8) = 3.8, p = 0.005$] and total symptom intensity [$t(8) = 3.8, p = 0.008$], and tended to show a greater increase in anxiety rating [$t(8) = 2.0, p = 0.08$]. We also subtracted the placebo day change from baseline scores from the pentagastrin day change from baseline scores for number of symptoms, total symptom intensity, and anxiety rating. These "difference of difference" scores reflect the response to pentagastrin over and above the response to placebo. Using these scores, patients and controls did not differ for any of the three dependent measures. In fact, control means were greater than patient means for increases in number of symptoms [controls = 9.1 ± 5.0, patients = 8.3 ± 4.9, $t(8) = 0.3$, $p = 0.80$] and anxiety ratings [controls = 0.5, patients = 0.3, $t(8) = 0.2$, $p = 0.83$]. For total symptom intensity the control mean was 15.5 ± 9.3 and the patient mean was 21.9 ± 10.4 [$t(8) = 1.0, p = 0.33$].

To examine the time course of the symptom responses to pentagastrin, we compared symptom levels at each postinfusion time point to mean baseline symptoms (using paired $t$-tests). There is some imprecision in this approach, as we did not collect continuous reports of symptoms as they occurred and the first "postpeak" ratings were not collected until 10 min after the infusion. Our subjects reported, however, that intense symptomatology lasted for 1 to 4 min. Symptom reports returned to baseline rapidly for control subjects and more slowly for patients. The analyses support these reports. For all subjects the immediate postinfusion symptom levels were substantially and highly significantly elevated above baseline ($p < 0.005$ for number of symptoms, total symptom intensity, and anxiety rating). For control subjects, all three symptom measures had returned to baseline levels by 10 min after the infusion ($p > 0.17$ for each measure). Patients had returned to baseline levels in anxiety ratings by 10 min after the infusion ($p > 0.80$), but were still reporting more symptoms and a greater total symptom intensity than at baseline ($p < 0.05$ for both). By 30 min after infusion symptom and anxiety levels for both groups were nearly identical to baseline levels. It appears then that patients had a more sustained symptom (but not anxiety) response to pentagastrin than did controls. The analyses produced identical results when performed on the smaller group of phase two subjects alone. Phase two patients also had mild, sustained symptom responses to the placebo infusion, however, and as with other analyses, when placebo day symptoms were subtracted from pentagastrin day symptoms, the patient–control differences disappeared, that is, both patients and controls had signifi-

Table 1. Intensity of Panic Symptoms Induced by Pentagastrin and Characteristic of Patients' "Typical" Attacks[a]

| | Pentagastrin | | Typical attack | Patients versus controls | | Pentagastrin versus typical | |
|---|---|---|---|---|---|---|---|
| | patients ($n=10$) | controls ($n=9$) | (patients only) ($n=9$) | $t$ | $p$ | $t$ | $p$ |
| Flushed/chilled | 2.5 ± 0.3 | 2.1 ± 0.2 | 2.0 ± 0.3 | 1.1 | 0.27 | 3.2 | 0.01 |
| Short of breath | 2.4 ± 0.3 | 0.8 ± 0.3 | 1.8 ± 0.2 | 3.6 | 0.002 | 1.5 | 0.18 |
| Anxious/worried | 2.2 ± 0.4 | 0.7 ± 0.2 | 2.7 ± 0.2 | 3.7 | 0.002 | 1.5 | 0.18 |
| Heart racing/pounding | 2.1 ± 0.3 | 1.2 ± 0.4 | 2.4 ± 0.2 | 1.7 | 0.10 | 1.5 | 0.17 |
| Nausea | 2.0 ± 0.4 | 2.1 ± 0.3 | 0.8 ± 0.4 | 0.2 | 0.82 | 2.6 | 0.03 |
| Sweating | 2.0 ± 0.4 | 0.9 ± 0.4 | 1.4 ± 0.4 | 2.1 | 0.05 | 1.5 | 0.18 |
| Dizzy/unsteady | 2.0 ± 0.3 | 0.7 ± 0.3 | 1.9 ± 0.4 | 3.0 | 0.008 | 0.0 | — |
| Faint | 1.9 ± 0.4 | 0.3 ± 0.2 | 1.8 ± 0.4 | 3.4 | 0.003 | 0.0 | — |
| Chest pain/discomfort | 1.8 ± 0.4 | 1.0 ± 0.4 | 1.1 ± 0.4 | 1.5 | 0.14 | 1.0 | 0.37 |
| Upset stomach | 1.5 ± 0.4 | 1.3 ± 0.4 | 1.3 ± 0.4 | 0.3 | 0.77 | 0.0 | — |
| Fear of losing control | 1.2 ± 0.4 | 0.4 ± 0.2 | 1.9 ± 0.4 | 1.6 | 0.12 | 1.3 | 0.22 |
| Shaking/trembling | 1.2 ± 0.3 | 0.3 ± 0.2 | 1.6 ± 0.3 | 2.3 | 0.04 | 0.7 | 0.51 |
| Fear of dying | 1.2 ± 0.4 | 0.0 ± 0.0 | 1.6 ± 0.5 | 2.6 | 0.02 | 0.4 | 0.73 |
| Choking | 1.0 ± 0.4 | 0.7 ± 0.4 | 0.4 ± 0.3 | 0 6 | 0.57 | 1.3 | 0.22 |
| Numbness/tingling | 0.6 ± 0.2 | 1.1 ± 0.4 | 1.3 ± 0.5 | 1.1 | 0.23 | 1.2 | 0.26 |
| Fear of going crazy | 0.5 ± 0.2 | 0.0 ± 0.0 | 1.1 ± 0.5 | 2.1 | 0.05 | 1.2 | 0.28 |
| Number of symptoms | 12.5 ± 0.9 | 8.2 ± 1.2 | 12.4 ± 0.6 | 2.8 | 0.01 | 0.2 | 0.85 |
| Total symptom intensity | 28.1 ± 2.9 | 14.3 ± 2.6 | 27.0 ± 1.7 | 3.5 | 0.003 | 0.2 | 0.83 |

[a] Values are mean ± SEM. Symptom intensity was rated on a 0 to 3 scale.

cantly elevated symptom levels only immediately following the infusion ($p < 0.05$ for both groups for number of symptoms and total symptom intensity) and had returned to baseline by 10 min later ($p > 0.28$ in all analyses).

### *Comparison to Typical Attacks*

Prior to entry into the experiment each patient (except 1) used our version of the API to provide symptom ratings for a recent "typical" panic attack, allowing us to compare pentagastrin-induced symptoms to the patients' usual attacks. We used paired $t$-tests to compare ratings of each symptom and Kendall's tau (Hayes 1981) to compare the symptom profiles (i.e., rank ordering of symptoms). For these and subsequent analyses we used only those symptoms from our version of the API that are also included in the DSM-III-R list of panic attack symptoms. Mean ratings and t-test results are presented in Table 1. Ratings differed significantly for only two symptoms, "flushed/chilled" and "nausea," both common pentagastrin side-effects. The number of symptoms and total symptom intensity reported following pentagastrin were nearly identical to those experienced with typical panic attacks (see Table 1). The symptom profiles were quite similar, tau = 0.47, $p < 0.01$.

### *Comparison to CCK-4 Responses*

Our protocol for infusing pentagastrin is essentially identical to that used by Bradwejn et al (1991) for their $CCK_4$ infusions. Our rating scale differed in that it did not include a rating of 4 (very severe), and there were minor differences in the wording and breakdown of the compounded DSM-III-R symptoms. To allow a direct comparison of symptom responses the Bradwejn group recalculated their means on a 0 to 3 scale, with their ratings of 3 and 4 lumped together as ratings of 3 (J. Bradwejn, personal communication, 1993). Recalculations were done for two cohorts of panic patients (Bradwejn et al 1991, 1992b) and one cohort of controls (Bradwejn et al 1991) who received 25 μg of $CCK_4$ and for one cohort each of patients and controls who received 50 μg of $CCK_4$ (Bradwejn et al 1991). We could then compare pentagastrin-induced symptoms to $CCK_4$-induced symptoms, using paired $t$-tests to compare ratings of each symptom and Kendall's tau (Hayes 1981) to compare the symptom profiles. Graphic comparison of patients' symptom profiles (pentagastrin versus 25 μg $CCK_4$) is presented in Figure 2. (graphic comparison with 50 μg $CCK_4$ available upon request).

Patients receiving 25 μg of $CCK_4$ did not differ from those receiving pentagastrin in their mean rating of any of 16 panic attack symptoms for either cohort [$t(19) < 1.71$, $p > 0.10$ in all 32 comparisons]. Control subjects receiving 25 μg of $CCK_4$ also did not differ from those receiving pentagastrin on any panic symptom rating [$t(22) < 1.64$, $p > 0.10$ for all 16 symptoms]. Panic patients receiving 50 μg of CCK, compared to patients receiving pentagastrin, reported significantly more numbness [$t(19) = 3.68$, $p < 0.05$] and tended to report less abdominal distress [$t(19) = 1.81$, $p < 0.10$], but did not differ on any other symptom [$t(19) < 1.64$, $p > 0.10$]. Controls receiving 50 μg of CCK, compared to controls receiving pentagastrin, reported sig-

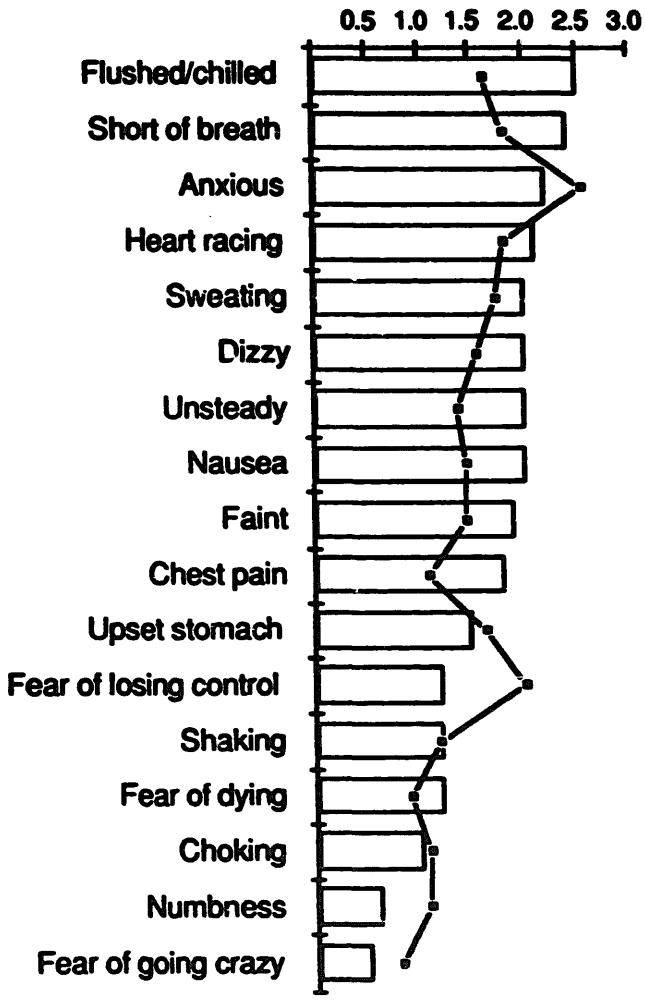


Figure 2. Mean ratings of symptom intensity (0 = none, 3 = severe) for the panic attack symptoms for which we had sufficiently comparable data to allow direct comparisions of pentagastrin-induced symptoms (bars) to $CCK_4$-induced symptoms (squares). The $CCK_4$ data are recalculated (Bradwejn, personal communication, 1993) from a cohort of panic patients given 25 μg of $CCK_4$ and initially described by Bradwejn et al (1991). The two drugs did not produce different ratings for any syumptom, even at a trend level ($p < 0.10$). Symptoms are arranged in descending order of intensity as experienced following pentagastrin to provide a cleaner graphical presentation.

nificantly more shortness of breath [$t(22) = 2.41, p < 0.05$] and tended to report more unsteadiness and faintness [$t(22) = 1.91$ and 2.03, respectively, $p < 0.10$ for both]. The rank orders of $CCK_4$-elicited symptoms as determined by patients' mean ratings were significantly related to the profile of pentagastrin-elicited symptoms for all three $CCK_4$ patient cohorts (tau = 0.56 and 0.57, $p < 0.008$ for the two 25 μg cohorts; tau = 0.52, $p < 0.008$ for the 50 μg cohort).

We also compared pentagastrin and $CCK_4$ in their ability to differentiate patients from control subjects, in individual symptom responses and in total number and intensity of symptoms experienced. The published examination of patient–control differences in individual symptom ratings following $CCK_4$ appeared in a dose response study that utilized 25 μg and 50 μg infusions of $CCK_4$ (Bradwejn et al 1991). Group differences (patient versus control) were reported for the overall effect of $CCK_4$, regardless of dose (no group-by-dose interactions were significant). Our group means, SEMs, and statistical tests appear in Table 1. Of the 16 symptoms that are comparable across studies, patients and controls differed significantly in their ratings of six following $CCK_4$ infusion and in their ratings of seven following pentagastrin infusion. In both cases patients and controls differed in the number of symptoms produced and the total symptom intensity. For all variables the patients were more symptomatic than controls. The items that differentiated patient and control groups with both $CCK_4$ and pentagastrin were shortness of breath, shaking, fear of going crazy, and anxiety.

### *Cardiovascular Responses*

As in the analyses of symptoms, the initial three-way ANOVAs for SBP and DBP found no significant effects involving phase (see Figure 3 for data collapsed across phase). For both SBP and DBP the main effect of time was significant [$F(4,60) = 9.7, p = 0.0001$ and $F(4,60) = 4.1, p = 0.005$, respectively], and the diagnosis-by-time interactions approached significance [$F(4,60) = 2.3$, $p = 0.065$ and $F(4,60) = 2.4$, $p = 0.056$, respectively]. Unpaired $t$-tests indicated that the trend toward patient–control differences in SBP response to pentagastrin was due to elevated SBP in patients 5 to 10 min [$t(17) = 2.7, p = 0.02$] and 10 to 30 min after the infusion [$t(17) = 2.2, p = 0.04$]. For DBP the group differences appeared only at 5 to 10 min after infusion [$t(17) = 2.7, p = 0.016$]. The groups did not differ significantly in SBP or DBP at baseline, immediately after the infusion, or 45 to 60 min later, although there was a trend for patients to have elevated DBP at baseline [$t(17) = 1.9, p = 0.068$].

The initial three-way ANOVA for HR showed a significant effect of time [$F(4,60) = 31.3, p = 0.0001$]. The main effects of diagnosis and phase were not significant, but all three interactions involving phase were significant [phase-by-diagnosis: $F(1,15) = 7.9$, $p = 0.013$; phase-by-time: $F(4,60) = 4.5, p = 0.003$; and phase-by-diagnosis-by-time: $F(4,60) = 2.7$, $p = 0.038$]. Examination of graphs showed these interaction effects to be due to lower HRs immediately following the infusion in panic patients in phase 1. Review of data sheets revealed that substantially fewer subjects from this group had recordings of heart rate obtained within the first 2 min following the infusion. This failure was a consequence of the highly symptomatic responses of patients, our lack of preparation for the strength of these responses early in phase 1, and a resultant failure to properly

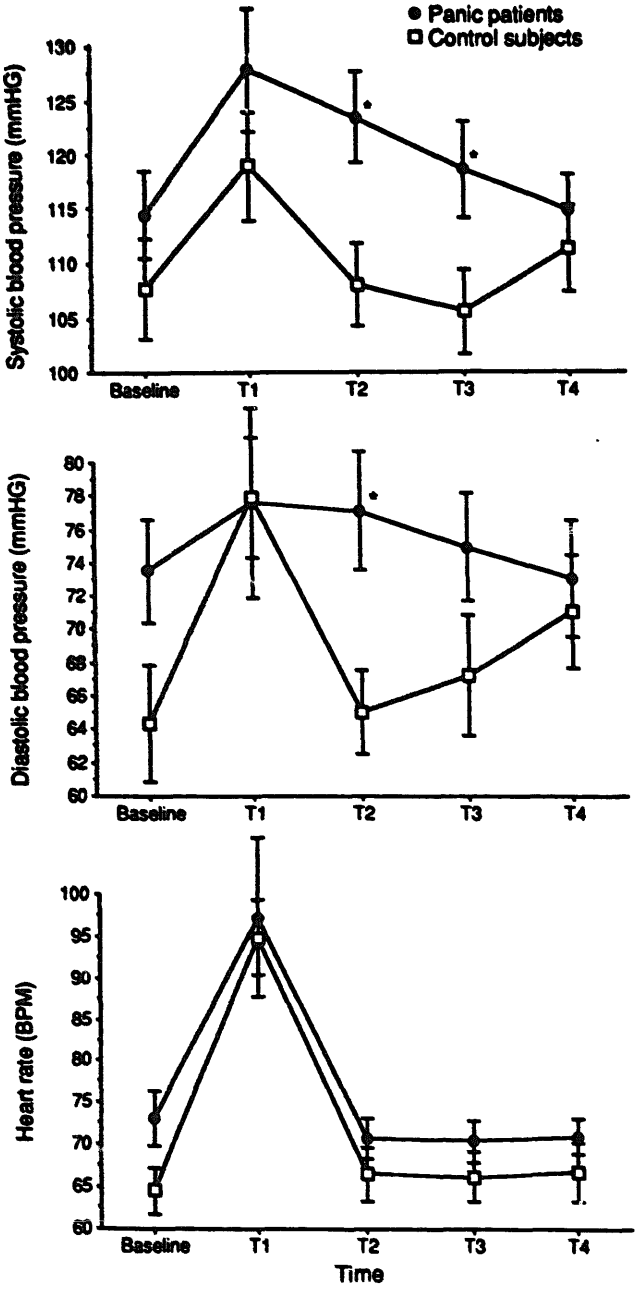


Figure 3. Cardiovascular responses (means ± SEM) to pentagastrin infusion in panic patients ($n = 10$) and normal controls ($n = 9$). The baseline measure is the mean of recordings made between 30 and 2 min before infusion; T1 represents a single measure recorded between 1 and 3 min after infusion; T2 to T4 represent the means of recordings made 5 to 10 min, 10 to 30 min, and 45 to 60 min after infusion, respectively. Asterisks mark time points at which patients and controls differed ($p < 0.05$).

trigger the automatic monitor to begin recording immediately after the infusion. A similar systematic failure to obtain immediate post-infusion HR measures did not occur in the control group, and the HR response curves for controls were identical for the two phases. The HR data is included in Figure 3, collapsed across phase. The peak HR for patients (time T1) is probably artificially reduced by the above problem.

As with symptom data, follow-up ANOVAs were applied to phase two data to control for placebo effects. Significant drug-by-time interactions for HR [$F(4,32) = 20.1$, $p = 0.0001$] and SBP [$F(4,32) = 4.5, p = 0.006$] indicated that pentagastrin produced greater rises in HR and SBP than did the act of being infused. The drug-by-time interaction for DBP did not reach significance [$F(4,32) = 2.1, p = 0.11$]; but the DBP response to pentagastrin appeared greater and more prolonged; and the main effect of drug [$F(1,8) = 5.3$, $p = 0.051$] and a significantly greater peak DBP response to pentagastrin than placebo [$t(9) = 4.1$, $p = 0.003$] support a differential effect. There was a significant main effect of diagnosis only for HR [$F(1,8) = 9.5, p = 0.015$]. No interactions involving diagnosis approached significance ($F < 2.34, p > 0.16$ or all two-way interactions; $F < 0.6, p > 0.65$ for all three-way interactions) for any of the three cardiovascular measures, suggesting no differential sensitivity to the active drug when controlled for placebo response differences.

In summary, the analyses suggest that HR, SBP, and DBP all increase in response to pentagastrin in patients and controls. The two groups do not appear to differ in their cardiovascular responses to the drug, but panic patients tended to have higher HRs throughout the procedures.

Correlational analysis revealed a significant relationship between anxiety levels following pentagastrin infusion and baseline HR ($r = +0.7$, $p = 0.001$). Furthermore, those patients who had panic attacks in response to pentagastrin had higher baseline HRs than those who did not panic [$t(8) = 3.8$, $p = 0.006$].

## Discussion

### *Pentagastrin and $CCK_4$*

Our findings demonstrate that pentagastrin produces symptom and cardiovascular responses similar to those produced by $CCK_4$ and thus provides a CCK-B receptor agonist model of panic that is available to researchers in the United States. There are inherent limitations in comparing data collected at different sites and using slightly different paradigms and instruments; but the similarities between pentagastrin and $CCK_4$ in symptom ratings, symptom profiles, and ability to differentiate panic patients from controls are striking. The time courses of symptom responses are also remarkably similar. Our impression that acute responses to pentagastrin lasted 1 to 4 min and our analyses, which indicate a return to baseline levels within 5 to 10 min for controls and within 20 to 30 min for patients, are in good accord with the durations reported for $CCK_4$ (acute responses lasted 2 min, with return to baseline in 10 min with

25 μg and 20 min with 50 μg) (Bradwejn et al 1991, 1992a). Though definitive conclusions cannot be reached without direct comparisons in a single laboratory, we believe the similarities are striking enough to support the viability of pentagastrin as an alternative to $CCK_4$ for the study of CCK-B receptor systems in human anxiety research.

We found some differences in the symptoms produced by the two agents and the frequency of panic attacks reported in response to $CCK_4$ is greater than we found for pentagastrin. Dose effects may be relevant as response to $CCK_4$ is dose dependent (Bradwejn et al 1992a). Whether this dose–response relationship is a function of CCK-B receptor occupancy is not known. It is therefore difficult to predict whether pentagastrin, with its slightly greater affinity for the CCK-B receptor but lower selectivity for the CCK-B over the CCK-A receptor, should be more or less potent in producing symptoms. Our average dose of pentagastrin was 41.5 μg. This dose produced a level of symptomatology that appeared to fall between the levels produced by 25 μg and 50 μg of $CCK_4$. It shared more similarities with the lower dose in symptom profile and individual symptom intensity ratings but was more similar to the higher dose in duration of symptoms. The precise role of specific receptor interactions in CCK symptom induction remains unclear, but variations in receptor affinity and selectivity may have contributed to the minor response differences between pentagastrin and $CCK_4$.

Sampling error also likely accounts for some of the differences between drugs, as even within Bradwejn's laboratory and using identical doses of $CCK_4$, different samples of panic patients produced somewhat differing symptom profiles (Bradwejn et al 1991; 1992b). Nonspecific aspects of the experimental setting and patient state (Cowley and Arana 1990) may also play a role, especially in the difference in frequency of panic attacks. Cognitive set can alter the panicogenic properties of provocative agents (Cowley and Arana 1990) and it is very possible that differences between laboratories, personnel, and study goals created different patient attitudes and expectancies at the two sites (see below).

### *Mechanisms of CCK/Pentagastrin-Induced Panic*

There is growing evidence that cognitive factors can mediate or modulate pharmacologically induced panic. Behavioral and cognitive manipulations which provide patients with a greater sense of control over provocative agents and that help them correctly attribute physical symptoms to known, short-lived, and safe effects of the agent can substantially reduce the frequency of panic attacks in laboratory models (Clark et al 1991; Rapee et al 1986; Sanderson et al 1989). Some have interpreted this data as suggesting that the mechanism of laboratory-induced panic involves catastrophic misinterpretation of somatic cues produced by provocative agents (Clark et al 1991). We did not actively engage in anxiety-reducing cognitive manipulations in this study; but because we were exploring a neuroendocrine probe rather than trying to develop a laboratory model of panic, we were not reluctant to offer our patients emotional support, reassurance, and comments that might help them avoid catastrophic misinterpretations of expectable and short-lived drug effects. We in fact offered considerable support to those subjects whose fear of drug-induced panic produced reluctance to participate in the study (see Methods). If cognitive factors can modulate drug-induced panic then differences in recruitment procedures and preparation of subjects for study could well have contributed to the difference in panic rates reported for pentagastrin and $CCK_4$. The fact that 41.5 μg of pentagastrin produced slightly higher ratings than 25 μg of $CCK_4$ on 9 of 12 physical symptoms but slightly lower ratings on three of the four fear/anxiety symptoms (see Figure 2), while producing very similar overall symptom profiles, is consistent with the possibility that cognitive factors were modulating fear/anxiety responses to the two agonists.

Alternatively, CCK agonists may produce anxiety directly through a highly specific receptor interaction within the brain; and $CCK_4$ may be slightly more active at the relevant receptor site than pentagastrin. If so, enhanced sensitivity of that receptor in panic patients could then explain patient–control differences in symptom/anxiety responses to either drug. The lack of patient–control differences in neuroendocrine responses to pentagastrin (Abelson et al 1994) argues against differences in receptor sensitivity. Other mechanisms of panic induction are capable of explaining patient–control differences in response to CCK agonism and both the similarities and differences between $CCK_4$ and pentagastrin. All proposed mechanisms remain purely speculative at this point, however, as the data is not yet strong enough to convincingly support any single hypothesis.

Though we did not design our study to specifically differentiate "cognitive" versus "pharmacological" mechanisms of panic induction, the consequences of correcting our subjects' responses to pentagastrin for their responses to the placebo infusion does support the hypothesis that nonpharmacological factors play a role in the anxiogenic activity of CCK agonism. Our initial analyses supported prior reports that panic patients, compared to controls, have enhanced symptom responses to CCK-B receptor agonists. In an effort to strengthen the argument that group differences reflect differences in pharmacological sensitivity to the provocative agent, we conducted additional analyses that controlled for nonspecific aspects of subjects' responses to the act of being infused. Because panic patients were also more symptomatically responsive to the placebo infusion, however, patient–control differences in symptom number,

symptom intensity, and anxiety ratings were substantially attenuated in the placebo-controlled analyses, though strong responses to pentagastrin remained. The initial group differences may therefore have been due to differing responsivity to nonspecific aspects of the infusion paradigm rather than to differing sensitivity to the active agent itself. The strength of this conclusion is weakened by two important caveats. One, it is possible that by statistically controlling for responses to placebo we were actually overly conservative as some of the placebo response may have been due to novelty effects (the placebo infusion was always given first and was therefore each subject's first experience with the act of being infused) that were no longer present when pentagastrin was given. Two, placebo-controlled analyses had very small sample sizes, low power to detect significant differences, and high risk of a Type II error. Replication with a larger sample and better control of novelty effects is clearly needed. Patients did have greater symptom responses to the placebo infusion than did controls, however, and the significance of this difference despite the low power of the analysis may suggest that it is a fairly robust finding. If confirmed, it strongly suggests that efforts to control nonspecific factors in panic-provocation models must be intensified. Hypersensitivity to nonpharmacological aspects of challenge paradigms will make demonstration of true pharmacological sensitivities in panic patients substantially more difficult.

### *Cardiovascular Responses*

Pentagastrin produced clear-cut, rapid, and substantial increases in heart rate and blood pressure. The heart rate response is likely even larger than we observed since our first recording was 2 min after infusion and rates recorded at 3 min postinfusion were already substantially reduced compared to the 2-min recordings. This impression is supported by Brandwejn's data (Bradwejn et al 1992a), which show somewhat larger heart rate responses than seen in our data, based on multiple recordings made within the first 2 min after infusion. More detailed comparison of cardiovascular responses between studies cannot be made because of the differences in time of measurement. The mechanism of this autonomic activation is not known, though we have pilot data showing a very brief but consistent epinephrine pulse appearing and disappearing within the first 3 min after pentagastrin infusion (Abelson et al 1994). Whether the cardiovascular responses are related in any way to the mechanism of the drug's anxiogenic activity can only be determined by further research. Further study will also be needed to determine if patients have abnormal cardiovascular reactivity to pentagastrin. Patients appeared to have prolonged blood pressure responses compared to controls, but placebo-controlled analyses did not confirm this finding. The placebo-controlled analyses had low statistical power due to small sample size, however. Correlational analyses indicated that (1) higher postpentagastrin anxiety levels were associated with higher baseline HR and (2) those patients who had panic attacks had higher baseline HR than those who did not, suggesting that whatever it is that sensitizes panic patients to CCK-B agonists may be linked to increase autonomic activity in the anticipatory phase.

### *Summary*

CCK-B receptor agonists are now firmly established as panicogenic agents. The two selective agonists available for human use, pentagastrin and $CCK_4$, induce very similar symptom profiles and both produce panic attacks that are similar to naturally occurring attacks. Symptom responses to the two agents also similarly differentiate panic patients from controls. It remains unclear, however, if this is a result of a specific pharmacological effect that would indicate CCK-B receptor mediation of human panic anxiety. Pentagastrin provides a readily available and potentially valuable tool for further exploring this possibility, for studying CCK systems in human subjects, and for studying panic attacks in a controlled laboratory setting.

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