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Dose Response of Adrenocorticotropin and Cortisol to the CCK-B Agonist Pentagastrin

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Cholecystokinin (CCK) is an abundant neurotransmitter in brain. Its functional significance in humans is incompletely understood, but it may modulate activity in the hypothalamic–pituitary-adrenal (HPA) axis. To explore this hypothesis, we examined the effects of varying doses (0 to 0.8 μg/kg) of the CCK-B agonist pentagastrin on adrenocorticotropin (ACTH) and cortisol release in healthy human subjects. We also examined anxiety, heart rate (HR), and blood pressure (BP) responses. Pentagastrin induced large (up to 520 % increase over baseline), significant and very rapid, dose-dependent elevations in ACTH and cortisol levels. Significant elevations in HR and BP were seen at all doses, without clear dose-response relationships. Anxious distress and symptom responses were also somewhat dose dependent; but hormonal responses were more robustly linked to pentagastrin dose than to these subjective measures. The HPA axis response to the CCK-B agonist pentagastrin may be a direct pharmacological effect. Further work is needed to determine the mechanisms and the physiological significance of CCK-mediated modulation of the human neuroendocrine stress axis. ***[Neuropsychopharmacology 21:485–494, 1999]*** *© 1999 American College of Neurospychopharmacology. Published by Elsevier Science Inc.*

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Cholecystokinin (CCK) is a peptide neurotransmitter widely distributed in the brain and abundant in cortex (Kreiger 1983). Two CCK receptor subtypes (designated A and B) have been identified. Both are found within the central nervous system (CNS), although CCK-B receptors are far more numerous and widely distributed there (Hill and Woodruff 1990; Saito et al. 1980; Woodruff and Hughes 1991). CCK or CCK receptors are found in brain areas involved with cognitive or emotional aspects of behavior, such as prefrontal cortex, cingulate, hippocampus, amygdala, and the locus coeruleus (Lindefors et al. 1993; Saito et al. 1980). CCK is also colocalized or interacts with neurotransmitters thought to be involved in anxiety and affective disorders, such as serotonergic (Brodin et al. 1989; Stallone et al. 1989), GABAergic (Kaneyuki et al. 1989; Sheehan and de Belleroche 1983), and monoaminergic systems (Beresford et al. 1988; Crawley 1991; Kaneyuki et al. 1989). Considerable work, in fact, supports a role for the CCK-B receptor in anxiety states and panic attacks (Abelson and Nesse 1994; Bradwejn and Koszycki 1994; Harro et al. 1993). There is also evidence that the CCK-B receptor may play a role in modulating activity within the hypothalamic–pituitary-adrenal (HPA) axis (Abelson et al. 1994). Thus, CCK may be a modulator of emotional behavior and stress responsiveness; however, its real functional significance in humans is still poorly understood.

Significant cross-species differences exist in the function and distribution of CCK receptors (Hinks et al. 1995; Woodruff et al. 1991). Therefore, development of human probes is critical for further insight into the

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functional biology of CCK in humans. CCK-4 has been well developed as a laboratory tool for studying human panic anxiety (Bradwejn and Koszycki 1994; Bradwejn et al. 1992), but access to this probe is limited, and its neuroendocrinology has not been extensively studied. Pentagastrin is an alternative, readily available CCK-B receptor agonist. It has a higher affinity for the CCK-B receptor than CCK-4; and although it is somewhat less selective for the B over the A receptor, it is a highly selective agonist with minimal CCK-A receptor activity in the dose range used in human studies (Hughes et al. 1990). Its anxiogenic properties in humans are similar to those of CCK-4 (Abelson and Nesse 1994).

A number of studies have now confirmed the ability of pentagastrin, as well as other CCK-B agonists, to activate the HPA axis in humans (Abelson et al. 1994; de Montigny 1989; Degli Uberti et al. 1983; Späth-Schwalbe et al. 1988), supporting a role in stress-response modulation. Animal work also supports the idea that CCK, in addition to having anxiogenic activity, does play a significant role in hypothalamic regulation of the pituitary–adrenal axis (Mezey et al. 1986; Micevych et al. 1987; Reisine and Jensen 1986; Vanderhaeghen et al. 1985). The limited available human data suggest that the anxiogenic and stress axis activating effects of CCK-B agonism may be unrelated and are, perhaps, mediated via different pathways (Abelson et al. 1994; Le Mellédo et al. 1997). However, additional work is needed to determine definitively whether the HPA axis response to CCK-B agonists represents a direct pharmacological effect, or a nonspecific, general stress response.

To develop pentagastrin further as a probe of the CCK-B receptor in human studies and to explore the role of CCK in HPA axis modulation, we sought to determine whether CCK-B receptor agonism stimulates adrenocorticotropin (ACTH) and cortisol secretion in a dose-dependent fashion. We initially determined the threshold dose needed to produce symptom reports and then examined the dose response across a full range, from just above the detectable level, as determined by the threshold finding study, to a dose expected, on the basis of prior work (McCann et al. 1995), to produce a maximal HPA axis response. A dose-dependent release of HPA axis hormones, not mediated by anxiety or subjective distress, would support the pharmacological and physiological relevance of the CCK-B receptor to human HPA axis activation and indicate a need for further work to define the mechanisms and functional significance of CCK-B receptor/HPA axis interactions.

METHODS

Subjects

Subjects were 19 female and 16 male healthy adults, with a mean age of 26.3 ± 7.7 years (range, 18–45 years), recruited through newspaper advertising and paid $100 for completion of the study. They were screened using the Structured Clinical Interview for DSM-IV (SCID) to ensure that none had any Axis I psychiatric disorders. Subjects received an abbreviated Family Informant Schedule and Criteria (FISC) interview (Mannuzza et al. 1985) to exclude those with first degree relatives with affective or anxiety disorders. All subjects provided written informed consent. All subjects were free of serious medical illness during the prior 3 months, had no history of DSM-IV alcohol or drug dependence, had no drug or alcohol abuse in the past 6 months, had no regular alcohol use during the past 6 months greater than four cans of beer per week or the equivalent, and did not smoke more than 20 cigarettes per day. All subjects were within −10% and +25% of ideal body weight, had a negative urine drug screen, and had normal screening laboratory tests (blood counts, electrolytes, glucose, liver and renal functions). Female subjects were premenopausal, were not taking birth control pills, and were studied within the first 10 days after the onset of menstruation (to preclude the possibility of pregnancy and control for effects of menstrual cycle on the HPA axis).

Threshold Finding Experiment

To determine the threshold dose for subjective symptom production, we conducted an "up-and-down" sensitivity trial (Dixon and Massey 1969). In this procedure, a priori decisions are made about a starting dose (we chose 0.05 μg/kg), a dose increment (also 0.05 μg/kg), and definition of a target response (we defined a symptom response as a 4-point rise from baseline in the postinfusion Acute Pain Inventory total score (described below) after correction for placebo injection symptom levels). The first subject is administered the starting dose. If a symptom response occurs, the next lower dose is tried in the next subject. If no symptom response occurs, then the next higher dose is used. This "up-and-down" dosing approach is repeated until six to eight subjects have been run, allowing fairly rapid and reliable determination of the threshold dose required to achieve the targeted response. We used seven subjects in this trial and examined ACTH responses to determine if any doses that were subthreshold for symptoms could produce at least a 20% increase in ACTH over basal levels.

Subjects were studied in the afternoon, with timing and procedures similar to that used in the full, dose-response study (see below), with the exception that a placebo injection (normal saline) was administered 1 hour before the pentagastrin. To detect pharmacologically meaningful symptom thresholds, symptoms elicited by placebo were subtracted from symptoms elicited by pentagastrin for each subject.

Procedures for Dose Response Experiment

Subjects were assigned to a placebo group or one of four dose groups (0.2, 0.4, 0.6, 0.8 μg/kg) by constrained random assignment (to give approximately equal age and gender distributions in the five groups). They were admitted for study to a Clinical Research Center (CRC) at 1:00 PM. Subjects and CRC staff were blind to group assignment. An intravenous catheter was inserted into a forearm or antecubital vein and kept open with a slow, normal saline drip. This line was used for both drug injection and blood sampling. Following IV insertion, subjects rested comfortably in bed for 2 hours to accommodate to the research setting. Baseline blood samples were obtained at 3:00 and 3:28 PM. The pentagastrin (commercially available Peptavlon™, Wyeth–Ayerst Laboratories, Philadelphia, PA) was prepared by the University of Michigan Investigational Drug Pharmacy by drawing the appropriate dosages into a syringe in a saline vehicle of less than 5 ml. Labeled syringes were delivered to the CRC 1 hour before infusion and kept refrigerated until used. The pentagastrin was injected by the study physician at 3:30 PM, via a port in the IV tubing, over 10 s, with the normal saline drip fully opened for rapid flow. Additional blood samples were obtained at 3, 5, 10, 20, 30, 45, and 60 min after injection. Samples for ACTH were drawn into vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) and those for cortisol were drawn into heparinized tubes. Samples were immediately placed on ice and were spun, separated, and frozen at −70°C within 30 min.

Additional Measures and Assays

At the time of screening, subjects completed a Beck Depression Inventory (BDI) (Beck 1978), the Anxiety Sensitivity Index (ASI) (Reiss et al. 1986), and the Speilberger State/Trait Anxiety Inventory (STAI) (Spielberger et al. 1969).

Heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured using an AirShields automated monitor. Recordings were made every 10 min from −30 minutes until −10 min before injection, every 2 min from −10 until +10 min after injection, and every 10 min thereafter until blood sampling was completed.

Physical and emotional symptoms were recorded, at the time of each blood sample, using a previously described (Abelson and Nesse 1994) version of the Acute Panic Inventory (API) (Dillon et al. 1987) and visual analog scales. The modified API provides ratings on a 4-point scale (none, mild, moderate, severe) of subjective and somatic symptoms of panic attacks as listed in DSM-IV. Subjects also give a yes or no response to the question, "Did you, or are you now having a panic attack?" We calculated two dependent variables from the API, the number of symptoms present (those rated at least mild), and the total symptom intensity (sum of individual symptom ratings). To determine the presence of panic attacks, we applied 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-IV's 4-symptom criterion and that there be at least a 2-point increase from baseline in the subject's rating of anxiety. For the visual analog scales (VAS), subjects rate themselves on 10 different feeling states (happy, drowsy, nervous, sad, calm, depressed, anxious, energetic, fearful, and angry) by marking a line on a 100-mm visual analog scale ranging from "not all" to "most ever." A composite measure of anxious distress was calculated by summing the VAS measurements for "anxious," "nervous," and "fearful," and subtracting the measure for "calm."

Cortisol was assayed using the direct, nonextraction, Coat-A-Count tube assay from Diagnostics Products Corporation (Los Angeles, CA). ACTH was assayed using the Allegro HS ACTH IRMA from Nicholas Institute (San Juan Capistrano, CA). Sensitivities were 6 pg/ml for ACTH and 0.2 μg/dl for cortisol, with coefficients of variation (CV) of less than 10%.

Analyses

In the threshold finding study, we followed the method of Dixon (Dixon and Massey 1969) to estimate the mean dose needed to produce a detectable symptom response. ACTH responses were calculated for each subject as percentage change from baseline (peak postinjection ACTH minus mean of two pre-injection values, multiplied by 100).

In the dose-response study, the five dose groups were first compared on screening and baseline measures using one-way analysis of variance (ANOVA). Hormonal data were log-transformed before analysis. ACTH and cortisol were initially analyzed using a repeated-measures ANOVA, with dose groups as a between-subject variable and time (two pre-injection and five postinjection measures) as a within-subjects variable. Dose group differences in response to pentagastrin would be reflected in a group-by-time interaction in these analyses. We also calculated an integrated response measure for each subject, for ACTH and cortisol, by calculating the area under the postinjection curve (using trapezoidal approximation) and subtracting the area under the pre-injection curve (multiplied by a constant to approximate the same time duration as the postinjection curve). We added a constant to the resultant difference (to avoid negative values) before log transformation. One-way ANOVAs were used to determine whether the dose groups differed on these response measures and Fisher's PLSD post hoc tests were

used to locate specifically the groups that differed when the group main effect was significant.

For cardiovascular measures, a mean baseline was calculated by averaging pre-injection values. Because all meaningful HR and BP fluctuations occurred in the first 10 min after pentagastrin, we calculated our response measure by averaging the values across this 10-min period and subtracting the mean baseline. We also calculated a peak-response measure by subtracting the mean baseline from the highest value obtained in the 10-min postinjection period. The effects of pentagastrin were verified using paired *t*-tests to compare postinjection values to pre-injection values in all groups combined. Dose group differences were tested using one-way ANOVAs and Fisher's post hocs, as described above for ACTH and cortisol.

For all subjects who received pentagastrin, symptom responses occurred within a minute of injection and waned within 3 min. Significant group differences in symptom measures appeared only in the 3-min, postinjection ratings, which reflected the maximal levels experienced in the prior few minutes. Pre-injection values were averaged to provide a baseline measure for each symptom variable (number of symptoms, symptom intensity, and subjective anxious distress, see above). Symptom response was calculated as the difference between the 3-min, postinjection rating and the mean baseline. Paired *t*-tests and one-way ANOVAs were used as described above for cardiovascular data. Relationships between variables, using the entire subject sample, were explored using Pearson product-moment correlation and multiple regression analyses.

RESULTS

Threshold Finding Study

The seven subjects were administered the following doses (+ indicates symptom response present, − indicates symptom response absent): 0.05 (−), 0.1 (−), 0.15 (+), 0.1 (+), 0.05 (−), 0.1 (−), and 0.15 (+) μg/kg. The estimated mean dose needed to produce a detectable symptom response was 0.11 μg/kg. The three subjects who had symptom responses (receiving 0.15, 0.15, and 0.1 μg/kg doses) had 72, 33, and 19% ACTH rises, respectively. The four subjects who did not have symptom responses showed minimal changes in ACTH levels, with a mean decline (−3% change) from baseline to postinjection.

Dose-Response Study

Based on the above study we evaluated 0, 0.2, 0.4, 0.6, and 0.8 μg/kg doses. The five dose groups did not differ significantly in mean age, sex distribution, height, weight, or scores on any of the screening inventories ($p > .22$ for all tests). The mean Beck Depression Inventory score was 3.1 (±3.4) and the State/Trait Anxiety scores were 5.5 (±0.9) and 7.6 (±1.3), respectively, consistent with a "normal" population with low levels of depression and anxiety. There were no significant group differences on any pre-injection (baseline) hormone, cardiovascular, or symptom measures. There were no differences between males and females on any hormonal, cardiovascular, or symptom responses to pentagastrin.

Hormone Data. Pentagastrin produced significant, dose-dependent elevations in ACTH and cortisol. Raw data graphs are presented in Figure 1. Highly significant main effects of time in the repeated measures ANOVAs ($F = 29.1$, df = 6, 180, $p < .0001$ for ACTH and $F = 38.2$, df = 6, 180, $p < .0001$, for cortisol) reflect the robust release of both hormones by pentagastrin. Significant group-by-time interactions ($F = 8.3$, df = 24, 180, $p < .0001$ and $F = 4.9$, df = 24, 180, $p < .0001$, respectively) support the dose-dependent nature of this response.

The dose effect is highlighted in the graphs and analyses of the net, integrated response data (see Figure 2). For cortisol, the main effect of group was significant

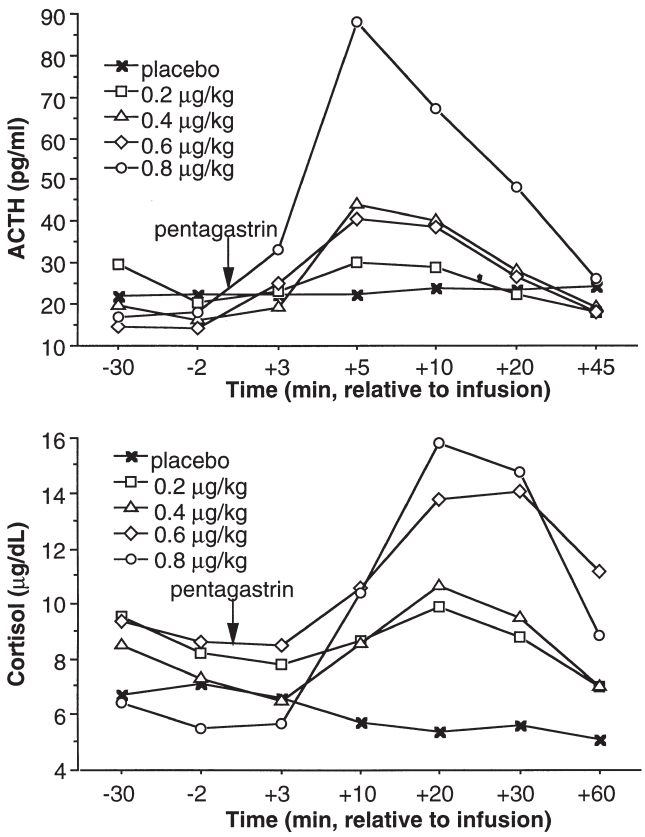

Figure 1. ACTH (top) and cortisol (bottom) responses to placebo and four different doses of pentagastrin; values are group means ($n = 7$ in each group).

(F = 10.6, df = 4, 30, $p < .0001$) and Fisher's PLSD post hoc tests showed that all four dose groups had significantly greater cortisol responses than the placebo group, that the higher dose groups (0.6 and 0.8 μg/kg) had significantly greater responses than the lowest dose group (0.2 μg/kg), and the 0.8 μg/kg group had a significantly greater response than the 0.4 μg/kg group ($p < .04$ for all comparisons). For ACTH, the main effect of group was significant (F = 6.7, df = 4, 30, $p = .0006$), and Fisher's PLSD post hoc tests showed that all of the dose groups except the lowest had significantly greater ACTH responses than the placebo group, and that the highest dose group (0.8 μg/kg) had significantly greater responses than the three lower (0.2, 0.4, and 0.6 μg/kg) dose groups ($p < .04$ for all comparisons).

Mean increases over baseline (postinjection peak minus mean baseline × 100%) in the five groups (listed in order of increasing dose) were 135, 163, 275, 353, and 521% for ACTH and 109, 141, 168, 170, and 314% for cortisol. There were no differences among the four pentagastrin groups in time from injection to hormone peak. The mean time to peak for all subjects receiving pentagastrin was 6.6 ± 3.3 min for ACTH and 24.0 ± 9.3 min for cortisol.

Cardiovascular Data. Pentagastrin produced significant rises in HR and BP. Paired *t*-tests for all groups combined showed significantly higher HR, SBP, and DBP during the 10 min after injection than during the pre-injection baseline ($t > 5.3$, df = 34, $p < .001$ for all three variables). As can be seen in Figure 3, there was no HR response to placebo, but HR rose similarly in all four pentagastrin dose groups. The group effect was significant in the ANOVA (F = 3.3, df = 4, 30, $p = .02$), and post hoc tests confirmed that each of the dose groups differed significantly in HR response from the placebo group ($p < .04$ for each), but none of the pentagastrin groups differed from each other ($p > .28$). SBP and DBP behaved similarly to HR (Figure 3), although the ANOVA group effects were not significant for ei-

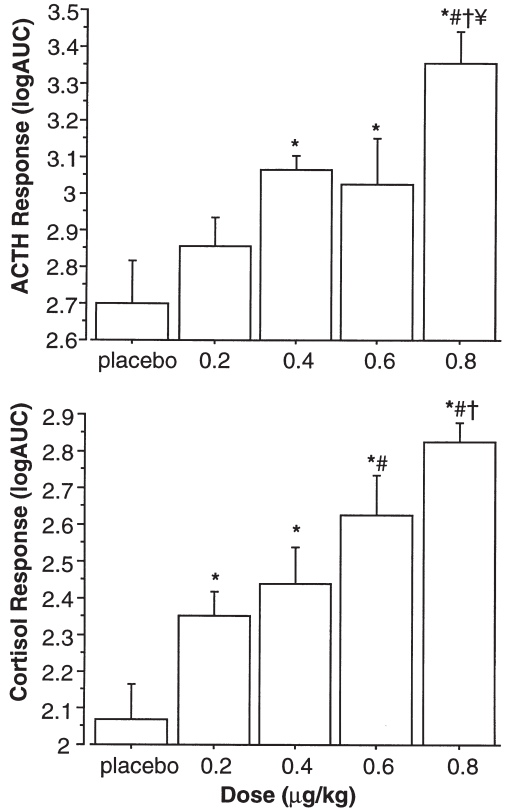

Figure 2. Net, integrated ACTH (top) and cortisol (bottom) responses (see text for derivation) to placebo and four different doses of pentagastrin; values are means ± SEM ($n = 7$ in each group); *significantly different from placebo ($p < .04$); #significantly different from 0.2 dose group ($p < .04$); †significantly different from 0.4 dose group ($p < .04$); ¥ significantly different from 0.6 dose group ($p < .02$).

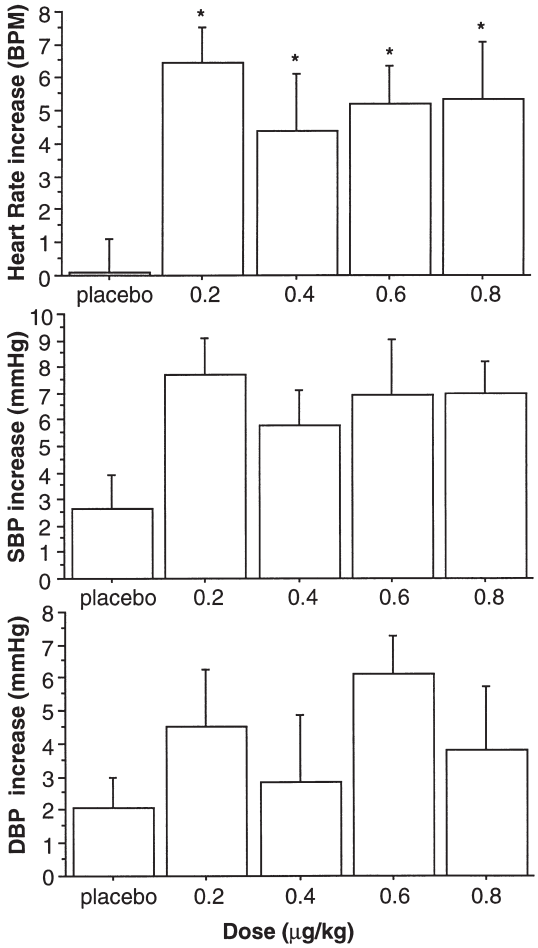

Figure 3. Heart rate and blood pressure responses to placebo and four different doses of pentagastrin; values are means ± SEM ($n = 7$ in each group); *significantly different from placebo ($p < .04$).

ther of these variables (F = 1.7, df = 4, 30, p = .16, and F = .9, df = 4, 30, p = .46, respectively). Identical results were obtained using peak response measures (maximum postinjection level minus mean baseline) for all three cardiovascular variables (data not shown).

Symptom Data. Pentagastrin produced significant increases in symptom reports. Paired t-tests for all groups combined showed significantly higher numbers of subjectively reported panic attack symptoms, greater symptom intensity, and elevated anxious distress 3 min after injection than at pre-injection baselines ($t > 6.9$, df = 34, $p < .0001$ for all three variables). The ANOVA group effects were significant for all three symptom variables (F = 4.3, df = 4, 30, p = .007; F = 12.2, df = 4, 30, $p < .0001$; and F = 8.1, df = 4, 30, p = .0002, respectively). The ANOVA and post hoc tests (see Figure 4) show that there were moderate symptom responses to the 0.2 μg/kg dose, but large responses in the three higher dose groups, all of which differed significantly from the placebo group. The three higher dose groups did not differ significantly from each other in symptom responses.

No subjects in the placebo group experienced a panic attack. Two subjects (28%) in each of the 0.2, 0.4, and 0.8 μg/kg dose groups experienced panic attacks. Three subjects (43%) in the 0.6 μg/kg dose group experienced a panic attack. There was no dose group effect on the rate of panic (Chi Square = 3.6, df = 4, p = .46).

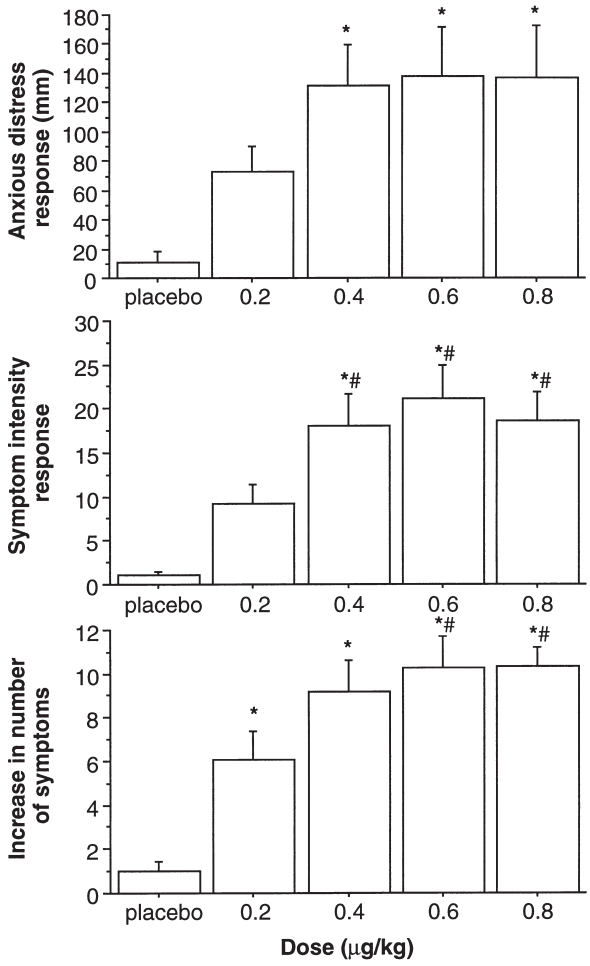

Figure 4. Panic symptom and anxious distress responses (see text for derivation) to placebo and four different doses of pentagastrin; values are means ± SEM (n = 7 in each group); *significantly different from placebo ($p < .004$); #significantly different from 0.2 dose group ($p < .05$).

Correlations. There were no significant relationships between hormone responses to pentagastrin and responses on any cardiovascular measure. Responses on the three cardiovascular measures (HR, SBP, DBP) were all highly correlated with each other (r = .5 to .7, $p < .001$, n = 34). The ACTH and cortisol responses were strongly correlated (r = .63, $p < .0001$, n = 34). Screening measures of depression and anxiety (BDI, ASI, STAI) did not significantly predict ACTH, cortisol, or cardiovascular responses to pentagastrin.

Simple correlations showed significant relationships between endocrine and symptom responses to pentagastrin, but both were also related to dose, so the relationships between these variables were examined in multiple regression analyses that allowed the dose effect to be partialled out. Dose, anxious distress, and symptom intensity together accounted for 53% of the variance in cortisol response, but in this multiple regression analysis (multiple r = .73, $p < .0001$; n = 35), only dose was a significant, independent predictor of cortisol response ($p < .0001$ for dose, p = .56 for anxious distress, and p = .83 for panic symptom intensity). Nearly identical results were found for ACTH. Dose, anxious distress, and symptom intensity together accounted for 42% of the variance in ACTH response; but in the multiple regression (multiple r = .65, $p < .0001$, n = 34) only dose was an independent, significant predictor of ACTH response (p = .006 for dose, p = .85 for anxious distress, and p = .52 for panic symptom intensity). There were no significant correlations between dose or symptoms and BP or HR responses to pentagastrin.

We also examined ACTH and cortisol responses to pentagastrin as a function of baseline cortisol levels [pre-injection cortisol area under the curve (AUC)]. In these analyses, we examined both integrated responses (AUC difference, as above) and peak responses (postinjection maximum minus pre-injection mean). Because we were exploring the ability of elevated baseline cortisol to inhibit activation of the HPA axis (Hermus et al. 1984), we included only the three higher dose groups because these groups had the clearest activation, with both ACTH and cortisol responses to pentagastrin, which were significantly different from placebo. We could find no significant relationship between baseline cortisol and any of the measures of ACTH or cortisol re-

sponse to activation, either in the three groups combined ($r < .2$, $p > .47$, $n = 21$), or in each dose group examined separately (correlations negligible or positive in each group).

DISCUSSION

We have demonstrated that the CCK-B receptor agonist pentagastrin produces rapid and robust activation of the HPA axis in humans, replicating prior work (Abelson et al. 1994; Degli Uberti et al. 1983; McCann et al. 1997). Earlier work has also demonstrated ACTH and cortisol release in response to other CCK-B receptor agonists (de Montigny 1989; Koszycki et al. 1996; Späth-Schwalbe et al. 1988), supporting CCK-B receptor modulation of the HPA axis in humans. The current data extend prior work by demonstrating that a CCK-B agonist stimulates release of ACTH and cortisol in a dose-dependent fashion. Pentagastrin infusion also reliably elevated heart rate and blood pressure in our subjects. We did not detect a clear dose effect in cardiovascular responses, but continuous monitoring of these variables may be necessary to detect any such dose effect, because maximal cardiovascular responses appear and disappear rapidly (Le Mellédo, personal communication) (Bradwejn et al. 1992). The data also confirm that CCK-B receptor agonism produces anxiety and panic-like symptoms, and even some panic attacks, in healthy human subjects (de Montigny 1989). The induction of panic attack symptoms and anxious distress is somewhat dose dependent, as has been previously reported in patients with panic disorder (Bradwejn et al. 1992). Our data suggest that the dose threshold for symptom provocation is the same as the threshold for HPA axis activation. However, we could detect no additional relationship between symptoms and ACTH/cortisol responses that were independent of dose effects.

One prior examination of pentagastrin dose effects on ACTH and cortisol release has been published (McCann et al. 1995). This study differed from ours in that pentagastrin was administered in the context of a social interaction challenge, but it also revealed little or no response to a 0.2 μg/kg dose, while equal, robust responses were seen to 0.6 and 1.0 μg/kg doses. The similarity in the size of the response to these two doses in the McCann et al. study suggested to us that 0.6 μg/kg might be a sufficient dose to produce a maximal HPA response. However, the large ACTH response in our data to a 0.8 μg/kg dose (520 % increase over baseline) indicates that further work is needed to define the maximal HPA response to CCK stimulation and the minimal dose capable of eliciting it. For receptor sensitivity studies, the 0.4 μg/kg dose may be useful, because it produces a clear HPA axis response that is detectable in a small sample study, but does not maximally stimulate the system and, thus, may be able to detect either increased or decreased sensitivity of CCK-B receptors.

Pentagastrin's ability to activate the HPA axis in a dose-dependent fashion suggests that the CCK-B receptor may play a pharmacological role in modulating release of ACTH and cortisol. Further work is needed to determine whether this is a direct effect or mediated by other mechanisms. Our data cannot rule out the possibility that symptom responses or anxious distress may play some role in mediating or modulating the HPA axis response to pentagastrin, but the correlational analyses clearly did not support strong behavioral mediation of the dose effect on hormonal responses. Separate mediation of behavioral and hormonal responses is supported by work using CCK-4, which has shown that both cardiovascular and symptom responses to CCK-B stimulation can be reduced with propranolol (Le Mellédo et al. 1998); whereas, an HPA axis response (arginine vasopressin or AVP) was not effected by the β-blocker (Le Mellédo et al. 1997). Follow-up studies with propranolol and pentagastrin are needed. Blockade of symptom responses without reduction in HPA axis responses would provide a more definitive test of the hypothesis that the hormonal response is a direct pharmacological effect and does not reflect a general stress response to sensations and symptoms produced by the drug.

Further work is also needed to determine whether CCK is a physiologically relevant modulator of the stress axis in humans, but animal work supports the presence of substantial interaction between the HPA axis and CCK systems. CCK is localized in components of the HPA axis and is co-localized with other HPA axis hormones (Kiss et al. 1984; Larsson and Rehfeld 1981; Mezey et al. 1986; Millington et al. 1992; Rehfeld 1978; Rehfeld and Larsson 1981; Rehfeld et al. 1987; Vanderhaeghen et al. 1985). The HPA axis is directly sensitive to CCK activation (Kamilaris et al. 1992; Matsumura et al. 1983; Mezey et al. 1986; Reisine and Jensen 1986). Central CCK systems are sensitive to levels of stress and circulating glucocorticoids (Mezey et al. 1986; Siegel et al. 1987) and peripheral CCK can regulate activity in central components of the HPA axis (Chen et al. 1993).

Although these animal data strongly support physiologically meaningful interactions between CCK and the HPA axis, the specific mediating mechanisms remain unclear. There is evidence to support HPA activation by CCK via peripheral pathways (Kamilaris et al. 1992), but direct effects via central or pituitary CCK receptors have also been implicated (Kamilaris et al. 1992; Millington et al. 1992; Reisine and Jensen 1986). Both the CCK-A receptor (Luckman et al. 1993; Millington et al. 1992) and a novel (non-A, non-B) receptor subtype may be involved (Parrott and Forsling 1992; Reisine and Jensen 1986), although the human work has shown highly selective CCK-B agonists to be potent ACTH

secretagogues (Abelson et al. 1994; de Montigny 1989; Koszycki et al. 1996). Both corticotropin-releasing hormone (CRH) (Biró et al. 1993; Kamilaris et al. 1992) and AVP (Bondy et al. 1989; DeBold et al. 1984; Mezey et al. 1986; Verbalis et al. 1987) have also been implicated as mediators in animal work. It would seem from this literature that there are important connections between CCK systems and the HPA axis and that multiple mechanisms and pathways may well be involved in their interactions. Multiple functions may be served by this complexity and both the pathways and functional significance of these interactions are likely to vary across species (Hinks et al. 1995; Woodruff et al. 1991). Clarification of mechanistic pathways and functional significance in humans will require human studies.

The present data can shed only limited light on mediating mechanisms, but pentagastrin elicited much more rapid ACTH responses than are reported with human CRH infusions [7 min to peak vs. about 30 min (Schürmeyer et al. 1984)], which argues against a CRH-mediated mechanism in our subjects and may support a direct anterior pituitary effect. Pentagastrin effects are also differentiated from CRH-induced ACTH release in that responses to CRH are inversely related to basal levels of cortisol (De Cherney et al. 1985; Hermus et al. 1984) because of negative feedback inhibition; but we could not detect evidence of similar negative feedback regulation of pentagastrin-induced ACTH release. We are examining further the relationship between ACTH response to pentagastrin and basal cortisol levels in a follow-up study by examining the response across the diurnal cycle.

The possible contribution of AVP to CCK-B induced ACTH response in humans also merits further study. CCK-8, which stimulates both CCK-A and CCK-B receptors, produces a dose-dependent release of AVP in humans, with a very rapid peak (at 2 min after injection) and a threshold dose similar to that seen in the present study with pentagastrin (Miaskiewicz et al. 1989). Although the vasopressin response to CCK may be mediated by the CCK-A receptor in some species (Parrott and Forsling 1992), it seems likely to be a CCK-B receptor effect in humans, because the selective CCK-B receptor agonist, CCK-4, produces a robust and very rapid AVP release in humans (Le Mellédo et al. 1997) which is quite similar to that seen with the non-selective agonist. Follow-up efforts to examine AVP levels in samples from this dose-response study and to correlate them with the ACTH responses are underway.

In conclusion, the clear dose-dependence of the HPA axis response to pentagastrin demonstrated in this study supports our hypothesis that this response is a pharmacological effect. The data are also consistent with our hypothesis that ACTH/cortisol release and symptom production are independent in this model. The CCK-B receptor may play a physiological role in modulating activity in the neuroendocrine stress axis. Pentagastrin is a useful probe for studying CCK-B receptor function in humans and its relevance to HPA axis activity.

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