Techniques & Methods

Plasma Adrenocorticotropin Responses to Opioid Blockade with Naloxone: Generating A Dose–Response Curve in a Single Session

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We examined two methods of generating a dose–response curve to the opioid receptor antagonist naloxone. In 15 healthy male subjects (18–25 years) plasma adrenocorticotropic (ACTH) responses to five doses of naloxone studied over 5 separate days were compared to plasma ACTH responses to five incremental doses of naloxone studied within a single session. There was a statistically significant positive correlation in ACTH responses (area under the curve and peak) between dosing methods. Furthermore, the doses of naloxone that produced half-maximal and maximal ACTH response were similar. The comparability of ACTH responses between the two naloxone dosing techniques, combined with the safety and ease associated with the single-session methodology, underscores the usefulness of the single-session technique for future investigations. Biol Psychiatry 2000;48:310–314 © 2000 Society of Biological Psychiatry

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Introduction

Studies employing opioid antagonists have been useful in helping to elucidate the role of the opioid system in central nervous system (CNS) function. In theory, if an opioid antagonist increases a response, it implies that endogenous opioid activity is a negative modulator of the response. In contrast, if a given response is decreased following administration of an opioid antagonist, the implication is that endogenous opioid activity is a stimulatory modulator of the response. Investigations with opioid antagonists have shown that CNS opioids impose tonic inhibition on the hypothalamic–pituitary–gonadal as well as the hypothalamic–pituitary–adrenal axes (Genazzani et al 1995; Inder et al 1995). Such studies have also suggested a role for the CNS opioid system in a number of neuropsychiatric disorders, including alcoholism (Wand et al 1998, 1999); autism (Campbell and Harris 1996); Gilles de la Tourette syndrome (Gillman and Sandyk 1985); obsessive-compulsive disorder (Keuler et al 1996); self-mutilation (Kars et al 1990; Lienemann and Walker 1989); trichotillomania (Carrion 1995); anorexia nervosa (Kaye et al 1987); and mood (Cohen et al 1983a, 1983b; Martin del Campo et al 1992, 1994). Moreover, antagonist studies have suggested that CNS opioids may help modulate learning and memory (Cohen et al 1983; Introini-Collison et al 1995; Izquierdo 1982; McGaugh et al 1986; Olson et al 1995).

Both single-dose and multiple-dose opioid antagonist studies have been conducted. Although information is gained by administering single doses of opioid antagonists, generating a response curve to multiple doses of an antagonist provides additional insight into endogenous opioid activity. For example, a leftward shift in a dose–response curve to naloxone implies enhanced sensitivity to the opioid receptor antagonist reflecting either low endogenous opioid activity or enhanced opioid receptor binding affinity (Wand et al 1998). This traditional dose–response method is conducted over several sessions, however, introducing unwanted variance as well as subject recruitment issues.

Our study explored the feasibility of generating an opioid antagonist dose–response curve within a single session. To date no study has compared in the same subject a dose–response curve conducted over separate sessions with a dose–response curve generated within a single session. In this study, adrenocorticotropic (ACTH) responses to five doses of naloxone generated within a single session are compared to responses generated over five separate sessions.

Methods and Materials

Men between the ages of 18 and 25 years were recruited by newspaper from the Baltimore area. Respondents gave informed consent to the Johns Hopkins University School of Medicine Internal Review Board approved protocol. Subjects completed medical history, physical examination, laboratory tests (complete blood cell counts, electrolytes, liver and renal function tests, and glucose) and underwent a semistructured diagnostic interview,
administered by a Masters level psychologist to insure the absence of DSM-IV Axis I disorders (including alcohol/drug dependence and abuse). Subjects with medical conditions, DSM-IV Axis I diagnosis, abnormal liver functions, maternal alcohol dependence, or undergoing pharmacotherapy with prescription medications were excluded from enrollment.

**Five-Session Protocol**

Subjects reported for sessions at 12:30 PM fasting since 9:00 AM breakfast. An intravenous catheter was inserted into a forearm vein at 1:00 PM. Subjects were supine for each of five separate naloxone dosing sessions (e.g., 0, 50, 125, 375, and 500 µg/kg) separated by greater than 48-hour wash-out period. Naloxone (dissolved in 0.9% saline) was randomized across the five sessions and administered double blind as a bolus. Blood for plasma ACTH was collected at −15, 0, +15, +30, +45, +60, +90, and +120 min. Naloxone dose range included a submaximal dose (Wand et al 1998).

**Single-Session Protocol**

At least 1 week following completion of the above protocol, subjects reported fasting for an additional session where an intravenous catheter was inserted into a forearm vein at 1:00 PM. One hour later, placebo (0.9% saline) was administered as a bolus. Subsequently, every 30 minutes, incremental doses of naloxone (50, 100, 200, and 400 µg/kg) dissolved in 0.9% saline were administered following the scheduled blood draw. Baseline blood samples were obtained 15 min before and immediately prior to placebo administration. Postplacebo blood samples were drawn every 15 min for 150 min.

**Neuroendocrine Assays**

Plasma concentrations of ACTH were assayed by a two-site IRMA (Nichols immunoradiometric assay). Intra-assay and interassay coefficients of variance are less than 9% (Waltman et al 1993; Wand et al 1999).

**Statistical Analysis**

Analyses were chosen to test the hypothesis that two different dosing methods used to generate a dose–response curve would result in a positive association in area under the plasma ACTH time curve and peak plasma ACTH response. Significance was evaluated at $p < .05$. All analyses were conducted with the Statistical Package for the Social Sciences. Mean ACTH response to opioid blockade during each of five individual dosing sessions was calculated and expressed as 1) area under the ACTH time curve (AUC) and 2) peak ACTH response. Repeated measures analyses of variance, with dose as the repeated measure and AUC or Peak as the dependent measure, was used to test the effects of dose on ACTH response across dosing sessions. Significant multivariate results were followed by contrast tests to examine 1) differences in ACTH response between placebo and each subsequent dose of naloxone; and 2) differences in ACTH response between ordinal doses (e.g., placebo vs. 50 µg/kg, 50 vs. 125, etc.). Maximum ACTH response to naloxone was defined as the highest ACTH value following baseline per session. For the single session protocol, mean ACTH responses to naloxone were calculated across 12 time points. A repeated measures analysis of variance with time as the repeated measure and plasma ACTH as the dependent measure was used to determine the effect of time (corresponding to dose) on ACTH response. Significant multivariate results were followed by contrast tests to examine 1) differences in ACTH response between baseline (time point 0) and each subsequent dose of naloxone; and 2) differences in ACTH response between ordinal time points (e.g., 0 vs. +15, 0 vs. +30, etc.).

**Results**

Figure 1A presents plasma ACTH across time, generated to five randomized doses of naloxone administered over 5 separate days. Figure 1B presents the data calculated as area under the ACTH time curve across dose. There was a significant within-subjects effect of dose $[F(4) = 9.77, p = .001]$. Adrenocorticotropin responses significantly differed from response at placebo at every naloxone dose: 50 $[F(1) = 6.70, p = .021]$; 125 $[F(1) = 13.65, p = .002]$; 375 $[F(1) = 31.60, p = .001]$; 500 $[F(1) = 13.05, p = .003]$. Also, there was a significant increase in ACTH levels between the 125 and 375 µg/kg dose $[F(1) = 6.18, p = .026]$. Maximal and half-maximal ACTH responses were observed at the 125 µg/kg and 375 µg/kg dose, respectively.

As shown in Figure 1C, there was a significant within-subjects effect of naloxone dose on peak ACTH response $[F(4) = 8.05, p = .001]$. Peak ACTH response at doses 125 $[F(1) = 8.04, p = .013]$, 375 $[F(1) = 17.60, p = .001]$, and 500 µg/kg $[F(1) = 9.62, p = .008]$ significantly differed from response at placebo. Peak ACTH response significantly increased between the 125 µg/kg and 375µg/kg dose $[F(1) = 6.11, p = .027]$. Maximal and half-maximal peak ACTH responses were observed at the 125 µg/kg and 375 µg/kg dose, respectively.

Figure 2 presents plasma ACTH response to five doses of naloxone generated within a single session. There was a significant within-subject effect for time $[F(11) = 7.992, p = .001]$. Plasma ACTH response at baseline (time 0) was significantly different than responses at the following time points: +45 $[F(1) = 12.17, p = .004]$; +60 $[F(1) = 11.04, p = .005]$; +75 $[F(1) = 11.16, p = .005]$; +90 $[F(1) = 14.90, p = .002]$; +105 $[F(1) = 15.71, p = .001]$; +120 $[F(1) = 9.32, p = .009]$, and +135 $[F(1) = 5.47, p = .035]$. In addition, there was a significant rise in ACTH response at time point 45 min relative to time point 30 min induced by the 50 µg/kg dose $[F(1) = 25.31, p =$
Similarly, there was a significant rise in ACTH response at time point 75 min relative to time point 60 min induced by the 100 \( \mu \)g/kg dose \( [F(1) = 6.40, p = .024] \). Half-maximal ACTH response was observed at time point 70 min following cumulative naloxone doses of 150 \( \mu \)g/kg. Maximal ACTH response was observed at time point 105 min following cumulative naloxone doses of 350 \( \mu \)g/kg.

Figure 3 shows the relationship for each subject between ACTH response using the two dosing methods. Plasma ACTH responses were highly correlated both calculated as area under the curve \( (r = .676, p = .006; \text{Fig 3A}) \) and peak ACTH \( (r = .698, p = .004; \text{Fig 3B}) \).

**Discussion**

In this study, we compared plasma ACTH response to five doses of naloxone generated within a single session to responses generated over five individual sessions. Our results show that the two techniques produce comparable findings. There was a strongly significant association in plasma ACTH response between the two methodologies. High ACTH responders to the five-session challenge were also high ACTH responders in the single-session challenge; conversely, low ACTH responders to the five-session challenge were low ACTH responders in the single-session challenge. Moreover, nearly identical doses of naloxone were required to generate maximal as well as half-maximal ACTH responses using both techniques. Finally the shape of the dose–response curve was compa-
rable under both procedures, with diminishing ACTH response at the highest naloxone dose.

Much of the earlier research in this field has been limited by the use of difficult-to-interpret single-dose studies, in which small, inconsistent, or no effects are found (Keuler 1996; Michelson et al 1996) or by dose–response curves generated over multiple sessions. A single session procedure has many advantages over traditional single dose or multisession approaches. The procedure combines the informative nature of multiple dosing techniques with absence of intersession variability provided by single-dose procedures. No longer is a lengthy time commitment required from subjects to complete repeated experimental sessions, which potentially jeopardizes enrollment. It is easier to study female subjects in a single phase of the menstrual cycle and avoid phase-related fluctuations in hormonal response. Other sources of between-session hormonal variance (e.g., alcohol/drug use, mood, stress, health, sleep) are eliminated. Finally, it introduces efficiency both for the investigator (e.g., cost,
recruitment, staff time, resources) and subject (e.g., absence from school, work, family).

One caveat associated with this study is that we employed somewhat different doses of naloxone for each method. Slightly lower doses for the single-session technique were chosen to guard against the induction of side effects following cumulative doses. Although naloxone blood levels were not obtained, the correlation in ACTH responses between methods was quite good suggesting that comparable doses were selected. No subject reported side effects during either procedure. Heart rate, blood pressure, and respiratory rates were not altered by naloxone.

In summary, it is possible to safely and accurately generate a comprehensive dose–response curve to naloxone within a single session. The abbreviated method will provide a useful adjunct in neuropsychoendocrine investigations of the opioid system.

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References


