Rett Syndrome: Controlled Study of an Oral Opiate Antagonist, Naltrexone

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Hypothesis: The opiate antagonist, naltrexone, will be beneficial in Rett syndrome. Subjects: Twenty-five individuals fulfilling the criteria for Rett syndrome. Method: Randomized, double-blind, placebo-controlled crossover trial with two treatment periods, 4 months each, and an intervening 1-month washout period. Clinical stage, motor and cognitive development, motor–behavioral analysis, neurophysiological parameters (computerized electroencephalographic analysis, breathing characteristics, quantification of stereotyped hand movements, and sleep characteristics), and cerebrospinal fluid β-endorphin measurements were evaluated at baseline and at the end of each treatment period. Results: Only data from the first period of this study were analyzed due to significant sequence effects in the crossover design. This analysis indicated positive effects on certain respiratory characteristics including decreased disorganized breathing during wakefulness. Four (40%) of the individuals receiving naltrexone progressed one or more clinical stages versus none of the individuals receiving placebo. The adjusted (for baseline value and Rett stage) end of treatment psychomotor test age (Bayley Scales) was significantly higher for the placebo group. There was no significant change for the other parameters. Conclusion: Naltrexone may modify some of the respiratory disturbance in Rett syndrome. Declines in motor function and more rapid progression of the disorder suggest a deleterious effect.

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Although Rett syndrome (RS) was described first in 1966 by Andreas Rett [1], general knowledge of the entity did not occur until 1983 with the report of Hagberg and colleagues [2] detailing their experience with 35 females with RS. During the past several years, our understanding of RS as a clinical entity has expanded greatly [3–5]. Rigorous diagnostic criteria [6, 7] and a useful staging system [8] have evolved during this time. However, the fundamental basis of RS remains to be revealed. The clinical characteristics of RS include mental retardation, movement and communication dysfunction, breathing irregularities, growth failure, and seizures. Treatment strategies have been directed toward symptomatic concerns but have not addressed the primary problems.

The purpose of this study was to test the hypothesis that the opiate antagonist, naltrexone, would be beneficial in RS. This hypothesis followed from reports of Brase and associates [9] that cerebrospinal fluid (CSF) β-endorphins (β-ENDs) are elevated significantly in RS. In their most recent report, Myer and collaborators [10] noted a more than twofold elevation of CSF β-ENDs over control values in greater than 90% of the 158 RS individuals. In addition, Myer (personal communication) evaluated CSF β-END levels in 46 girls or women with RS from the Baylor Rett Syndrome Program Project and found the mean value in the subset to be virtually identical with his larger series. Further, β-ENDs were measured in three brain regions from a single postmortem examination and elevated values were noted specifically in the thalamus and to a lesser extent in the cerebellum compared with an age-matched control [11]. Brase and associates [9] pointed out that the intraventricular administration of endorphins in animals has produced naloxone-reversible characteristics that are very much reminiscent of RS, namely motor dysfunction, stereotypic behavior, seizures, and breathing irregularities. Based on this information the present study was undertaken. Portions of this study have been reported previously [12].

Methods and Subjects

Study Design

The study was designed as a randomized, double-blind, placebo-controlled, crossover trial. The only inclusion criteria

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were that the participant have RS and be classified as stage II or III. The presence of the syndrome and staging were determined through the use of careful objective assessment [6, 7]. The only exclusion criterion was inability to comply with follow-up visits. All aspects of the study were conducted under informed consent. The protocols were approved by the Baylor Institutional Review Board.

For the first 4-month period of the study one-half of the participants were randomized to a placebo and one-half to the drug naltrexone. The first treatment period was followed by a 1-month washout period, which was followed in turn by a second 4-month treatment period. For the second treatment period the participants crossed over to placebo if they took naltrexone in treatment period 1 and to naltrexone if they took placebo in treatment period 1. Each of the parameters listed below were evaluated at baseline (just prior to the first treatment period) and during the last week of each of the two 4-month treatment periods. Both naltrexone (Trexan) and placebo were kindly provided by Du Pont (Wilmington, DE). Naltrexone was utilized under IND (Investigational New Drug) approval from the Food and Drug Administration. Naltrexone, which was given as a single daily oral dose, was limited to 1 mg per kilogram of body weight per day under the conditions of this IND.

Subjects

Twenty-five individuals who fulfilled the clinical criteria for RS [6, 7] were enrolled in this study. Twenty individuals completed both treatment periods; 1 died just before the end of the second treatment period; 3 dropped out during the first treatment period; 1 dropped out during the second treatment period. Clinical stage of RS was determined based on previously reported criteria [7, 8].

Objective Parameters

Objective parameters were as follows:

(1) Change of RS clinical stage during the study: Determination of clinical stage was according to the staging format established by Hagberg and Witt-Engerström [7, 8].

(2) The following evaluations were assessed by a developmental pediatrician (D.W.) and a psychologist: Bayley Scales of Infant Developmental Mental and Psychomotor Tests, Peabody Fine Motor Developmental Schedules, Gesell Gross and Fine Motor Assessment Scales, and Vineland Adaptive Behavior Scales for Gross and Fine Motor Scales.

(3) Motor–behavior analysis: Motor characteristics were rated on a motor–behavioral assessment scale previously described [13] and were rated as follows: 0, normal or never; 1, mild or rare; 2, moderate or occasional; 3, marked or frequent; and 4, very severe or constant. The score for motor characteristics was determined by the rating of the following parameters: hand stereotypies, ataxia, bradykinesia, dystonia, hypomimia, hypertonia/rigidity, and hyperreflexia. A behavioral assessment composite score was determined by rating the following: speech disturbance, bruxism, breath holding, hyperventilation, expulsion/drooling, mouthing of hands and objects, and biting self or others.

(4) Neurophysiological parameters: Patients underwent 12- to 24-hour video–electroencephalographic (video/EEG) polygraphic studies [14] that allowed the determination of the following: sleep characteristics (percent time of rapid eye movement [REM] sleep, percent time non-REM sleep, and percent total sleep time); respiratory characteristics including minimum O_2 saturation value during wakefulness, maximum Pco_2 value, percent time of disorganized breathing during wakefulness, and number of drops in O_2 saturation below 90% during wakefulness; and quantification of hand stereotypies (average number of hand movements per minute of wakefulness). Computerized analysis of EEG background activity by previously reported methodology [15] allowed determination of alpha frequency and amplitude of delta (less than 3.5 Hz), theta (3.5–7.5 Hz), and beta (greater than 13 Hz) activity.

(5) CSF assessment: CSF β-END levels were determined according to previously described methodology [10]. All CSF was collected between 9:00 and 11:00 A.M. and immediately stored at −80°C.

Data Analyses

This study was designed as a two-period crossover trial. The first step in data analysis was to determine if the random assignment to the sequence of drug administration had produced two groups of Rett patients (those patients assigned to the naltrexone for the first treatment period and placebo for the second treatment period versus those patients assigned to the placebo for the first treatment period and naltrexone for the second treatment period) that were similar with respect to baseline measurements. These analyses were done using the t test (or its nonparametric equivalent, the Kruskal–Wallis test) for continuous variables and the χ^2 test for categorical variables [16]. Any variables determined to be significantly different between the two groups at baseline were adjusted for subsequent analyses (e.g., used as covariates).

The second step in the analysis plan was to determine if there was a treatment-by-period interaction, sometimes called a sequence effect (indicative of a between-subject, residual drug or carry-over effect) or a drug-by-sequence interaction (indicative of within-subject effects that depend on whether the subject received naltrexone followed by placebo or placebo followed by naltrexone). The existence of such an interaction invalidates the two-period crossover analysis [17]. Using repeated-measures analysis of variance for the continuous variables, it was determined that there was a significant period-by-treatment interaction [18]. The variables that showed a significant ($p < 0.05$) sequence effect were psychomotor test age, minimum O_2 saturation value during wakefulness and Rett stages. The variable that showed a significant drug-by-sequence effect was Rett stage. This finding necessitated using only the data from the first 4-month treatment period to determine if there was a difference between the patients treated with placebo and those treated with naltrexone. Therefore, the analyses presented resulted from the first 4-month treatment period of the crossover study being analyzed as though it were a simple two-group (the patients assigned to naltrexone for the first period versus the patients assigned to placebo for the first period) randomized trial [17]. For each variable, analysis of covariance was used to compare

the placebo and naltrexone groups with the measurement of the variable obtained at the end of the first treatment period as the outcome and the baseline values of the same variable used as a covariate [18]. The Rett stage of the patients at baseline was also used as a covariate. Only a few of the variables were found to differ between the two groups at baseline and several others had uncorrected p values between 0.05 and 0.10. However, since the small sample sizes provided little power to detect such differences, it was decided to correct all analyses for the baseline value.

To adjust for the fact that many statistical tests had been performed, the Bonferroni correction was used [16]. The correction was applied not to the total number of tests but to groups of similar types of variables (e.g., the four motor–behavioral variables were considered as a group so that to be considered significant, the p values had to be smaller than $0.05/4 = 0.0125$).

Results

Twenty-two of the 25 RS individuals originally enrolled in the study completed the first treatment period. The 3 not completing the first treatment period included 1 patient in clinical stage II randomized to naltrexone, 1 patient in clinical stage III randomized to naltrexone, and 1 patient in clinical stage III randomized to placebo. These patients were not brought back to follow-up by their caretakers and their assigned drug was stopped after a variable time or not started by their caretakers. No specific reasons were offered other than inability to keep appointments. Adverse or positive effects while receiving the assigned drug were not reported. Other than decreasing the sample size, these dropouts did not appear to otherwise affect the analysis. There were 12 patients in the placebo group and 10 in the naltrexone group. Of the 12 patients in the placebo group, 4 were clinical stage II and 8 were clinical stage III (see Table 1). The ages in years of the 12 placebo patients ranged from 2.0 to 15.7 with a mean of 8.1 and standard deviation of 4.6. Of the 10 patients in the naltrexone group, 7 were clinical stage II and 3 were clinical stage III. The ages in years of the 10 naltrexone patients ranged from 3.5 to 11.6 with a mean and standard deviation of 5.8 and 2.7, respectively. The two groups (placebo and naltrexone) did not differ with respect to clinical stage at baseline ($\chi^2 = 2.93$, degrees of freedom [DF] $= 1$, $p = 0.09$) or age (Kruskal–Wallis $\chi^2 = 0.85$, $DF = 1$, $p = 0.36$). However, since the p value for baseline clinical stage was between 0.05 and 0.10, this variable was used as a covariate in analyses comparing end of treatment values between the two groups.

Table 1. Rett Stage Change

	Placebo Group		Naltrexone Group	
	Baseline No. of Patients	End of Treatment No. of Patients	Baseline No. of Patients	End of Treatment No. of Patients
Clinical stage[a]				
II	4	4	7	5
III	8	8	3	3
IV	0	0	0	2

[a]In the placebo group, no patient changed clinical stage. In the naltrexone group, 4 patients advanced one clinical stage ($p < 0.05$).

Stage Change

Naltrexone had a significant negative effect on Rett stage (see Table 1). None of the individuals on the placebo advanced a clinical stage during the first treatment period. Four of the individuals in the naltrexone group had advanced at least one clinical stage by the end of the first treatment phase with naltrexone. Two individuals advanced from stage II to stage III; 2 individuals advanced from stage III to stage IV. The percentage advancing a stage in the naltrexone group (40%) is statistically greater than the percentage advancing a stage in the placebo group (0%) ($\chi^2 = 5.87$, $DF = 1$, $p < 0.05$).

Developmental Assessment

Child developmental assessment revealed a significant negative effect on motor development (see Table 2). The adjusted (for baseline value and Rett stage) end of treatment psychomotor test age was significantly higher for the placebo group than for the naltrexone group (Bonferroni adjusted, $p = 0.005$). Though not achieving significance, a similar relationship was seen for the adjusted means on the Peabody Developmental Schedule for Fine Motor Skills and Gesell Gross Motor and Fine Motor assessments. This trend was reflected in parental reporting of gross and fine motor skills on the Vineland Adaptive Behavior Scales.

Motor Behavioral Analysis

Composite scores of measures of motor and behavioral characteristics indicated no difference between the placebo and naltrexone groups (see Table 3).

Cerebrospinal Fluid

Measurements of CSF levels of the β-END revealed no significant differences between placebo and naltrexone groups (see Table 4).

Neurophysiological Parameters

A significant positive effect of naltrexone on respiratory parameters was observed (see Table 5). The individuals receiving naltrexone were significantly different from the placebo group with respect to awake O_2 saturation values, percent time spent in disorganized breathing, and maximum CO_2 value. The impact of

Table 2. Development Assessment

	Placebo Group		Naltrexone Group	
	Baseline	End of Treatment	Baseline	End of Treatment
Bayley Scale of Infant Development				
Mental Mean Test Age (mo)	5.5 (2.0–13.8) n = 12	5.6 (3.0–10.0) n = 12	4.3 (1.0–9.0) n = 10	4.5 (1.0–7.0) n = 10
Psychomotor Test Age (mo)[a]	10.4 (4.5–19.0) n = 12	11.3 (4.5–19.0) n = 12	10.3 (4.0–16.0) n = 10	9.9 (4.0–16.0) n = 10
Peabody Development Schedules				
Fine Motor Age Equivalent (mo)	5.3 (1.0–10.0) n = 10	6.0 (1.0–10.0) n = 10	3.3 (1.0–7.0) n = 9	3.4 (1.0–6.0) n = 9
Percentiles Total Score	48.7 (13.0–90.0) n = 6	52.7 (16.0–87.0) n = 6	38.5 (20.0–70.0) n = 4	31.3 (20.0–42.0) n = 4
Gessell				
Gross Motor Level Quotient (mo)	13.9 (8.0–29.0) n = 12	15.7 (8.0–35.0) n = 12	22.4 (6.7–40.0) n = 9	23.3 (6.0–48.0) n = 9
Fine Motor Assessment Age Equivalent (mo)	25.4 (4.0–44.0) n = 10	27.2 (8.0–44.0) n = 10	16.0 (4.0–36.0) n = 9	16.2 (4.0–28.0) n = 9
Vineland Adaptive Behavior Scales				
Gross Motor Skills Age Equivalent (mo)	8.6 (2.0–18.0) n = 10	9.4 (3.0–18.0) n = 10	9.8 (3.0–19.0) n = 10	9.6 (2.0–18.0) n = 10
Fine Motor Skills Age Equivalent (mo)	5.1 (0.0–12.0) n = 9	5.8 (2.0–12.0) n = 9	4.0 (0.0–12.0) n = 10	4.3 (0.0–10.0) n = 10

Not all children could complete all testing. Values are expressed as unadjusted means with minimum and maximum in parentheses. Comparisons were made for end of treatment values, placebo group vs naltrexone group, corrected for baseline values and Rett stage.

[a] $p = 0.005$.

Table 3. Results of Motor–Behavioral Assessment

	Placebo Group		Naltrexone Group	
	Baseline	End of Treatment	Baseline	End of Treatment
Composite Behavioral Assessment Score	15.6 (9.0–24.0) n = 12	14.7 (7.0–21.0) n = 12	14.0 (9.0–21.0) n = 9	12.6 (7.0–21.0) n = 9
Composite Motor Assessment Score	20.4 (11.0–31.0) n = 12	20.1 (12.0–33.0) n = 12	18.0 (9.0–30.0) n = 9	19.8 (7.0–35.0) n = 9
Hand Stereotypies	2.8 (1.0–4.0) n = 12	3.0 (2.0–4.0) n = 12	2.9 (1.0–4.0) n = 9	2.9 (1.0–4.0) n = 9
Vasomotor Disturbance	1.0 (0.0–2.0) n = 10	1.0 (0.0–2.0) n = 10	1.2 (0.0–4.0) n = 9	1.2 (0.0–4.0) n = 9

Not all children could complete all testing. Values are expressed as unadjusted means with minimum and maximum in parentheses. Comparisons were made for end of treatment values, placebo group vs naltrexone group, corrected for baseline values and Rett stage.

Table 4. Results of Cerebrospinal Fluid Analysis

	Placebo Group		Naltrexone Group	
	Baseline	End of Treatment	Baseline	End of Treatment
β-Endorphins	111.9 (46.0–276.0) n = 9	130.6 (57.0–261.0) n = 9	97.3 (53.0–147.0) n = 10	116.5 (60.0–204.0) n = 10

Not all patients could complete all testing. Values are expressed as unadjusted means (picograms per milliliter) with minimum and maximum in parentheses. Comparisons were made for end of treatment values, placebo group vs naltrexone group, corrected for baseline values and Rett stage.

Table 5. Results of Neurophysiological Parameter Analysis

	Placebo Group		Naltrexone Group	
	Baseline	End of Treatment	Baseline	End of Treatment
Electroencephalography				
Alpha frequency (Hz)	8.8 (7.6–9.3) n = 10	8.8 (7.5–9.8) n = 10	8.9 (8.2–9.9) n = 8	9.0 (8.0–9.8) n = 8
Delta amplitude (μV)	49.1 (20.2–125.4) n = 10	45.7 (11.4–109.8) n = 10	36.2 (14.8–70.0) n = 8	52.8 (16.2–162.2) n = 8
Theta amplitude (μV)	28.4 (12.3–65.5) n = 10	28.9 (11.6–59.1) n = 10	24.8 (11.0–44.8) n = 8	34.6 (12.5–86.4) n = 8
Beta amplitude (μV)	10.2 (7.7–15.4) n = 10	10.7 (7.9–15.4) n = 10	11.1 (7.3–17.5) n = 8	12.8 (8.9–19.6) n = 8
Sleep characteristics				
Percent total sleep time	67.9 (0.0–95.0) n = 12	77.8 (65.0–95.0) n = 12	71.6 (16.0–91.0) n = 10	71.0 (22.0–89.0) n = 10
Percent REM sleep	8.3 (0.0–24.0) n = 12	14.1 (2.0–29.0) n = 12	10.2 (0.0–24.0) n = 10	13.5 (5.0–24.0) n = 10
Percent non-REM sleep	83.4 (0.0–100.0) n = 12	85.9 (71.0–98.0) n = 12	89.8 (76.0–100.0) n = 10	86.5 (76.0–95.0) n = 10
Respiratory characteristics				
Low O_2%[a]	72.7 (27.0–90.0) n = 12	71.6 (43.0–91.0) n = 12	85.2 (73.0–90.0) n = 10	87.7 (70.0–90.0) n = 10
Maximum P_{CO_2} mm Hg[b]	49.0 (40.0–68.0) n = 12	47.1 (40.0–60.0) n = 12	48.6 (45.0–55.0) n = 10	51.4 (45.0–61.0) n = 10
% of time awake with disorganized breathing[b]	12.1 (0.0–25.1) n = 12	11.7 (0.0–33.0) n = 12	7.3 (0.0–31.0) n = 9	1.9 (0.0–6.5) n = 9
Number of O_2 drops <90% per hour awake	9.5 (0.0–30.7) n = 12	7.9 (0.0–24.0) n = 12	5.9 (0.0–41.0) n = 10	1.8 (0.0–18.0) n = 10
Movement				
Number of stereotypied hand movements per minute	17.7 (4.9–71.5) n = 11	15.8 (4.3–73.0) n = 11	16.7 (1.2–31.5) n = 9	21.7 (4.7–64.5) n = 9

Not all children could complete all testing. Values are expressed as unadjusted means with minimum and maximum in parentheses. Comparisons were made for end of treatment values, placebo group vs naltrexone group, corrected for baseline values and Rett stage.

REM = rapid eye movement.

[a] $p = 0.03$; [b] $p = 0.02$.

naltrexone on the number of drops in O_2 saturation below 90% per hour of wakefulness was similar to that on the other respiratory parameters but was not significant. The naltrexone group had a significantly higher awake minimum O_2 saturation value ($p = 0.03$) and had less percent time spent with disorganized breathing during wakefulness ($p = 0.02$) in comparison with their baseline values. However, subjects receiving naltrexone had an elevated end tidal carbon dioxide value that was significantly higher than that observed in those who received placebo ($p = 0.02$).

Other physiological parameters including EEG measures (alpha frequency and amplitude of delta, theta, and beta activity) and sleep characteristics were not significantly different between the placebo and naltrexone groups. The average number of hand movements per minute of wakefulness was not significantly different between the two groups.

Discussion

The pervasive developmental disabilities associated with Rett syndrome have failed to respond to previous therapeutic interventions. The single consistent neuropathological finding in Rett syndrome has been the reduction in melanin pigmentation in the substantia nigra [19–21]. This finding along with reports of biogenic amine reductions in various brain regions [22] led to clinical trials, generally uncontrolled, of dopamine precursors or agonists [23–25]. Except for two reports of efficacy of the dopamine agonist, bromocriptine [24, 25], these clinical trials have provided little encouragement. Our hypothesis for therapeutic intervention developed from the findings of significant elevation of β-ENDs in CSF in individuals with Rett syndrome [10], from the regional elevation of β-ENDs in brain from 1 child with Rett syndrome [11], and from the evidence that intraventricular administration of β-

ENDs in animals produced naloxone-reversible effects similar to the behaviors typically seen in individuals with Rett syndrome [9]. The availability of the oral opiate antagonist, naltrexone, allowed us to implement the present treatment paradigm. We recognized that because of their severe neurological impairments, evaluation of these individuals would be challenging. However, most parameters were consistently measured across our RS subjects. Therefore, we believe our findings are valid and show that naltrexone had both positive and negative effects on the symptomatology of Rett syndrome.

We observed beneficial effects on the awake respiratory disturbance that has been well characterized in Rett syndrome [14]. The girls and women with Rett syndrome frequently demonstrate a pattern of disorganized breathing (periods of apparent breath holding [central or obstructive apnea] and/or hyperventilation) alternating with periods of regular, normal breathing while awake. While asleep, these individuals typically have a normal breathing pattern. During this study we observed that naltrexone tended to be associated with an improvement in this abnormal awake breathing pattern as indicated by a higher minimum O_2 saturation value during wakefulness, fewer episodes of oxygen desaturation, and less time spent during wakefulness with periods of disorganized breathing. This is similar to reports of the effect of the opiate antagonist naloxone to reduce apnea/respiratory pauses in other disorders such as infant apnea [26, 27] and in an animal model [28]. We also observed a significantly higher maximum carbon dioxide value in those individuals treated with naltrexone. This may reflect an alteration in chemoreceptor sensitivity by naltrexone.

In contrast to the positive effects on oxygenation, we have documented significant negative effects on developmental parameters. Despite subjective parental reports of improvement in our subjects, once the blind was broken, this improvement was evenly divided between naltrexone and placebo. Developmental evaluation performed blind as to whether an individual subject was on placebo or naltrexone, indicated negative effects on motor development. This negative effect was further suggested by progression of at least one clinical stage at the end of the 4-month treatment with naltrexone in 4 of the individuals versus no change in clinical stage in any of the individuals receiving placebo. The failure to demonstrate objective improvement, and indeed the demonstration of a negative effect with regard to motor performance and advancement of clinical Rett stage, suggests that the increased β-END levels in CSF in RS are unrelated to the primary abnormality in RS. However, it remains possible that an aberrant endorphin receptor would be incapable of responding to the antagonist. This consideration seems less likely in that significant effects were noted with regard to the respiratory parameters. Our results raise the possibility that increased β-END levels in the CSF may reflect a protective role of β-ENDs in retarding the progression of motor deterioration and/or clinical stage advancement at the expense of awake breathing abnormalities.

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