Pentagastrin Infusions in Patients with Panic Disorder II. Neuroendocrinology

James L. Abelson, Randolph M. Nesse, and Aaron I. Vinik

Cholecystokinin (CCK) has well-documented anxiogenic effects in animals and normal people, and panicogenic effects in patients with panic disorder, but little is known about its neuroendocrine profile. We examined neuroendocrine responses to intravenous infusions of pentagastrin, a selective CCK-B receptor agonist, in 10 patients with panic disorder and 10 normal control subjects. Pentagastrin potently activated the hypothalamic–pituitary–adrenal (HPA) axis, but did not release growth hormone or any of several vasoactive peptides (neurokinin A, substance P, vasoactive intestinal peptide). The HPA axis response was unrelated to increases in symptoms. Panic patients did not differ from controls in neuroendocrine responses to the CCK agonist. Differential sensitivity to novelty stress accounted for the only patient–control differences in neuroendocrine profiles. The data suggest that CCK may help modulate normal HPA axis activity, but its anxiogenic effects are unrelated to its stimulatory effects on the HPA axis. Pentagastrin provides a safe and readily available probe for further study of CCK receptor systems in humans.

Key Words: Pentagastrin, cholecystokinin, panic disorder, corticotropin, cortisol, growth hormone

Introduction

Cholecystokinin (CCK) may be the most abundant peptide neurotransmitter in the brain (Rehfeld 1985). Its receptors are widely distributed throughout the central nervous system (CNS), with high densities in the hypothalamus, limbic system, basal ganglia, hippocampus, and cortex (Woodruff and Hughes 1991). CCK is co-localized with or interacts dopaminergic (Crawley 1991), noradrenergic (Beresford et al 1988; Kaneyuki et al 1989), GABAergic (gamma aminobutyric acid) (Kaneyuki et al 1989; Sheehan and de Belleroche 1983), and serotonergic (Brodin et al 1989; Stallone et al 1989) neurotransmitter systems. It may co-modulate hypothalamic–pituitary–adrenal (HPA) axis (Abelson et al 1991) and hypothalamic–pituitary–somotrophic (HPS) axis activity (Karashima et al 1984; Spencer et al 1991). Its functional roles are not yet fully defined, but it appears to mediate anxiety (Singh et al 1991) and it may participate in satiety (Silver and Morley 1991), alcohol satiation (Kulkosky et al 1989), psychosis (Crawley 1991), nociception (Baber et al 1989), and drug withdrawal (Hughes et al 1991; Singh et al 1991).

Despite a neuroanatomy, neurophysiology, and functional significance that establish CCK as a neurotransmitter of potential importance in a number of psychiatric dis-

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orders, it has received relatively little attention in the psychiatric research literature. The dearth of clinical psychiatric research on CCK likely results from the early focus of basic research on its gastrointestinal functions and from a lack of CCK probes that can be used in humans. Recent identification of two types of CCK receptors (CCK-A and CCK-B) and increased awareness of their distributions within both the peripheral and central nervous systems, however, have greatly expanded the range of potential therapeutic applications of CCK active drugs (Dethloff and De La Iglesia 1992) and has led to increased commercial interest in agents with subtype-specific receptor activity and with psychiatric applications. New antagonists with increased specificity for the CCK-B receptor, which is the predominant subtype within the central nervous system, have now shown therapeutic potential in animal models of anxiety disorders and drug abuse (Woodruff and Hughes 1991). These new agents and the possibility that CCK may play a key role in alerting/alarm circuits (Hughes et al 1991) make more detailed understanding of human CCK systems of great importance. Cross-species variations in the distribution and function of CCK receptors (Woodruff and Hughes 1991) make it difficult, however, to generalize to humans from the animal literature. The availability of selective CCK-B receptor antagonists for human research and their use in conjunction with already available selective agonists will be critical to expanding what we know about CCK receptor function in humans.

Pentagastrin is a 5-amino acid synthetic peptide that has been used as a provocative agent in tests for endocrine tumors (Ahlman et al 1985; O'Connell et al 1990; Oberg et al 1989; Vinik et al 1990). It is a highly selective CCK-B receptor agonist (Woodruff and Hughes 1991) that offers an established and readily available neuroendocrine probe for human use. We initiated study of pentagastrin infusions in patients with panic disorder because the relevance of the CCK-B receptor to anxiety disorders in general, and panic in particular, has been clearly suggested by both animal and human studies (Bradwejn et al 1991; Singh et al 1991). The selective CCK-B receptor agonist, CCK_4, is now well-established as a panicogenic agent (Bradwejn et al 1991) and CCK-B receptor antagonists may provide a novel approach to the treatment of panic disorder (Bradwejn et al 1993). In a separate article we report that pentagastrin is comparable to CCK_4 as a panicogenic agent (Abelson and Nesse 1994). The present report examines the neuroendocrinology of the CCK-B receptor system in humans, focusing on pentagastrin's effects on neuroendocrine systems that have been implicated in the pathophysiology of panic disorder. Because blunted corticotropin [adrenocorticotropic hormone (ACTH)] responses to corticotropin-releasing hormone (CRH) provide evidence of an HPA axis abnormality in panic (Roy-Byrne et al 1986) and because CCK agonists can stimulate ACTH release (Degli Uberti et al 1983), ACTH and cortisol responses are of particular interest. Panic patients also have blunted growth hormone (GH) responses to clonidine challenge, which supports an hypothesized abnormality in monoaminergic systems (Abelson et al 1992), so we also examined GH and catecholamine responses. Finally, it remains unclear if anxiety induced by CCK is due to central or peripheral effects. Pentagastrin can stimulate peripheral release of vasoactive peptides (Ahlman et al 1985; Oberg et al 1989; Vinik et al 1990), which could mediate or mimic peripheral somatic symptoms of panic. We therefore also examined vasoactive intestinal peptide (VIP), neurokinin A (NKA), and substance P. Previously published preliminary analyses of a subset of our ACTH and cortisol data (Abelson et al 1991) demonstrated that pentagastrin stimulated the release of ACTH and cortisol and suggested that panic patients might have increased HPA axis responsivity to pentagastrin. These analyses were based on very small samples and were not controlled for placebo responses. We now report the full analyses of all neuroendocrine data collected in a two phase study.

Methods

Subjects

All 20 subjects gave informed consent and were medically healthy as determined by medical 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). Panic patients met DSM-III-R criteria for panic disorder ($n = 4$) or panic disorder with agoraphobia ($n = 6$). They did not meet criteria for current (past 6 months) major depression or substance abuse and did not have any history of primary depression or psychosis. Control subjects were recruited from the community using newspaper advertising. They were age matched and gender matched to the patients and did not meet criteria for any Axis I disorder. All women ($n = 16$, 8 in each group) had normal menstrual cycles and were studied within 10 days of onset of menses (to insure they were not pregnant and to avoid the effects of the preovulatory estrogen surge on hormonal measures). All subjects were free of psychotropic medication for at least 2 weeks prior to study. Only two patients had received daily pharmacological treatment for panic disorder in the months prior to study (one was taking buspirone and another alprazolam), but both discontinued their medication more than a month before the study. A third patient had used occasional doses of lorazepam (2–3 times/week) up until 3 weeks prior to study.

Design and Procedures

The study was conducted in two phases in a Clinical Research Center (CRC). In the first phase, five patients and

five controls were each admitted once and given a single infusion of pentagastrin. Blood sampling at 30, 15, and 1 min before infusion, and approximately 1, 3, 5, 7, 10, 15, 20, 30, 45, and 60 min after infusion allowed detailed initial examinations of hormonal response patterns. These initial results allowed reduced sampling frequency in phase 2, which permitted addition of a placebo infusion without exceeding blood volume limits. An additional five patients and five controls were recruited. Phase 2 subjects were admitted on two occasions a week apart, receiving a saline placebo infusion on visit 1 and pentagastrin on visit 2 (administered in a single-blind fashion). Samples were obtained at 30 and 1 min before infusion, and 3, 5, 10, 20, 30, and 45 min after infusion. We used a fixed order of administration, with active substance second, because panic patients are especially reactive to novel situations and therefore stress-reactive measures are likely to be closer to true baselines during a second visit to the CRC. Phase 2 was identical to phase 1 except for the addition of the placebo infusion and the reduced blood sampling frequency.

Subjects were admitted to the CRC the night prior to study. They were awakened at 7:30 AM. At 8 AM an indwelling heparin lock catheter was inserted into an antecubital vein. Baseline blood samples were drawn between 8:30 AM and 8:59 AM. At 9 AM saline placebo or pentagastrin (commercially available as Peptavlon, Wyeth-Ayerst Laboratories, Philadelphia, PA) was infused into the heparin lock, in view of the patient, in less than 1 min. The pentagastrin dose was 0.6 μg/kg, in a saline vehicle of less than 1 ml. Blood samples were drawn into chilled tubes according to the schedule described above. Samples were spun, separated, and frozen at −70°C within 30 min of being drawn. Details of instructions given to subjects and monitoring of symptom and cardiovascular measures are described elsewhere (Abelson and Nesse 1994).

Biochemical Assays

Samples for ACTH, GH, substance P, and NKA were drawn into tubes containing ethylenediaminetetraacetic acid (EDTA). ACTH was measured by radioimmunoassay, with a sensitivity of 6 pg/ml and intraassay and interassay coefficients of variation (CVs) of less than 10%. GH, substance P, and NKA were measured by a double-antibody radioimmunoassay. For GH the sensitivity was 0.8 ng/ml and intraassay and interassay CVs were 18.3% and 14.5%, respectively. For substance P the sensitivity was 10 pg/ml and intraassay and interassay CVs were 1.4% and 15.8%, respectively. For NKA the sensitivity was 0.15 pg/ml and CVs were less than 10%. Samples for cortisol and catecholamines were drawn into tubes containing heparin. Cortisol and catecholamines were measured by high-performance liquid chromatography. For cortisol the sensitivity was 0.1 μg/dl and CVs were less than 6%. For epinephrine, norepinephrine, and dopamine the sensitivities were 17 pg/ml, 11 pg/ml, and 22 pg/ml, respectively. The CVs were all less than 12%. VIP was drawn into tubes containing EDTA and aprotinin. It was measured using radioimmunoassay with a sensitivity of 11 pg/hml and intraassay and interassay CVs of 2.5% and 21%, respectively.

Data Analyses

Two sets of three-way, repeated measures analyses of variance (ANOVAs) were used for the primary analyses. In order to confirm, with placebo-controlled data, that pentagastrin releases ACTH and cortisol, the initial analyses used phase-2 data alone to examine the effects of drug (pentagastrin versus placebo), diagnosis (patients versus controls), and time. The effects of pentagastrin on hormone release are reflected in the drug effect and the drug-by-time interaction in these analyses. The drug-by-time-by-diagnosis interaction reflects the degree to which patients and controls responded differently to the 2 conditions (placebo versus pentagastrin) and thus tests whether patients were more sensitive than controls to specific pharmacological effects of the drug.

The second set of analyses utilized pentagastrin-day data from both phases in three-way ANOVAs (phase-by-diagnosis-by-time). Because the entire sample was included, these analyses had greater power to detect patient–control differences. Phase was included as a between-group main effect in these analyses to determine if prior experience with the infusion procedure (placebo session) altered responses to pentagastrin for phase-2 subjects. Patient–control differences are reflected in the the effect of diagnosis and its interactions. The time-by-diagnosis interaction reflects patient–control group differences in responsivity to pentagastrin. Time is a within-group factor, utilizing baseline and postinfusion measures and reflecting response to the infusion.

All hormone data was log-transformed prior to analysis to normalize distributions. In analyses that utilized data from both phase 1 and 2 (ACTH, cortisol, GH, and NKA), only time points at which samples were collected in both phases were included. When significant effects were found in the primary ANOVAs, follow-up *t*-tests were used to further dissect the findings. Additional comparisons were carried out on total preinfusion and postinfusion hormone secretion, as measured by area under the preinfusion and postinfusion curves (AUCs) calculated by trapezoidal approximation, and on peak responses, calculated by subtracting mean baseline levels from the postinfusion peak level.

Results

One control subject in phase 1 had a resting norepinephrine level of over 1000 pg/ml, suggesting a neuroendocrine ab-

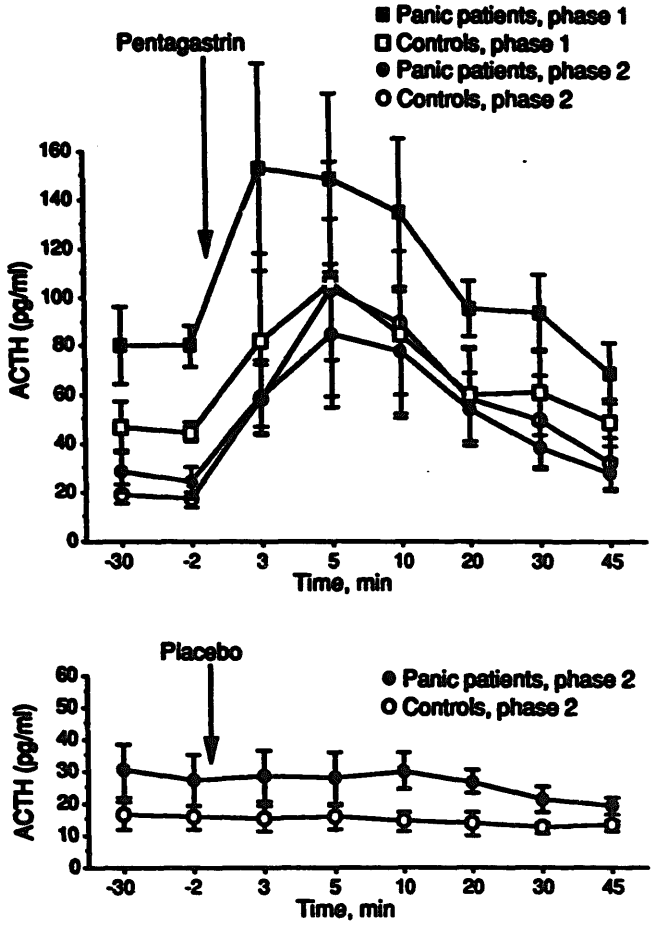

Figure 1. Corticotropin (ACTH) responses to pentagastrin (upper graph) and placebo (lower graph) in patients with panic disorder (closed symbols) and controls (open symbols). Subjects in phase 1 (squares) received only pentagastrin infusion. Subjects in phase 2 (circles) received both pentagastrin and placebo. Group means and SEMs are plotted at each sampling point before and after infusion (arrows). The x-axis was plotted with equal distance between sampling points rather than scaled to actual time to make the graphs more readable. See text for analyse.

normality, and he was dropped from all analyses. Characteristics of the patient and control groups have been presented elsewhere (Abelson and Nesse 1994).

ACTH and Cortisol

ACTH responses to pentagastrin (phases 1 and 2) and placebo (phase 2) are presented in Figure 1. Analysis of placebo-controlled data (phase 2 alone, see Table 1) confirmed the striking effect of pentagastrin on ACTH secretion that is evident in the graphs. ACTH levels doubled following pentagastrin infusion but did not respond at all to placebo (effects of drug, time, and their interaction were all highly significant). There were no significant differences between patients and controls in these placebo-controlled analyses, though the drug-by-diagnosis interaction approached significance (Table 1). This trend appears due to placebo day differences between patients and controls that disappeared under the influence of pentagastrin. On placebo day patients had significantly higher peak ACTH levels [patient mean = 34.2 ± 14.8; control mean = 17.4 ± 8.26; $t(8) = 2.59$, $p = 0.032$] and nearly significantly higher total ACTH secretion [patient mean = 1931 ± 940; control mean = 1070 ± 459; $t(8) = 2.1$, $p = 0.066$], compared to controls; but the groups did not differ in peak ACTH levels following pentagastrin [patient mean = 84.8 ± 56.45; control mean = 106.4 ± 63.87; $t(8) = 0.5$, $p = 0.63$] and they had nearly identical levels of total ACTH secretion (AUC) on pentagastrin infusion day [patient mean = 3045 ± 1648; control mean = 3170 ± 1907; $t(8) = 0.1$, $p = 0.94$].

Table 1. Diagnosis (Patients versus Controls) by Drug (Pentagastrin versus Placebo) by Time ANOVAs of Placebo-Controlled Phase 2 Data for Corticotropin (ACTH), Cortisol, and Growth Hormone (GH)

	ACTH		Cortisol		GH	
	F	*p*	*F*	*p*	*F*	*p*
Diagnosis	1.04	NS	0.82	NS	5.5	0.058
Drug	27.62	0.0008	1.71	NS	1.68	NS
Time	12.16	0.0001	4.74	0.0003	1.53	NS
Drug-by-time	16.31	0.0001	12.33	0.0001	1.88	NS
Diagnosis-by-time	0.39	NS	0.55	NS	4.15	0.002
Diagnosis-by-drug	4.31	0.07	0.03	NS	0.41	NS
Diagnosis-by-drug-by-time	0.69	NS	0.41	NS	1.05	NS

Patient–control differences should be more evident in analyses of pentagastrin-day data from the entire sample (phases combined), but the overall ANOVA (Table 2) revealed no significant ACTH differences between patients and controls. The highly significant effect of time in this analysis again reflects the striking rise in ACTH following pentagastrin infusion. Paired *t*-tests showed ACTH levels to be significantly elevated above baseline levels at 3, 5, 10, 20, and 30 min after infusion [$t(18) = 5.4$, $p = 0.0001$; $t(18) = 5.6$, $p = 0.0001$; $t(18) = 5.0$, $p = 0.0001$; $t(18) = 3.5$, $p =$

Table 2. Diagnosis (Patients versus Controls) by Phase (1 versus 2) by Time ANOVAs of Corticotropin (ACTH), Cortisol, and Growth Hormone (GH) Responses to Pentagastrin for All Subjects

	ACTH		Cortisol		GH	
	F	*p*	*F*	*p*	*F*	*p*
Diagnosis	1.31	NS	0.31	NS	4.85	0.048
Phase	7.25	0.02	0.17	NS	5.07	0.04
Time	23.26	0.0001	18.52	0.0001	1.18	NS
Diagnosis-by-phase	1.20	NS	3.83	0.07	1.07	NS
Diagnosis-by-time	0.42	NS	0.19	NS	1.21	NS
Phase-by-time	3.95	0.0007	1.34	NS	0.92	NS
Diagnosis-by-phase-by-time	0.35	NS	0.37	NS	1.15	NS

0.003; and $t(18) = 2.8$, $p = 0.011$, respectively]. There were significant differences between phase 1 and phase 2 (reflected in the phase effect and phase-by-time interaction), primarily because of elevated baseline ACTH levels in phase 1. For all subjects, preinfusion ACTH secretion (AUC) was significantly higher in phase 1 than in phase 2 [$t(17) = 4.9$, $p = 0.0001$]. This phase effect on baseline ACTH levels remained significant for patients and controls analyzed separately [$t(8) = 3.7$, $p = 0.006$; and $t(7) = 3.3.59$, $p = 0.009$, respectively]. A phase effect on *post*infusion ACTH secretion was present only for patients [phase 1 > phase 2, $t(8) = 2.51$, $p = 0.036$ for patients; $t(7) = 0.47$, $p = 0.65$ for controls]. Phase 2 patients look identical to controls in either phase on post-pentagastrin secretion, whereas phase 1 patients hypersecreted ACTH before and after infusion (see Figure 1).

Because of the striking effect of phase on baseline ACTH we further examined baseline levels in a two-way ANOVA that utilized mean baseline ACTH during subjects' first visit to the CRC (first and only visit for phase 1 subjects, first of two visits for phase 2 subjects) as the dependent variable. The main effects examined were diagnosis and phase. Both main effects were significant. For all subjects, during their first experience with the infusion paradigm preinfusion ACTH levels were higher in phase 1 than phase 2 [$F(1,15) = 32.7$, $p = 0.0001$]. Regardless of phase, patients had higher pre-infusion ACTH levels than controls during the first CRC visit [$F(1,15) = 8.2$, $p = 0.012$]. The phase-by-diagnosis interaction was not significant [$F(1,15) < 1$, $p = 0.99$].

Cortisol responses to pentagastrin (phases 1 and 2) and placebo (phase 2) are presented in Figure 2. Analysis of placebo-controlled cortisol data (phase 2 alone, see Table 1) confirmed the striking effect of pentagastrin on HPA activity that was seen in the ACTH analyses and is evident in the cortisol graphs. A normal diurnal decline in cortisol levels is seen following placebo infusions but a clear rise is seen following pentagastrin infusions (effect of time and the drug-by-time interaction were both highly significant). There were no significant differences between patients and controls in placebo-controlled analyses of cortisol data.

Analyses of pentagastrin-day cortisol data from the combined sample (Table 2) also revealed no significant differences between patients and controls. There was a highly significant effect of time in this analysis, again reflecting the striking HPA response to pentagastrin infusion. Cortisol levels declined from 30 min before infusion to 3 min after infusion, then rose to a peak at 20 min after pentagastrin. Paired *t*-tests showed cortisol levels to be significantly below baseline levels at 3 min after infusion [$t(18) = 4.9$, $p = 0.0001$] and significantly elevated above baseline levels at 10, 20, and 30 min after infusion [$t(18) = 5.1$, $p = 0.0001$; $t(18) = 6.0$, $p = 0.0001$; and $t(18) = 3.9$, $p = 0.001$, respectively].

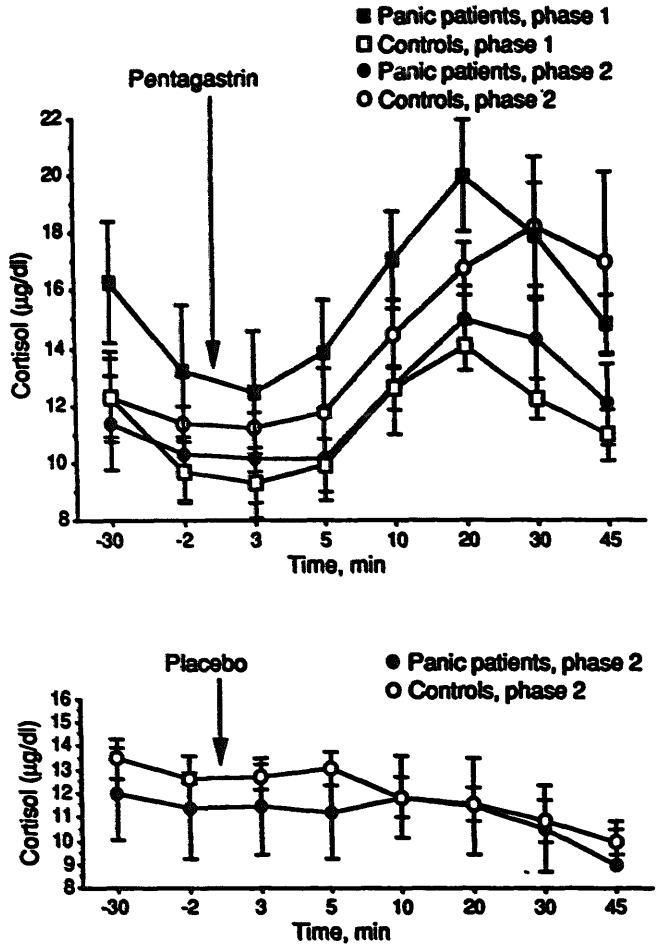

Figure 2. Cortisol responses to pentagastrin (upper graph) and placebo (lower graph) in patients with panic disorder (closed symbols) and controls (open symbols). Subjects in phase 1 (squares) received only pentagastrin infusion. Subjects in phase 2 (circles) received both pentagastrin and placebo. Group means and SEMs are plotted at each sampling point before and after infusion (arrows). The x-axis was plotted as in Figure 1. See text for analyses.

The phase effect was less evident in the cortisol data than in the ACTH data, but there was a trend towards a significant diagnosis-by-phase interaction (Table 2). This interaction reflected the patients' higher cortisol levels throughout phase 1 as compared to phase 2, whereas controls had higher levels throughout phase 2 than they did in phase 1.

To search further for possible patient–control group differences, patients and controls were directly compared on preinfusion and postinfusion cortisol secretion, and on peak response, first for the total groups and then separately for each phase. The total patient group ($n = 10$) did not differ from the total control group ($n = 9$) on any of these measures ($p > 0.60$ for each). In phase 1 patients had greater postpentagastrin cortisol secretion [$t(7) = 2.8$, $p = 0.026$] and higher cortisol peaks [$t(7) = 2.8$, $p = 0.025$] than did controls, but

did not differ from controls in baseline secretion [$t(7) = 1.8$, $p = 0.12$]. In phase 2 patients and controls were nearly identical on all 3 measures ($p > 0.20$ for each).

Pearson product–moment correlation coefficients were used to examine relationships between ACTH and cortisol responses and baseline levels. Baseline ACTH and baseline cortisol were significantly related to each other ($r = 0.6$, $p = 0.006$) and postpentagastrin ACTH secretion (AUC) was significantly related to baseline ACTH secretion ($r = 0.6$, $p = 0.005$). Postpentagastrin ACTH secretion was not related to baseline cortisol secretion ($r = 0.2$, $p = 0.51$) and the ACTH peak response was not related to either baseline cortisol ($r = 0.1$, $p = 0.58$) or baseline ACTH ($r = 0.2$, $p = 0.50$), however. The relationships between both postpentagastrin ACTH secretion and ACTH peak response and baseline cortisol levels remained small and nonsignificant for patient and control groups examined separately.

Pearson correlations were also used to search for relationships between ACTH secretion and baseline and postinfusion symptom levels. There were no relationships between the number of symptoms, total symptom intensity, and anxiety levels experienced before or after pentagastrin and either the peak ACTH response or total postinfusion ACTH secretion, for the total group and for controls examined separately ($r < 0.3$, $p > 0.23$ in every case). When panic patients were examined separately there were inverse relationships (trends) between prepentagastrin anxiety rating and postpentagastrin ACTH secretion ($r = -0.6$, $p = 0.059$); and between the number of symptoms experienced in response to pentagastrin and peak ACTH response ($r = -0.6$, $p = 0.07$). Those panic patients who were most anxious prior the infusion and experienced the greatest number of symptoms in response to it tended to have smaller ACTH responses. A trend in the opposite direction was seen for the total group (patients and controls) receiving placebo ($n = 10$), with a positive relationship between the number of symptoms experienced after the infusion and peak ACTH levels ($r = 0.6$, $p = 0.05$).

The data were also examined for relationships between the HPA axis and clinical or demographic variables. We detected no effects of age, weight, age of panic onset, duration of illness, panic attack frequency, or presence or absence of agoraphobia on any measure of HPA axis activity or responsivity to pentagastrin. There were too few men in the study to allow assessment of gender effects.

Growth Hormone

GH responses to pentagastrin (phases 1 and 2) and placebo (phase 2) are presented in Figure 3. One control subject and one patient were dropped from GH analyses due to elevated baseline GH levels (>5 μg/L). Another patient was dropped due to insufficient sample volumes. In contrast to analyses of ACTH and cortisol data, analysis of placebo-controlled

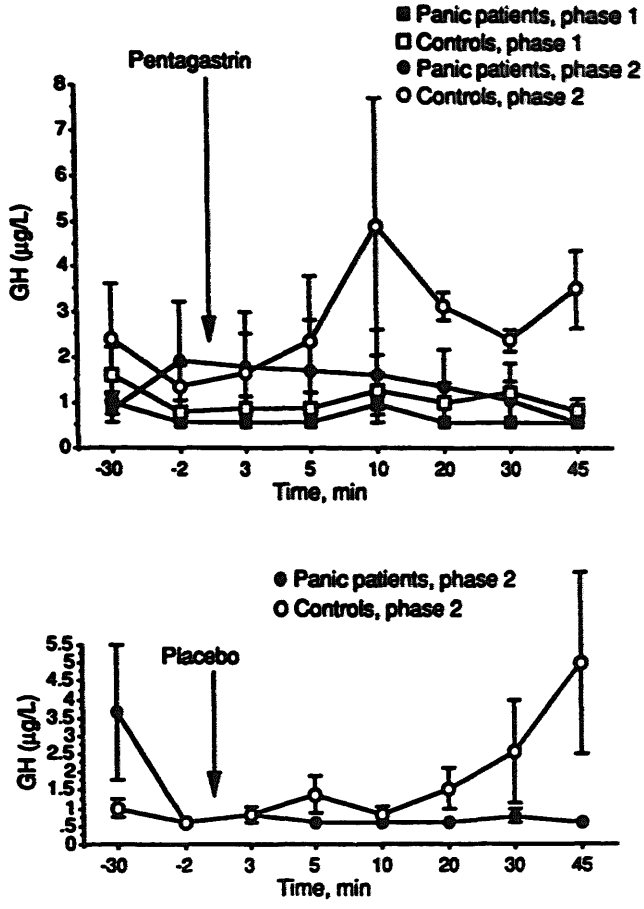

Figure 3. Growth hormone (GH) responses to pentagastrin (upper graph) and placebo (lower graph) in patients with panic disorder (closed symbols) and controls (open symbols). Subjects in phase 1 (squares) received only pentagastrin infusion. Subjects in phase 2 (circles) received both pentagastrin and placebo. Group means and SEMs are plotted at each sampling point before and after infusion (arrows). The x-axis was plotted as in Figure 1. See text for analyses.

GH data revealed no response to pentagastrin (Table 1). Patient–control differences are suggested by the nearly significant effect of diagnosis and the significant diagnosis-by-time interaction. The interaction effect was due to the rising GH levels seen at 30 and 45 min following both placebo and pentagastrin in controls, while patients' levels remained flat (see Figure 3).

Patient–control differences are slightly more evident in analyses of pentagastrin-day data from the combined sample (Table 2). Here the main effect of diagnosis was significant. Patients had lower overall mean GH levels than controls (1.02 ± 1.22 μg/L versus 1.90 ± 2.04 μg/L). The main effect of phase also reached significance; overall GH levels were lower in phase 1 than phase 2 (0.87 ± 0.62 μg/L versus 2.04 ± 2.22 μg/L). The lack of any time effects confirms the lack of GH response to pentagastrin infusion. GH levels were extremely low throughout the procedures for nearly all

subjects. There were few secretory spikes seen and a possible response to pentagastrin was seen for only one subject, a control in phase 2 who went from a baseline of 1.7 μg/L just prior to pentagastrin infusion to a peak of 13.3 μg/L at 10 min after pentagastrin.

Overall mean GH levels were inversely related to pentagastrin day baseline ACTH levels ($r = -0.8$, $p = 0.0002$). This relationship remained strong for patients and controls examined separately ($r \leq -0.8$, $p < 0.05$ for both) and for phase 1 and phase 2 data examined separately ($r = -0.7$, $p < 0.06$ for both).

Neurokinin A

Samples for NKA assay were available from all 10 subjects in phase 2, but only from 3 controls and 4 patients from phase 1. The two three-way ANOVAs produced only a significant main effect of phase [$F(1,13) = 20.299$, $p = 0.0006$], and a nearly significant effect of diagnosis [$F(1,13) = 4.221$, $p = 0.061$]. No effects involving time and no interaction effects approached significance. NKA levels were higher in phase 1 than in phase 2 and tended to be higher in patients than in controls. These differences appeared primarily in baseline levels on pentagastrin day. There was no discernable response to pentagastrin. Baseline NKA levels on pentagastrin day were positively related to baseline ACTH levels ($r = 0.7$, $p = 0.001$).

Catecholamines, VIP, and Substance P

Data on additional hormones are only available from phase 1, so the main analysis was two-way ANOVA (diagnosis-by-time). Useful data was not available from all subjects and the resultant small sample sizes made patient–control comparisons unlikely to be meaningfully interpretable. Our interest centered on "time" effects, which might reflect responsivity to the infusion. The main effect of time was significant in the two-way ANOVAs for epinephrine [$F(9,45) = 2.5$, $p = 0.019$] and VIP [$F(12,72) = 2.0$, $p = 0.034$], approached significance for norepinephrine [$F(9,63) = 1.978$, $p = 0.057$], and was not significant for dopamine [$F(9,54) = 0.679$, $p = 0.72$] and substance P [$F(12,84) = 1.2$, $p = 0.27$]. Epinephrine showed a striking, consistent, and brief response, with a spike present 1 min after infusion but gone at 3 min after infusion in six of seven subjects (see Figure 4). Epinephrine levels were significantly elevated above baseline levels only at the +1 min time point [$t(6) = 3.9$, $p = 0.008$]. VIP levels fell slightly over the baseline period to a nadir at 1 min after infusion, and then rose to a peak that was inconsistently maintained from 7 to 20 min after infusion before falling again. VIP levels at 7 and 20 min after infusion were significantly elevated above the +1 min trough [$t(7) > 2.6$, $p < 0.05$ for both]. The nearly significant effect of time for NE was due to slowly rising

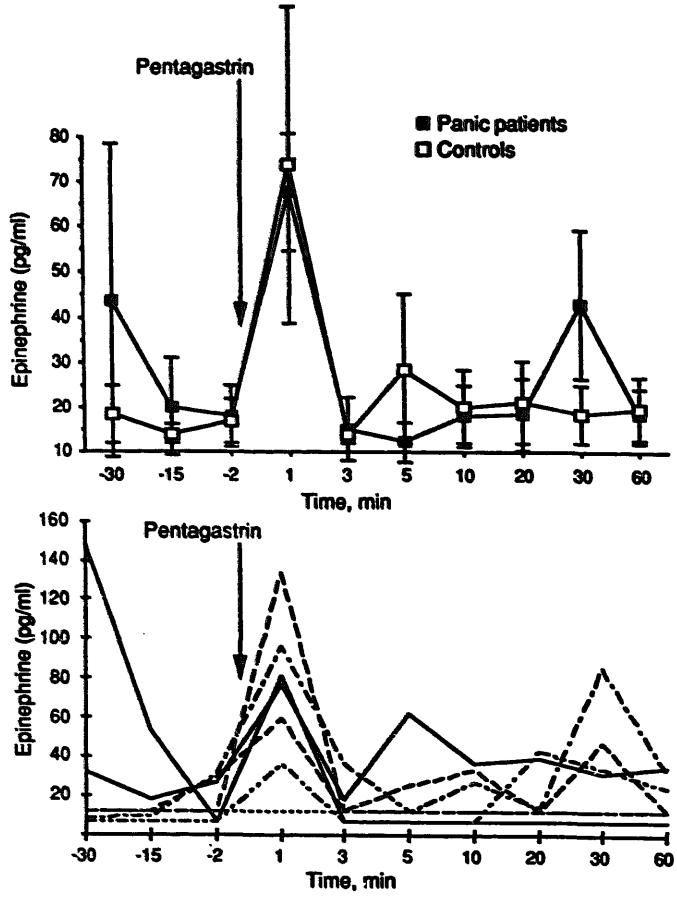

Figure 4. Epinephrine responses to pentagastrin in seven subjects from phase 1. Group means and SEMs are presented in the upper graph. Individual subjects' data are presented in the lower graph (controls are represented by solid lines and patients by broken lines). The point of infusion is marked with arrows. The x-axis was plotted with equal distance between sampling points rather than scaled to actual time to make the graphs more readable. See text for analyses.

levels from the beginning to end of the sampling period with no consistent response to the infusion itself.

Epinephrine levels at 1 min after infusion (peak) were strongly related to baseline heart rate ($r = 0.78$, $p < 0.05$) but not to postinfusion heart rate or to blood pressure. There were no significant relationships between epinephrine and any measures of symptoms or HPA axis activity.

Discussion

CCK and HPA Axis

As far as we know, this data provides the first placebo-controlled confirmation of an early report that pentagastrin potently activates the HPA axis in humans (Degli Uberti et al 1983). Data from our enlarged samples support our earlier impression (Abelson et al 1991) that this response is a direct pharmacological effect and is not mediated by anxiety

symptoms or a nonspecific stress response. We found no relationship between the ACTH or cortisol responses to pentagastrin and the symptoms produced. After placebo infusion there was a positive correlation between ACTH levels and the number of symptoms experienced, indicating that HPA activity is sensitive to nonspecific stressors in our experimental paradigm, but under the apparently potent stimulus of pentagastrin this positive association disappears. There is even a hint that for panic patients it inverts; the patients with higher levels of anticipatory anxiety and more symptomatic responses to pentagastrin tended to have smaller ACTH responses. Further support for the specificity of the ACTH response is provided by the lack of GH or NKA responses. GH is known to be a stress responsive hormone (Breier et al 1988), but did not respond to pentagastrin despite the substantial subjective distress produced. NKA levels were strongly related to ACTH levels prior to the pentagastrin infusion, perhaps linked by shared responsivity to the stress of the procedure, but NKA levels did not rise following the infusion, indicating pharmacological specificity in pentagastrin's ability to release ACTH. It is possible that pentagastrin has direct inhibitory effects on the release of GH (see below) or NKA, which block a stress response that might otherwise be seen, but this is still consistent with pharmacologically specific neuroendocrine effects.

Our data are consistent with the few other studies that have examined CCK-HPA interactions in humans. In the only other published study of HPA response to pentagastrin in humans (Degli Uberti et al 1983), a dose of 0.5 μg/kg produced ACTH and cortisol response curves nearly identical to ours. Cerulein, a naturally occurring CCK agonist, stimulates ACTH and cortisol responses in humans nearly identical to those elicited by CRH (Späth-Schwalbe et al 1988). Elevation in cortisol following CCK_4 infusion has also been reported (de Montigny 1989).

Neuroanatomical and pharmacological data from basic studies are consistent with our findings and support a physiological role for CCK in modulating HPA axis activity. CCK/gastrins are found within the pituitary and in parvocellular neurons in the paraventricular nucleus (PVN) in the hypothalamus and are co-localized with ACTH and CRH (Larsson and Rehfeld 1981; Rehfeld 1978; Rehfeld and Larsson 1981; Rehfeld et al 1987; Vanderhaeghen et al 1985). CCK in the hypothalamus is regulated by adrenal activity (Mezey et al 1986). CCK stimulates ACTH and β-endorphin release in vitro as well as in vivo (Matsumura et al 1983; Mezey et al 1986; Reisine and Jensen 1986).

The mechanism by which CCK agonism releases ACTH has not yet been determined. There is some in vitro evidence that it is not mediated by CRH (Reisine and Jensen 1986). Our data are consistent with this possibility in that the ACTH response we observed is comparable in size but considerably more rapid than that seen in response to either human or ovine CRH (Bardeleben and Holsboer 1988; Roy-Byrne et al 1986). Peak ACTH levels occur within 5 min of pentagastrin infusion but not until about 30 min after CRH infusion. Even when intensive effort is made to insure very rapid infusion of a CRH bolus, the peak does not appear until 10–15 min after infusion (Young et al 1990). The rapidity of the CCK-induced ACTH response is strikingly consistent across studies (Degli Uberti et al 1983; Späth-Schwalbe et al 1988) and clearly different from human CRH even in direct comparison (Späth-Schwalbe et al 1988). The lack of a relationship in our data between the ACTH response to pentagastrin and baseline cortisol levels also differentiates CCK-mediated HPA activation from CRH-mediated activation and suggests that the CCK-mediated response may be relatively insensitive to cortisol feedback inhibition. In vitro, however, the CCK-induced response has been suppressible with dexamethasone, like CRH-stimulated ACTH release (Matsumura et al 1983; Reisine and Jensen 1986). The relative sensitivity of the two activators to glucocorticoid inhibition has not been directly examined in animal models in vivo. Clarification of the roles of CRH and of cortisol feedback inhibition in CCK-induced HPA activation will require further study.

Whether the ACTH response we observed is mediated by the CCK-B receptor also remains unknown. Animal evidence suggests that stimulation of the corticotroph by CCK is not mediated by either the CCK-A or CCK-B receptor, but may involve a third, novel CCK receptor subtype (Matsumura et al 1983; Reisine and Jensen 1986). Substantial cross-species variation in the central distribution and function of the A and B receptor types (Woodruff et al 1991) makes it difficult to draw cross-species conclusions about CCK receptor subtype function, however. The intracellular mechanism by which CCK stimulates ACTH release is also not yet established but, in contrast to CRH, CCK-stimulated ACTH release does not appear to involve cyclic adenosine monophosphate (cAMP) (Reisine and Jensen 1986).

HPA Axis and Panic Disorder

Our data suggest that panic patients do not differ from normal control subjects in their ACTH or cortisol responses to pentagastrin. Interpretation is complicated by the effect of phase on HPA axis activity. Discussion of patient–control comparisons in analyses that separate the two phases are speculative and preliminary, because cell sizes become quite small in these analyses. Phase 1 data did raise the possibility that patients had greater HPA responses to pentagastrin, however, reflected in overall higher ACTH levels and greater postpentagastrin cortisol secretion. Because the elevated postpentagastrin cortisol levels could be due to elevated pre-infusion ACTH levels, it remained unclear whether patient–control differences reflected differences in

responsivity to pentagastrin or in responsivity to the experimental situation. Phase 2 data suggests that it is more related to situational factors than to drug effects since patients and controls looked nearly identical when studied after prior experience in the experimental situation. For baseline ACTH secretion, placebo responses, and postpentagastrin ACTH secretion, differences between patients and controls appeared only during the subjects' first experience in the CRC. This phase effect is consistent with other reports that panic patients are hyperresponsive to "novel" stimuli, such as laboratory situations (Roth et al 1992), and with evidence that the HPA axis is responsive to novelty stress (Breier et al 1987; Davis et al 1981; Voigt et al 1990; Young et al 1990). Apparent abnormalities in HPA axis activity in panic patients could thus be due to a secondary response of the axis to, for example, an abnormality in an arousal/vigilance system that creates hypersensitivity to experimental situations, rather than a primary pathophysiological defect within the HPA axis itself. Our evidence that pentagastrin is less abnormally activating of the HPA axis in panic patients when they have had a prior benign experience in the experimental situation supports this possibility. Increased anticipatory anxiety in "novel" environments or situations could account for many of the apparent HPA axis abnormalities thus far reported in panic patients (Abelson et al 1991; Carr et al 1986; Goldstein et al 1987; Gurguis et al 1991; Nesse et al 1984; Roy-Byrne et al 1986). Basal elevations in ACTH levels, for example, appear more likely to be reported when they are measured just prior to first exposure to a complex procedure (Roy-Byrne et al 1986) than when they are measured after more prolonged exposure to the laboratory situation (Holsboer et al 1987) or in the context of a simple blood draw (Gurguis et al 1991).

The strongest evidence for a more primary HPA axis abnormality comes from two reports of blunted ACTH responses to CRH in panic patients (Holsboer et al 1987; Roy-Byrne et al 1986). In one of these studies (Roy-Byrne et al 1986), however, the blunted ACTH response could be secondary to situational factors. Baseline levels of ACTH and cortisol were elevated, perhaps due to an early secretory response to entry into the experimental situation. This acute stress response prior to the CRH challenge could then be responsible for the blunting following challenge, since both human (Young et al 1990) and animal data (Rivier and Vale 1983; Young and Akil 1985) suggest that acute stress can be followed by an acute subsensitivity of pituitary corticotrophs to CRH challenge. The patient–control difference could therefore be due to events occurring before the infusion and not to differential responsivity to the CRH itself. This type of explanation applies less well to the CRH stimulation test data from the European group (Holsboer et al 1987) as subjects in their setting had a 6-hr period of accommodation to the laboratory situation, and the blood-drawing and infusion procedures were done out of view of the subjects. Perhaps the reduced novelty and stressfulness of this paradigm accounts for the normal baseline levels of ACTH and cortisol seen, but ACTH responses to CRH remained blunted. Unfortunately, this group's published reports (Bardeleben and Holsboer 1988; Holsboer et al 1987) do not facilitate full assessment of the possible effects of situational reactivity on their results. Procedures are not described in detail. Patients' responses to the accommodation phase and ACTH secretory activity during it are not reported and could account for some of the subsequent blunting after CRH stimulation.

Though the effects of novelty sensitivity on panic patients' HPA responses to pentagastrin seemed clear in our data, follow-up studies explicitly designed to study this phenomenon are needed before definitive conclusions can be drawn. In retrospect it appeared to us that pentagastrin was given in a "high novelty/anticipatory anxiety" condition in phase 1 but in a lower "novelty/anticipatory anxiety" condition in phase 2. The conditions were not explicitly designed to create differential levels of stress or anxiety and subjects were not randomly assigned to condition, however. If appropriately designed studies confirm that differential sensitivity to novelty stress can contribute to differential neuroendocrine responsivity when panic patients are compared to controls, then study of receptor level sensitivities using pharmacological probes in this patient population will be much more difficult. The circumstances under which a neuroendocrine response is measured may markedly affect results and levels of novelty and anticipatory anxiety will need to be carefully controlled and monitored. Panic patients may need to be desensitized to the nonspecific stressors of the research setting before the pharmacological probe is applied if receptor level sensitivity to specific pharmacological effects of the probe are of primary interest. It was with this possibility in mind that we decided to use a fixed order, active-substance-second design in phase 2. The results suggest that this strategy may be appropriate in studies of this sort. A randomized-order design in phase 2 may have led us to the erroneous conclusion that exaggerated cortisol responses to pentagastrin are present in panic. Our novelty-effect findings must, however, be considered preliminary. The ultimate appropriateness of fixed or randomized order designs in neuroendocrine infusion studies in panic patients can only be determined by larger follow-up studies that utilize both designs to directly examine novelty effects.

We believe that further development and utilization of pentagastrin as a probe of the stress axis in panic patients, including manipulation and closer monitoring of preinfusion situational stressors, could help illuminate the nature of the apparent HPA axis dysregulation seen in some studies of panic disorder. Test–retest paradigms will be needed to

examine the stability of the HPA response to pentagastrin over time and are especially important in light of panic patients' supersensitivity to first exposure effects. Further studies with CRH are also needed, but must include fuller descriptions of preinfusion activity in the axis.

CCK and Other Systems

The absence of GH response to pentagastrin in either patients or controls highlights the pharmacological specificity of the ACTH response. The fact that our panic patients had significantly lower GH levels overall than controls also adds to the growing body of evidence that patients with panic disorder have a regulatory abnormality in their GH axis (Uhde et al 1992). Given the anxiety and subjective distress produced by pentagastrin (Abelson and Nesse 1994), we might have expected some elevation in GH levels. There is both animal (Karashima et al 1984; Spencer et al 1991) and human data, however (Nair et al 1984), that suggest that CCK agonism can inhibit stimulated GH secretion. This raises the possibility that CCK might play a role in the consistently blunted GH response to clonidine seen in patients with panic disorder (Abelson et al 1992). Given that CCK agonism may play a role in anxiogenesis (Bradwejn et al 1991), the possibility that it may also inhibit GH secretion is extremely intriguing, as CCK could therefore play a role both in symptom production in panic disorder and in the most robust neuroendocrine abnormality thus far identified in panic patients. The animal data on the effects of CCK on GH are not entirely consistent with an inhibitory effect (Matsumura et al 1984), but because of species variability in CCK function more human data is needed. Pentagastrin provides a useful tool to explore CCK-GH interactions in humans. Its ability to inhibit stimulated GH secretion in both normal controls and panic patients should be examined, as should the ability of CCK antagonists to reverse the blunted GH response to clonidine seen in patients with panic disorder.

The strong inverse relationship between baseline ACTH levels on pentagastrin infusion day and overall GH levels is intriguing. It suggests that those subjects whose HPA axes were most reactive to anticipation of the experimental procedure had the greatest tonic inhibition in their GH axes. There is some evidence that CRH can in fact inhibit GH release in animals (Rivier and Vale 1984); and some human data suggest that HPA axis hormones can modulate GH release patterns (Wiedemann et al 1991). The overactivity of the HPA axis and underactivity in the HPS axis noted in some psychiatric disorders, including panic, may thus not represent entirely independent phenomena (Wiedemann et al 1991). Our data provide one of the first demonstrations of a correlation between increased HPA reactivity and decreased HPS activity within a single group of panic patients. Discovery of the linkage mechanism could provide important new information on GH and stress axis abnormalities in psychiatric disorders. Because CCK appears capable of both activating the HPA axis and inhibiting GH release, it could mediate the link between the two systems.

The brief but consistent epinephrine response is also quite intriguing. We unfortunately did not collect +1 min samples in phase 2 so we do not know if it is a response to the pentagastrin or to the act of being infused. It appeared simultaneously with or just before the appearance of symptoms but was not correlated with symptoms. Its rapidity, brevity, and consistency across subjects suggest that it may have been a first-pass pharmacological response of the adrenal medulla to the CCK-B agonist. We are not aware of any studies examining the adrenal medulla for the presence of CCK-B receptors. The brevity of the response suggests that continuous sampling techniques may be critical for meaningful study of catecholamines in patients with anxiety disorders (Dimsdale and Moss 1980).

Our data do not support the hypothesis that symptoms induced by peripheral release of vasoactive peptides such as NKA, VIP, or substance P might account for pentagastrin's panicogenic effects. The lack of peripherally detectable vasoactive peptide release may indicate that activity at or above the level of the pituitary is more relevant to the role of CCK in anxiety disorders than activity in the periphery. NKA, substance P, and VIP are all found in the central nervous system as well as peripherally and all have actions and neuroendocrine interactions that make them of potential interest to psychoneuroendocrine research (Brodin et al 1989; Gjerris et al 1984; Panza et al 1992; Reichlin 1988; Shen and North 1992; Tschope et al 1992). Our data shed little light on their possible relevance to panic disorder; but the rise in VIP following pentagastrin infusion and the association between basal NKA and ACTH levels (raising the possibility that NKA is stress responsive) are findings that merit further exploration. Our data did provide a hint that a subset of patients might have elevated levels of substance P or VIP (data not presented), but there were too few subjects and too much variance to draw any real conclusions. Further study of these peptides in panic patients may still be warranted.

Implications

If follow-up studies support the hypotheses that CCK modulates HPA axis activity independently of CRH, via different intracellular mechanisms, and with differing sensitivity to glucocorticoid feedback inhibition, this would be of great theoretical interest to biological psychiatry. Multihormonal control of the HPA axis is clearly supported by available data and likely reflects the critical importance of the stress axis in mammalian adaptation (Axelrod and Reisine 1984). Multihormonal control of the axis may allow its continued responsivity to acute stressors even when chronic stress or

other factors may have reduced its responsivity to some activators (Reisine and Jensen 1986). CCK could participate in acute stress axis activation in psychiatric patients who have reduced responsivity to CRH. Evidence that panic patients have normal ACTH responses to pentagastrin but blunted responses to CRH (Bardeleben and Holsboer 1988; Roy-Byrne et al 1986) is consistent with this possibility, although other factors may be involved for panic patients (see above). Application of the pentagastrin stimulation test to other psychiatric disorders that demonstrate blunted ACTH responses to CRH will provide additional tests of this hypothesis. The possibility that psychiatric patients might have differing ACTH and cortisol responses to two ACTH secretogogues that work through differing mechanisms and with differing sensitivities to feedback inhibition creates intriguing new avenues for study of stress axis regulation in these populations. The roles of hypercortisolemia or cAMP (Charney et al 1989; Smith et al 1989) in HPA axis dysregulation, for example, could be explored using combined infusions of pentagastrin and CRH.

Our data caution us to be careful about the conclusions we draw from neuroendocrine studies in patients with panic disorder because nonpharmacological aspects of experimental paradigms may influence endocrine responses to phramacological probes. An apparent abnormality in regulation of the HPA axis in panic patients (Holsboer et al 1987; Roy-Byrne et al 1986), for example, could be due to an interaction between an abnormality in arousal/attentional control systems (noradrenergic, locus coeruleus?) and experimental situations rather than any intrinsic dysregulation within the HPA axis itself.

Similarly, some have speculated that the sensitivity of panic patients to the anxiogenic capacity of CCK could be due to increased sensitivity of the CCK-B receptor in patients with panic disorder (Brambilla et al 1993). Nonpharmacological aspects of the infusion experience could contribute to the patients' behavioral sensitivity, however. The normal neuroendocrine responses of panic patients to pentagastrin argues against abnormal CCK-B receptor sensitivity, though low dose infusion studies are needed before definitive conclusions about receptor sensitivity can be reached.

Clearly much work remains to be done. An expanding collection of receptor-specific agonist and antagonist neuroendocrine probes will greatly facilitate that work. Pentagastrin is a useful addition to that collection, providing a safe and readily available tool for studying the psychoneuroendocrinology of CCK-B receptor systems in humans.

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References

Abelson JL, Nesse RM (1994): Pentagastrin infusions in patients with panic disorder I. Symptoms and cardiovascular responses. *Biol Psychiatry* 36:73–83.

Abelson JL, Nesse RM, Vinik A (1991): Stimulation of corticotropin release by pentagastrin in normal subjects and patients with panic disorder. *Biol Psychiatry* 29:1220–1223.

Abelson JL, Glitz D, Cameron OG, Lee MA, Bronzo M, Curtis GC (1992): Endocrine, cardiovascular, and behavioral responses to clonidine in patients with panic disorder. *Biol Psychiatry* 32:18–25.

Ahlman H, Dahlstrom A, Gronstad K, et al (1985): The pentagastrin test in the diagnosis of the carcinoid syndrome. Blockade of gastrointestinal symptoms by ketanserin. *Ann Surg* 201:81–6.

Axelrod J, Reisine TD (1984): Stress hormones: Their interaction and regulation. *Science* 224:452–459.

Baber NS, Dourish CT, Hill DR (1989): The role of CCK, caerulein, and CCK antagonists in nociception. *Pain* 39:307–328.

Bardeleben U von, Holsboer F (1988): Human corticotropin releasing hormone: Clinical studies in patients with affective disorders, alcoholism, panic disorder and in normal controls. *Prog Neuropsychopharmacol Biol Psychiatry* 12:S165–S187.

Beresford IJM, Hall MD, Clark CR, Hill RG, Hughes J (1988): Cholecystokinin modulation of [3H]noradrenaline release from superfused hypothalamic slices. *Neurosci Lett* 88:227–232.

Bradwejn J, Koszycki D, Shriqui C (1991): Enhanced sensitivity to cholecystokinin tetrapeptide in panic disorder. *Arch Gen Psychiatry* 48:603–610.

Bradwejn J, Koszycki D, Dutertre AC, et al (1993): L-365,260, a CCK-B antagonist, blocks CCK-4 in panic disorder. Presented at Anxiety Disorders Association of America Annual Meeting, Charleston, South Carolina, March 20, 1993.

Brambilla F, Bellodi L, Perna G, Garberi A, Panerai A, Sacerdote P (1993): Lymphocyte cholecystokinin concentrations in panic disorder. *Am J Psychiatry* 150:1111–1113.

Breier A, Albus M, Pickar D, Zahn TP, Wolkowitz OM, Paul SM (1987): Controllable and uncontrollable stress in humans: Alterations in mood and neuroendocrine and psychophysiological function. *Am J Psychiatry* 144:1419–1425.

Breier A, Wolkowitz OM, Doran AR, Bellar S, Pickar D (1988): Neurobiological effects of lumbar puncture stress in psychiatric patients and healthy volunteers. *Psychiatry Res* 25:187–94.

Brodin K, Rosen A, Iwarsson K, Ogren SO, Brodin E (1989): Increased levels of substance P and cholecystokinin in rat cerebral cortex following repeated electroconvulsive shock and subchronic treatment with a serotonin reuptake inhibitor. *Acta Physiol Scand* 136:613–614.

Carr DB, Sheehan DV, Surman OS, et al (1986): Neuroendocrine correlates of lactate-induced anxiety and their response to chronic alprazolam therapy. *Am J Psychiatry* 143:483–494.

Charney DS, Innis RB, Duman RS, Woods SW, Heninger GR (1989): Platelet alpha-2-receptor binding and adenylate cyclase activity in panic disorder. *Psychopharmacology (Berl)* 98:102–107.

Crawley JN (1991): Cholecystokinin-dopamine interactions. *Trends Pharmacol Sci* 12:232–236.

Davis HA, Gass GC, Bassett JR (1981): Serum cortisol response to incremental work in experienced and naive subjects. *Psychosom Med* 43:127–132.

de Montigny C (1989): Cholecystokinin tetrapeptide induces panic-like attacks in healthy volunteers. *Arch Gen Psychiatry* 46:511–517.

Degli Uberti EC, Transforini G, Margutti AR, Rotola CA, Pansini R (1983): Effect of pentagastrin on adrenocorticotropin hormone and thyroid-stimulating hormone release in normal subjects. *Horm Res* 17:74–77.

Dethloff LA, De La Iglesia FA (1992): Cholecystokinin antagonists—A toxicologic perspective. *Drug Metab Rev* 24:267–293.

Dimsdale JE, Moss J (1980): Short-term catecholamine response to psychological stress. *Psychosom Med* 42:493–497.

Gjerris A, Rafaelsen OJ, Vendsborg P, Fahrenkrug J, Rehfeld JF (1984): Vasoactive intestinal peptide decreased in cerebrospinal fluid (CSF) in atypical depression. *J Affective Disord* 7:325–337.

Goldstein S, Halbreich U, Asnis G, Endicott J, Alvir J (1987): The hypothalamic–pituitary–adrenal system in panic disorder. *Am J Psychiatry* 144:1320–1323.

Gurguis GNM, Mefford IN, Uhde TW (1991): Hypothalamic-pituitary–adrenocortical activity in panic disorder: Relationship to plasma catecholamine metabolites. *Biol Psychiatry* 30:502–506.

Holsboer F, Bardeleben U von, Buller R, Heuser I, A S (1987): Stimulation response to corticotropin-releasing hormone (CRH) patients in with depression, alcoholism and panic disorder. *Horm Metab Res* [suppl] 16:80–88.

Hughes J, Hunter JC, Woodruff GN (1991): Neurochemical actions of CCK underlying the therapeutic potential of CCK-B antagonists. *Neuropeptides* 19(suppl):85–89.

Kaneyuki T, Morimasa T, Shohmori T (1989): Action of peripherally administered cholecystokinin on monoaminergic and GABAergic neurons in the rat brain. *Acta Med Okayama* 43:153–159.

Karashima T, Okajima T, Kato K, Ibayashi H (1984): Suppressive effects of cholecystokinin and bombesin on growth hormone and prolactin secretion in urethaneanesthetized rats. *Endocrinol Jpn* 31:539–47.

Kulkosky PJ, Sanchez MR, Foderaro MA, Chiu N (1989): Cholecystokinin and satiation with alcohol. *Alcohol* 6:395–402.

Larsson LI, Rehfeld JF (1981): Pituitary gastrins occur in corticotrophs and melanotrophs. *Science* 213:768–770.

Matsumura M, Yamanoi A, Yamamoto S, Saito S (1983): In vivo and in vitro effects of cholecystokinin octapeptide on the release of β-endorphin-like-immunoreactivity. *Neuroendocrinology* 36:443–448.

Matsumura M, Yamanoi A, Yamamoto S, Mori H, Saito S (1984): In vivo and in vitro effects of cholecystokinin octapeptide on the release of growth hormone in rats. *Horm Metab Res* 16:626–630.

Mezey E, Reisine TD, Skirboll L, Beinfeld M, Kiss JZ (1986): Role of cholecystokinin in corticotropin release: Coexistence with vasopressin and corticotropin-releasing factor in cells of the rat hypothalamic paraventricular nucleus. *Proc Natl Acad Sci* 83:3510–3512.

Nair NP, Lal S, Thavundayil JX, Wood PL, Etienne P, Guyda H (1984): CCK-33 antagonizes apomorphine-induced growth hormone secretion and increases basal prolactin levels in man. *Neuropeptides* 4:281–91.

Nesse RM, Cameron OG, Curtis GC, McCann DS, Huber-Smith MJ (1984): Adrenergic function in patients with panic anxiety. *Arch Gen Psychiatry* 41:771–776.

Oberg K, Norheim I, Theodorsson E, Ahlman H, Lundqvist G, Wide L (1989): The effects of octreotide on basal and stimulated hormone levels in patients with carcinoid syndrome. *J Clin Endocrinol Metab* 68:796–800.

O'Connell JE, Dominiczak AF, Isles CG, et al (1990): A comparison of calcium pentagastrin and TRH tests in screening for medullary carcinoma of the thyroid in MEN IIA. *Clin Endocrinol* 32:417–421.

Panza G, Monzani E, Sacerdote P, Penati G, Panerai AE (1992): Beta-endorphin, vasoactive intestinal peptide and cholecystokinin in peripheral blood mononuclear cells from healthy subjects and from drug-free and haloperidol-treated schizophrenic patients. *Acta Psychiatr Scand* 85:207–10.

Rehfeld JF (1978): Localization of gastrins to neuro- and adenohypophysis. *Nature (Lond)* 271:771–773.

Rehfeld JF (1985): Neuronal cholecystokinin: one or multiple transmitters? *J Neurochem* 44:1–10.

Rehfeld JF, Larsson LI (1981): Pituitary gastrins: Different processing in corticotrophs and melanotrophs. *J Biol Chem* 256:10426–10429.

Rehfeld JF, Lindholm J, Andersen BN, Bardram L, Cantor P, Fenger M, Lüdecke DK (1987): Pituitary tumors containing cholecystokinin. *N Engl J Med* 316:1244–1247.

Reichlin S (1988): Neuroendocrine significance of vasoactive intestinal polypeptide. *Ann NY Acad Sci* 527:431–449.

Reisine T, Jensen R (1986): Cholecystokinin-8 stimulates adrenocorticotropin release from anterior pituitary cells. *J Pharmacol Exp Ther* 236:621–626.

Rivier C, Vale W (1983): Influence of the frequency of ovine corticotropin releasing factor administration on adrenocorticotropin and corticosterone secretion in rat. *Endocrinology* 113:1422–1426.

Rivier C, Vale W (1984): Ovine corticotropin releasing factor (CRF) acts centrally to inhibit growth hormone secretion in the rat. *Endocrinology* 114:2409–2411.

Roth WT, Margraf J, Ehlers A, et al (1992): Stress test reactivity in panic disorder. *Arch Gen Psychiatry* 49:301–310.

Roy-Byrne PP, Uhde TW, Post RM, Gallucci W, Chrousos GP, Gold PW (1986): The corticotropin-releasing hormone stimulation test in patients with panic disorders. *Am J Psychiatry* 143:896–899.

Sheehan MJ, de Belleroche J (1983): Facilitation of GABA release by cholecystokinin and caerulein in rat cerebral cortex. *Neuropeptides* 3:429–434.

Shen KZ, North RA (1992): Substance P opens cation channels and closes potassium channels in rat locus coeruleus neurons. *Neuroscience* 50:345–354.

Silver AJ, Morley JE (1991): Role of CCK in regulation of food intake. *Prog Neurobiol* 36:23–34.

Singh L, Lewis AS, Field MJ, Hughes J, Woodruff GN (1991): Evidence for an involvement of the brain cholecystokinin B receptor in anxiety. *Proc Natl Acad Sci USA* 88:1130–1133.

Smith MA, Davidson J, Ritchie JC, et al (1989): The corticotropin-releasing hormone test in patients with posttraumatic stress disorder. *Biol Psychiatry* 26:349–355.

Späth-Schwalbe E, Piroth L, Pietrowsky R, Born J, Lorenz Fehm H (1988): Stimulation of the pituitary adrenocortical system in man by cerulein, a cholecystokinin-8-like peptide. *Clin Physiol Biochem* 6:316–320.

Spencer GS, Berry C, Johnston S (1991): Neuroendocrine regulation of growth hormone secretion in sheep. IV. Central and peripheral cholecystokinin. *Domest Anim Endocrinol* 8:555–563.

Spitzer RL, Williams JBW (1986): *Structured Clinical Interview for DSM-III-R-Upjohn Version-Revised.* Biometrics Research Department, New York State Psychiatric Institute.

Stallone D, Nicolaidis S, Gibbs J (1989): Cholecystokinin-induced anorexia depends on seritoninergic function. *Am J Physiol* 256:R1138–1141.

Tschope C, Picard P, Culman J, et al (1992): Use of selective antagonists to dissociate the central cardiovascular and behavioral effects of tachykinins on NK1 and NK2 receptors in rat. *Br J Pharmacol* 107:750–755.

Uhde TW, Tancer ME, Rubinow DR, et al (1992): Evidence for hypothalamic-growth hormone dysfunction in panic disorder: profile of growth hormone (GH) responses to clonidine, yohimbine, caffeine, glucose, GRF and TRH in panic disorder patients versus healthy volunteers. *Neuropsychopharmacology* 6:101–118.

Vanderhaeghen JJ, Goldman S, Lotstra F, et al (1985): Co-existence of cholecystokinin- or gastrin-like peptides with other peptides in the hypophysis and hypothalamus. *Ann NY Acad Sci* 448:334–344.

Vinik AI, Gonin J, England BG, Jackson T, McLeod MK, Cho K (1990): Plasma substance-P in neuroendocrine tumors and idiopathic flushing: the value of pentagastrin stimulation tests and the effects of somatostatin analog. *J Clin Endocrinol Metab* 70:1702–9.

Voigt K, Ziegler M, Grünert-Fuchs M, Bickel U, Fehm-Wolfsdorf G (1990): Hormonal responses to exhausting physical exercise: The role of predictability and controllability of the situation. *Psychoneuroendocrinology* 15:173–184.

Wiedemann K, von Bardeleben U, Holsboer F (1991): Influence of human corticotropin-releasing hormone and adrenocorticotropin upon spontaneous growth hormone secretion. *Neuroendocrinology* 54:462–468.

Woodruff GN, Hughes J (1991): Cholecystokinin antagonists. *Annu Rev Pharmacol Toxicol* 31:469–501.

Woodruff GN, Hill DR, Boden P, Pinnock R, Singh L, Hughes J (1991): Functional role of brain CCK receptors. *Neuropeptides* 19[suppl]:45–56.

Young EA, Akil H (1985): CRF stimulation of ACTH/β-endorphin release: effects of acute and chronic stress. *Endocrinology* 117:23.

Young EA, Watson SJ, Kotun J, et al (1990): β-Lipotropin-β-endorphin response to low-dose ovine corticotropin releasing factor in endogenous depression. *Arch Gen Psychiatry* 47:449–457.