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Effect of time of preparation on pentagastrin-induced symptom, endocrine and cardiovascular responses

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Abstract

Pentagastrin is a cholecystokinin (CCK)-B agonist and laboratory panicogen producing endocrine (ACTH and cortisol), symptom (anxiety, panic) and cardiovascular (heart rate) responses. Although *in vitro* data supports its chemical stability, preliminary data suggested that increasing time between drug preparation and drug infusion could reduce the magnitude of endocrine and symptom responses. The current study examined this possibility. Twenty-one healthy subjects presented at the University of Michigan General Clinical Research Center (GCRC) and had an intravenous catheter inserted. Heart rate, cortisol levels and subjective anxiety were measured before and after pentagastrin and placebo injections. Pentagastrin was prepared either within 60 minutes of IV infusion (Normal Preparation group) or at least 3.5 h prior to infusion (Early Preparation group). Relative to the Normal Preparation group, Early Preparation subjects had similar heart rate responses but significantly smaller cortisol and subjective anxiety responses. Early preparation of pentagastrin thus appears to weaken endocrine and subjective anxiety responses, highlighting the importance of attending to often overlooked procedural variables (e.g., time between preparation and administration) in studies of this type. The sensitivity of cortisol and anxiety responses to preparation time, but insensitivity of heart rate, is consistent with previous studies suggesting different thresholds of activation for the three response modalities. These differential sensitivities may suggest different and separable CCK-B stimulated pathways for each response, which combine to produce panic, rather than a single, unified CCK-B mediated panicogenic response.

Keywords

Pentagastrin; CCK; HPA; cortisol; anxiety; panic

1. Introduction

Pentagastrin is a laboratory panicogen and agonist for cholecystokinin (CCK) B-receptors. Animal and human studies show that CCK-B agonists (pentagastrin and the closely related peptide CCK-4) produce anxiogenic effects, stimulate cardiovascular activity and activate the hypothalamic-pituitary-adrenal (HPA) axis (Abelson and Liberzon, 1999; Zwanzger et al., 2003; Rotzinger and Vaccarino, 2003; Khan et al., 2004a). In panic disorder patients, CCK-B agonists also produce most of the classic symptoms of a panic attack (Bradwejn, et al. 1990; Abelson and Nesse, 1994; Koszycki et al., 1998; Abelson et al., 1999). Pentagastrin thus

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provides an opportunity to measure endocrine, physiological and psychological symptoms following CCK-B stimulation, and the relationship among these responses in the context of stress and anxiety. Pentagastrin studies have been used to demonstrate the role of CCK-B receptors in stimulating panic symptoms, and to explore linkages between biological responses to CCK-B stimulation and panic symptoms, other psychological variables, and contextual factors. We have shown, for example, that the HPA response to pentagastrin is fully independent of its anxiogenic properties (Khan et al., 2004a; Khan et al, 2004b), but that brief manipulations of psychological expectancies can significantly alter the HPA response to this pharmacological activator (Abelson et al., 2005).

The relationships among the various response channels in pentagastrin and CCK-4 challenge models have bearing on the interpretation of data from studies that use them as laboratory models of panic. If CCK-B receptor activation plays an underlying role in the pathophysiology of panic attacks in panic disorder (Bradwejn & Koszycki, 2001) then we would expect CCK-B agonists to produce the full set of psychological and biological phenomena associated with panic in a coherent and interrelated pattern. If CCK-B agonists produce only some of the symptoms associated with panic attacks, or if the components produced are separable, then it seems less likely that they are producing a clinical panic attack through a CCK-B mediated mechanism that is directly relevant to the pathophysiology of spontaneous attacks.

Our own dose response data in healthy subjects have suggested that the anxiogenic, cardiovascular, and HPA effects of pentagastrin have different thresholds of activation and are not tightly interconnected in a way suggesting a single, coherent “event” (Abelson and Liberzon, 1999). If the response measures have different sensitivities to CCK-B activation, it may suggest different CCK-B-stimulated pathways mediating their effects, and a potential separability of the three responses. We recently were able to further explore different patterns of activation for pentagastrin responses and their relationship to one another, when we inadvertently discovered that reconstitution of lyophilized pentagastrin into solution a few hours prior to administration led to an unexpected decrease in HPA axis response, though the magnitude of response in other channels was preserved. This phenomenon is important to report in its own right, since the manufacturer’s stability data suggested that no such decrease in response magnitude should occur. Other investigations using peptidergic or hormonal agents may be subject to similar effects, which could unknowingly but significantly alter results. It also offered an opportunity to analyze the effects of different pentagastrin preparations on this agent’s various response modalities (cardiovascular, endocrine, subjective anxiety).

2. Methods

2.1 Subjects

Subjects were 21 healthy adults (mean age = 25.6 ± 0.8 years) recruited through newspaper advertising, screened using the Structured Clinical Interview for DSM-IV (SCID) to ensure absence of psychiatric disorders, and paid \$100. Subjects were of normal weight, medically healthy and with low levels of alcohol (< 5 drinks/week) use. They had no recent history (past 6 months) of drug or alcohol abuse or dependence. Two of the 21 subjects were smokers (≤ 15 cigarettes/day). Urine drug screens were negative and blood tests (cell counts, electrolytes, glucose, liver and renal function) were normal. Females were pre-menopausal, not using birth control pills, and studied within the first 10 days after onset of menstruation. All subjects provided written, informed consent.

2.2 Procedures

Evaluations were completed 1 week before initiation of study. Subjects reported at 1 PM for two experimental sessions, on separate days within one week of each other. Use of food and

tobacco was prohibited during the sessions. Each session began with a 5-minute audio tape that fully described the apparatus and procedures and listed the common side effects of pentagastrin. The experimental procedures were conducted in a General Clinical Research Center (GCRC), where an intravenous catheter (saline drip) was inserted into an antecubital vein at approximately 1:30 PM. Subjects rested in bed and accommodated to the setting for 1.5 hours. Baseline blood samples were obtained at 3 PM and 3:28 PM. At 3:30 PM, the investigator injected placebo or pentagastrin (0.6 µg/kg; Calbiochem-Novabiochem, Laufelfingen, Switzerland) through the intravenous catheter over 15 sec. All subjects received placebo on first visit and pentagastrin on the second. Pentagastrin was prepared (i.e. lyophilized powder was dissolved in normal saline according to manufacturer's instructions and drawn into a syringe) by pharmacists in the hospital's Investigational Drug Pharmacy, delivered to the GCRC and kept refrigerated until just before use. In one group (n=11; 5 males, 6 females) preparation occurred at least 3.5 h prior to IV infusion (Early Preparation). In the second group (n=10; 5 males, 5 females) preparation occurred exactly 60 min prior to infusion (Normal Preparation). Subjects were blind to whether placebo or pentagastrin was being administered. Subjects in the Early Preparation condition were run first. The study was then repeated in an identical fashion, with a later preparation, when the reduced cortisol responses were noted, thus subjects were not randomly assigned to condition.

Blood samples were obtained at 3, 5, 10, 20, 30, 45, and 60 minutes after the administration of pentagastrin, drawn into vacuum tubes containing heparin and placed on ice. They were spun in a refrigerated centrifuge within 5 minutes; plasma was separated and stored at -70°C.

2.3 Measures and Assays

Physical and emotional symptoms were recorded at the time of each blood sample using a visual analog scale. This scale measured feeling states on 100-mm visual analog lines ("not at all" to "most ever"). The primary dependent variable was subjective anxious distress (sum of visual analog scale ratings of "anxious," "nervous," and "fearful" minus a rating of "calm").

Heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using Airshields Automated Monitors. Recordings were made every 10 min from 30 min before to 10 min before pentagastrin injection, every 2 min from then until 10 min after injection, and then every 10 min until completion of the study. Cortisol was assayed using the Coat-A-Count assay from Diagnostic Products Corporation (Los Angeles, Calif), with sensitivity of 0.2 µg/dL and coefficients of variation of less than 10%.

2.4 Analyses

Primary analyses involved a two-factor (Time X Group) repeated measures analysis of variance (RM-ANOVA). The impact of pentagastrin on subjective anxiety, heart rate and logtransformed cortisol response is seen in the main effect of Time. The impact of time of preparation is seen in the main effect of Group and the Time X Group interaction. Pentagastrin effects were also analyzed by comparing peak responses, which capture the immediate impact of pentagastrin on magnitude of response by subtracting mean baseline from post-pentagastrin maximum. Using multivariate ANOVA, we compared Early and Normal Preparation groups on endocrine, symptom and heart rate responses. Inter-relationships between the three responses in the Early Preparation group were also explored using Pearson product-moment correlations.

3. Results

A significant effect of Time was observed for cortisol ($F(6,108)=10.35$, $P<.001$), subjective anxiety ($F(4,76)=15.47$, $p<.001$) and heart rate ($F(16,272)=17.42$, $P<.001$), confirming

pentagastrin stimulation of these responses. No significant Group effects were found for measures of cortisol ($F(1,18)=.722, P=.407$), subjective anxiety ($F(1,19)=3.2, P=.089$) or heart rate ($F(1,17)=1.88, P=.118$). However, significant Time X Group interactions were observed for cortisol $F(6,108)=5.796, P<.001$ and subjective anxiety ($F(4,76)=6.09, P<.001$), but not for heart rate ($F(16,249)=.499, P=.947$). The interaction effects were due to reduced response magnitude in the early preparation group for cortisol and anxiety, while response magnitude for heart rate was identical between the two groups (see Figure 1–Figure 3).

Peak response analyses confirmed time of preparation effects on cortisol and subjective anxiety responses, but not on heart rate response, with significantly larger responses for Normal compared to Early Preparation groups for cortisol ($F(1,20)=5.48, P=0.03$) and subjective anxiety ($F(1,20)=11.58, P=.003$), and identical responses across the two groups for heart rate ($F(1,20)=0.27, P=.607$). In the Early Preparation group, Pearson product-moment correlations revealed a significant relationship between heart rate and subjective anxiety responses ($r=0.711, P=.014$), but not between heart rate and cortisol responses ($r=0.509, P=.11$). There was also a correlation between cortisol and subjective anxiety ($0.730, P=.011$). None of these relationships were significant within the Normal Preparation group ($P > .15$).

4. Discussion

Assessment of hypothalamic-pituitary-adrenal (HPA) axis function in human stress studies is frequently carried out by measuring the profile of increment in ACTH and/or cortisol levels in plasma in response to peptidergic or hormonal administration (e.g. pentagastrin, CCK-4, CRH). Our results highlight the importance of attending to potentially uncontrolled variables (i.e., time between preparation and administration) in these studies. Pentagastrin that was prepared more than 3.5 hours prior to infusion produced significantly lower cortisol and anxious symptom responses than pentagastrin prepared within 60 minutes of infusion. Thus, despite manufacturer's data demonstrating chemical stability over the time spans used, our data suggest declining psychobiological response magnitudes. If time of preparation can alter biological impacts of a provocative agent and is not carefully controlled across subjects and groups in studies examining peptidergic/hormonal stimulation of anxiety and endocrine responses, erroneous conclusions could result. We have reviewed all of our own past studies with pentagastrin, and all used our standard preparation, 60 minutes prior to administration. We discovered the effect of early preparation only when our Investigational Drug Pharmacy began preparing the drug earlier for their own convenience, during the study reported here, and we saw the loss of biological signal in cortisol responses.

In this study, all of our response measures were not equally sensitive to the time-related decrease in response magnitude. This is consistent with a previous pentagastrin dose-response study that suggested each outcome measure had a different dose threshold for producing a maximal response. Thus, heart rate ($0.2 \mu\text{g/kg}$), panic symptom ($0.4 \mu\text{g/kg}$) and cortisol ($0.6 \mu\text{g/kg}$) responses were fully activated by pentagastrin at incrementally higher doses, suggesting that each response has a different threshold for maximal activation following CCK-B stimulation (Abelson and Liberzon, 1999). In this study, earlier preparation appears to have acted like a reduction in dose, which we interpret as a loss of potency. The reduced cortisol and anxiety responses indicate that our "active dose" in this study now fell below their maximal activation thresholds, whereas our "active dose" was still above the $0.2 \mu\text{g/kg}$ threshold required to produce a maximal heart rate response.

Different thresholds of activation may suggest that the three responses represent distinct CCK-B mediated effects rather than a single, cohesive response. More specifically, different sensitivities to CCK-B activation suggest that different pathways may mediate their effects, allowing for separability of these response modalities. Indeed, several studies have shown that

pentagastrin-induced response modalities can be separated by various manipulations. For instance, the beta-adrenergic antagonist propranolol inhibited the heart rate response to pentagastrin, while leaving the subjective anxiety response unaffected and producing a slight enhancement of the cortisol response (Khan et al., 2004b). Challenging subjects with pentagastrin on two successive visits produced a small but significant habituation of the cortisol and anxious symptom response to repeat infusion, but no decline in cardiovascular measures (Khan et al., 2004a). A brief manipulation of cognitive set and expectancies can significantly reduce the cortisol response to pentagastrin, without affecting psychological symptoms (Abelson et al., 2005).

Accumulating data thus suggest that CCK-B stimulation may produce a number of separable effects, potentially mediated by different pathways, which when assembled together produce a 'panic-like picture.' This suggests to us that the laboratory panic generated in CCK-B models may not necessarily represent a single, coherent, and "true" panic, produced through a single, direct, CCK-B mediated mechanism. The observation that many agents with widely varying biological actions can all generate panic attacks in the laboratory is consistent with this possibility (Charney, et al., 1985; Hollander et al., 1989; Peskind et al., 1998; Sinha et al., 1999). Either different biological pathways to panic exist within the brain, or secondary effects of each agent are what create their panicogenic potency. In either case, a single unified mechanism involving direct CCK-B stimulation appears unlikely.

This interpretation requires careful empirical testing in future studies of laboratory panic. Although speculative, this distinction is critical to the future use of laboratory panic models. If our interpretation is correct, then future studies should be directed at finding the pathways shared in common by effective laboratory panicogens, rather than focusing on their primary biological effects. The fact that the final picture is similar across different agents suggests that there may be a "final, common pathway" in these models, which when characterized will tell us important things about naturally occurring panic attacks. Additionally, though we can separate response modalities in our studies, there are still meaningful interconnections between response measures; examination of linkage patterns between psychobiological outcome channels across panicogenic agents may provide information about what components are most central to the phenomena of panic attacks. In the present data, in the early preparation group, we were able to find linkages between heart rate and anxious symptom intensity, as well as between cortisol and anxiety, despite the reduced potency for cortisol and symptom production. Although these results suggest relationships between some of the response intensities, the meaning of the relationships is difficult to interpret given the weak symptom and endocrine responses. The Normal Preparation group, with stronger symptom and endocrine responses, did not show similar relationships. Previous pentagastrin studies with robust symptom and cortisol responses also failed to show a correlation between them. One possibility is that initially, at lower intensities, these responses share more common mechanisms, and are thus more closely related. As the intensities increase in response to exogenous activators, separate secondary pathways may be recruited, and the responses dissociate. Correlations between psychological and biological variables in challenge paradigms thus may or may not reflect "normal" links between the phenomena of interest.

Some caution is needed in interpreting our results. This study was not specifically designed to test the impact of time of preparation on potency, so groups were not created by random assignment. This was also a study of healthy control subjects, not of panic patients, and response patterns may differ in patients. However, we believe that these data do shed light on the "normal" psychobiological effects of pentagastrin that is relevant to its use as a laboratory model of panic. We also believe that our experience provides an important, cautionary tale for all investigators who are using peptidergic or hormonal challenge agents in human studies, about the potential for unexpected confounds in their data, such as time of preparation.

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Figure 1.
Cortisol levels, before and after pentagastrin infusion, in subjects receiving normal and early preparations of pentagastrin (means $\pm$ SE).

Figure 2. Subjective anxiety levels, before and after pentagastrin infusion, in subjects receiving normal and early preparations of pentagastrin (means $\pm$ SE).

Figure 3.

Heart rate before and after pentagastrin infusion, in subjects receiving normal and early preparations of pentagastrin (means $\pm$ SE).