

Low-dose naltrexone effects on plasma chemistries and clinical symptoms in autism: a double-blind, placebo-controlled study

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Abstract

The effect of month-long naltrexone (NTX) treatment at a daily oral dose of 0.5 mg/kg/day was contrasted with placebo (PLC) in a double-blind study with conjoint clinical and biochemical evaluations of therapeutic effects. Modest clinical benefits were achieved with both PLC and NTX, with marginally better overall results following NTX, and degree of improvement appeared to be related to plasma chemical profiles. Massively elevated levels of β -endorphin were observed in all children with assays using C-terminal antibody but not with an N-terminal antibody assay. In addition, 70% of the children exhibited abnormally low levels of adrenocorticotrophic hormone, and smaller subsets exhibited elevated norepinephrine (60%), arginine-vasopressin (50%), and serotonin (20%). The best clinical responders exhibited the clearest normalization of the elevated plasma chemistries, especially in C-terminal- β -endorphin and serotonin. There was some evidence of therapeutic carry-over effects in both clinical and biochemical measures in those children who received NTX before PLC. The results suggest that NTX only benefits a subgroup of autistic children, who may be identified by the presence of certain plasma abnormalities. These results suggest a possible linkage between abnormal plasma chemistries, especially those related to the pro-opiomelanocortin system, and autistic symptoms.

Keywords: Child psychiatry; β -Endorphin; Opioids, endogenous; Vasopressin; Norepinephrine; Serotonin

Recent evidence suggests that one neurochemical vector that may promote autistic symptoms is excessive brain opioid activity (see Leboyer et al.,

1990; Herman, 1991; Panksepp et al., 1991). Preliminary biochemical evidence of abnormalities in levels of endogenous opioids in autism has been reported (Gillberg et al., 1985, 1990; Ross et al., 1987; Weizman et al., 1988; Sandman et al., 1991). The opioid-excess hypothesis of autism (Deutsch,

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1986; Sahley and Panksepp, 1987; Sandman, 1988; Herman, 1991), first proposed on the basis of symptom similarities between autistic symptoms and behavioral changes induced in animals treated with opiate receptor agonists (Panksepp, 1979, 1981), provides one rationale for the treatment of autistic children with opioid antagonists such as naltrexone (Panksepp and Sahley, 1987). Naltrexone (NTX), which antagonizes opioid receptors (particularly μ type), has a rapid onset of action and long-lasting effects approaching 24 h after moderate oral doses (Verebey et al., 1976).

In several uncontrolled open trials, orally administered NTX has been found to reduce various autistic symptoms, including hyperactivity, self-injurious behavior (SIB), stereotyped and other compulsive behaviors (Campbell et al., 1988, 1990; Leboyer et al., 1988; Herman et al., 1987; see summaries in Herman [1991] and Panksepp and Lensing [1991] for SIB studies, many in nonautistic individuals). NTX has also occasionally been reported to promote positive social tendencies, such as eye contact, social solicitation, and attempts to communicate under socially stimulating circumstances (Leboyer et al., 1988; Panksepp and Lensing, 1991; Walters et al., 1990). Some have noted reductions in temper tantrums and irritability, and increases in object play, friendly social interactions, attention, and clarity of communication (Lensing et al., 1992). However, substantial variation exists from one study to the next in the doses of NTX that seem most effective in producing desired behavioral changes, and only a subset of children appears to respond well to the medication. Also, alleviation of different symptoms may require different doses (Panksepp et al., 1991). Low doses such as 0.5 mg/kg given every few days may be optimal for positive social effects (Panksepp and Lensing, 1991; Leboyer et al., 1992; Lensing et al., 1992), while higher doses such as 1.5 mg/kg appear to yield maximal reduction of SIB (Herman et al., 1987; Leboyer et al., 1988).

Many aspects of NTX therapy still need to be evaluated, especially the magnitude of therapeutic effects under double-blind, placebo-controlled (DB-PC) conditions and biochemical changes that result from the therapy. In the first DB-PC study to evaluate the effects of 1 mg/kg of NTX in 18

children (Campbell et al., 1990) and a subsequent study with 41 children (Campbell et al., 1993), reliable reductions of excessive motor activity were observed following medication in children affected with autism, but many other measures exhibited only marginal trends in the desired direction. In a small DB-PC study with four subjects, Leboyer et al. (1990, 1992) observed stronger therapeutic effects on both behavioral and plasma neurobiological abnormalities, but the behavioral effects were highly dose-dependent, with 0.5 and 2 mg/kg/day for a week yielding better therapeutic effects than 1 mg/kg/day. Most strikingly, the elevated plasma levels of β -endorphin-like activity (*C*-terminal antibody), norepinephrine (NE), and arginine-vasopressin (AVP) were normalized by all doses of NTX in the clinical responders. On the other hand, Schifo et al. (1991) observed reliable clinical benefits in 12 autistic children treated for three successive 5-week periods at dosages of 0.5, 1.0, and 1.5 mg/kg, with no clear relationship to plasma levels.

The aim of this study was to evaluate the plasma chemical changes in autistic children as a function of low-dose NTX treatment administered in a DB-PC design and to determine if any predictive trends would be evident in clinical changes and plasma parameters, especially in β -endorphin (β -END), AVP, and biogenic amine levels (Leboyer et al., 1992). The present work placed special emphasis on measures of carboxy-terminal β -END, which has been found to be massively elevated in more than 80% of autistic children (Leboyer et al., 1994). Past work suggests that all autistic children do not exhibit improvements during NTX treatment, and it was of special interest to determine whether any plasma profile could be identified that had the potential to predict clinical benefits in a group of children not characterized by SIB. The work also highlights methodological issues that need to be considered in future studies, especially the presence of positive long-term carry-over effects of NTX to subsequent placebo (PLC) conditions that may statistically obscure some of the therapeutic effects of the drug in studies such as this one which did not include adequate washout periods between successive phases of evaluation. A preliminary summary of some of the behavioral

data was included in Panksepp et al. (1991) and an overview of the biochemical results has been presented as an extended abstract for the 1993 International Narcotic Research Conference summary (Panksepp et al., 1994).

2. Methods

2.1. Design

This was a DB-PC crossover study that compared one daily dose of NTX (0.5 mg/kg/day) and PLC. The sequence of treatment was randomly distributed so that in half of the children, the first period was PLC and the second was NTX (group PLC-NTX), and for the other half, the sequence was reversed (group NTX-PLC). Each period of treatment was given for 4 weeks. The trial was preceded by a set of baseline measurements.

2.2. Subjects

Ten children (five boys and five girls), aged 5–14 years (mean = 9.5), were included in the study. Children were systematically recruited from an outpatient university clinic for children with autism in Paris (Hôpital Robert Debré). All children met *DSM-III-R* criteria for autistic disorder, full syndrome present (American Psychiatric Association, 1987). In addition, all parents were interviewed with the French version of the Autistic Diagnostic Interview (ADI; Leboyer et al., 1988; Le Couteur et al., 1989) by one of us (M.L.) to check that the ICD-10 algorithm of the ADI was fulfilled. Within the 10 children, six had no language (two boys and four girls), while four (three boys and one girl) had verbal abilities. Parents were asked if the child ever showed SIB (head banging, head hitting, hitting other parts of the body, biting or scratching himself or herself). Six children were rated positively as having had mild SIB in the past, but none displayed SIB at study intake. Absence of any organic disease was checked by a systematic screening procedure that included skin and neurological examination, karyotype in a medium depleted of folic acid, and computed tomography. No child was receiving any ongoing psychotropic treatment. All children were outpatients and studied with parental consent.

2.3. Medication and clinical evaluation

To accustom the children to the experimental conditions, an initial clinical assessment was performed 15 days before the beginning of the first treatment period. Subsequently, evaluations were done just before the start of the treatment, and at the mid-points and ends of each of the two successive 30-day counterbalanced phases of treatment with NTX and PLC (data for the mid-point are not presented, as all effects are adequately represented by the end-of-month measures). A final posttest evaluation was conducted 15 days after the end of the counterbalanced NTX-PLC phase. NTX (Nalorex®) or comparable PLC was administered orally in tablets every day at 09:00 h. At each evaluation period, three major behavioral evaluation tools were employed: (a) The 29 items of the Childhood Psychiatric Rating Scale (CPRS), which were divided into the following components: psychoticism, activity, compulsivity, anxiety, hostility, behavioral restraint, sleep, and enuresis (see Fish, 1985); (b) the 20 items of the Behavioral Summarized Evaluation (BSE; Barthélémy et al., 1990), which was divided into the following factors: sociability, communication, object relations, motor abnormalities, aggression/emotionality, feeding problems, and attention; (c) the abbreviated 10-item version of the Conners Parent Teacher Rating Scale (Goyette et al., 1978). The CPRS and BSE were rated on the basis of parents' reports and the Conners scale on the basis of clinical observations of the child.

2.4. Biochemical measurements

At the end of each phase (baseline and the first and second periods of treatment), blood samples were drawn at 10:00 h into tubes containing ethylenediaminetetra-acetic acid and aprotinin. Platelet-poor plasma (containing about 0.1% of the original platelet count as assessed by contrast phase microscopy) was obtained within 2 h by centrifugation (2,000g, 4°C, 15 min) and frozen as 0.5-ml aliquots at –80°C until assayed (blind procedures used). The possible impact of NTX on some neurohumoral parameters was analyzed by the measurement of the following: (a) whole blood serotonin (5-hydroxytryptamine, 5-HT) content by radioenzymology (Walker et al., 1983) after an

ethanol/acetone (v/v) extraction (Launay et al., 1983); (b) plasma levels of unconjugated catecholamines (dopamine [DA], NE, and epinephrine [E]), also by radioenzymology (Da Prada and Zurcher, 1979); (c) plasma levels of four neuropeptides (adrenocorticotrophic hormone [ACTH], AVP, β -END, and substance P [SP] by radioimmunoassay). AVP was determined as previously described (Vittet et al., 1989). Commercially available (INCSTAR, Minnesota, USA) kits were used for ACTH, SP, and *N*-terminal β -END. The β -END was first extracted and concentrated from plasma with a Sep-Pak C-18 cartridge. The eluted fraction was tested by radioimmunoassay with two antisera. The recognition site of one antiserum is toward the C-terminal region (Asn²⁰-His²⁷) (Guillemin et al., 1977). The *N*-terminal β -END antiserum used has only 5% immunoreactivity with β -lipotropin, and the antigenic determinant recognized is in the 1-23 region of β -END (see Kerdelhué et al., 1982; Leboyer et al., 1994). NTX and PLC were administered without knowledge of treatment condition. Biochemical levels were not available to clinicians until completion of the study.

2.5. Statistical analysis

Comparison of the two counterbalanced groups (NTX-PLC and PLC-NTX) did not reveal any statistical difference, either in clinical baseline ratings as evaluated by the three scales or in sex ratios (three boys and two girls in the NTX-PLC group vs. two boys and three girls in the PLC-NTX group). Statistical analyzes consisted of separate analyses of variance (ANOVAs) for the four phases of the whole trial as well as for just the counterbalanced NTX and PLC periods. Correlational effects were evaluated by *t* tests.

3. Results

3.1. Clinical findings

No untoward effects of either NTX or PLC or gender effects were observed during the course of the trial. The clinical measures provided marginal evidence for selective improvement during the NTX treatment as compared with PLC, with the magnitude of the overall effects being small and

potentially obscured by carry-over effects, especially from NTX to PLC and NTX to post-treatment periods. Only a subgroup of the children appeared to exhibit substantial clinical responses. On the basis of two independent post hoc clinical raters — one who made direct observations of the children (M.L.) and one who performed numerical evaluation of the behavioral results (J.P.) — yielding 90% agreement, it was estimated that six to seven of the children experienced some clinical benefits. Of those, only four were deemed strong responders, two to three were weak responders, and three to four were considered by both raters to be nonresponders. As a result, some of the biochemical data will be summarized with a split group of four strong and six weak or nonresponders. Before presentation of the biochemical results, which are deemed to be key findings of the study, we briefly summarize the overall clinical observations. As shown in Fig. 1, all three clinical measures yielded reliable overall improvements when the four phases of the study (baseline, NTX, PLC, and posttreatment) were all considered (largely because of a decline of symptoms from initial baseline levels). The statistical trends were weak when only the counterbalanced NTX and PLC phases were considered.

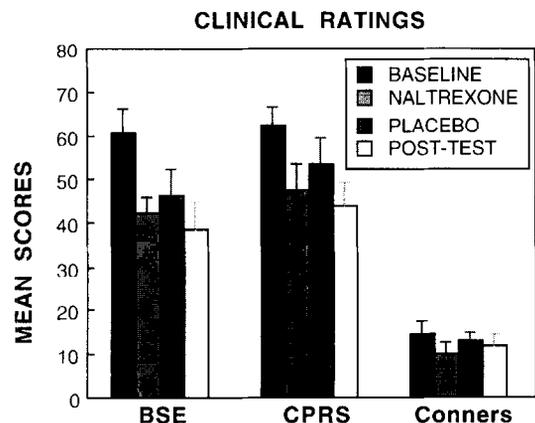


Fig. 1. Mean \pm SE scores for the Behavioral Summarized Evaluation (BSE), the Childhood Psychiatric Rating Scale (CPRS), and the Conners Parent Teacher Rating Scale at baseline assessment, at the end of counterbalanced month-long naltrexone and placebo phases, and 15 days post-test.

The overall ANOVAs for the four phases of testing were as follows: there was an overall decline in symptoms as measured by the CPRS ($F = 9.47$; $df = 3, 27$; $P < 0.001$) and the BSE ($F = 10.05$; $df = 3, 27$; $P < 0.0001$), and a marginal decline as measured by the Conners scale ($F = 2.69$; $df = 3, 27$; $P < 0.07$). In all cases, these effects were due to declines from baseline scores ($P_s < 0.05$, all post hoc comparisons with the Scheffé test), with no clear differences among the NTX, PLC, and posttreatment scores, although the Conners scale did yield a marginal overall indication of improvement, from 12.8 for PLC to 10.1 for NTX ($P = 0.07$); the CPRS showed a similar trend ($P < 0.09$), but no trend was seen for the BSE ($F = 1.42$; $df = 1, 9$; $P = 0.26$).

Subscale analyses of the CPRS indicated that the largest therapeutic trends were evident in three factors — namely, hyperactivity ($F = 1.42$; $df = 3, 27$; $P < 0.0001$), hostility ($F = 3.79$; $df = 3, 27$; $P < 0.03$), and restraint ($F = 5.3$; $df = 3, 27$; $P < 0.01$), but none of the respective NTX-PLC differences were statistically significant. Likewise, a subscale analysis of the factors of the BSE indicated that NTX had the largest beneficial effect on sociability ($F = 8.68$; $df = 3, 27$; $P < 0.001$), communication ($F = 6.42$; $df = 3, 27$; $P < 0.005$), object relations ($F = 6.04$; $df = 3, 27$; $P < 0.005$), and attention ($F = 8.7$; $df = 3, 27$; $P < 0.001$), changes that again largely reflected improvements from initial baseline levels. The restricted NTX versus PLC comparisons for these factors did not yield reliable effects except for attentional problems, where the mean score following NTX of 3.0 was reliably better than the PLC score of 4.4 ($F = 4.5$; $df = 1, 9$; $P = 0.05$).

Although the overall ANOVAs of the counter-balanced NTX versus PLC conditions revealed only marginal therapeutic trends, this may have partially been due to sustained carry-over therapeutic effects of NTX in the NTX-PLC subjects. For instance, this was evident for overall BSE scores, which yielded a reliable interaction between test period and treatment ($F = 7.61$; $df = 1, 9$; $P < 0.03$), reflecting the fact that children receiving NTX first exhibited a 21.3% decline from baseline symptoms and then during the subsequent PLC period exhibited an additional 14.4% decline

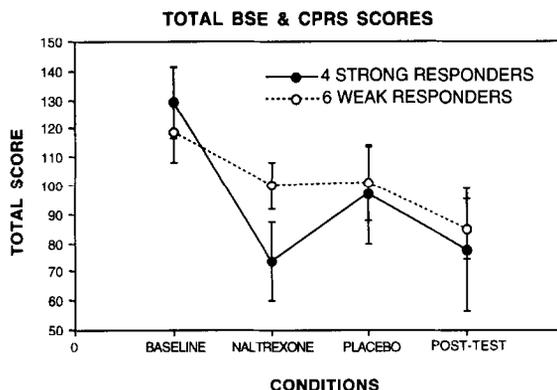


Fig. 2. Mean \pm SE scores for combined results for the Behavioral Summarized Evaluation (BSE) and the Childhood Psychiatric Rating Scale (CPRS) in the four best clinical responders compared with the remaining subjects.

in symptom severity. In the PLC-NTX group, the initial PLC-related change was only a 12.4% improvement, which was followed during the NTX phase by a 32.5% improvement.

As mentioned before, it was evident that only some of the children exhibited strong clinical responses. The combined CPRS and BSE scores (see Fig. 2), as well as the scores for the individual scales, paralleled these results. On all measures, the four strong responders showed a marked decline in symptomatology, while the remaining six subjects exhibited only a marginal sustained decline during the course of the trial. The four responders also exhibited a more striking normalization of plasma chemistries.

3.2. Biochemical findings

Fig. 3 shows the range of baseline values. The whole group of autistic children exhibited markedly elevated C-terminal β -END concentrations (mean value = 107 pg/ml), which are on the average four times higher than the upper control values (25 pg/ml). However, the N-term β -endorphin was not statistically different from control values in these autistic children. In addition, six children exhibited elevated NE, five elevated AVP, while only two of the children exhibited 5-HT concentrations exceeding the normal range. SP, DA, and E were within the normal range for all the autistic children. By contrast, the mean value of ACTH was

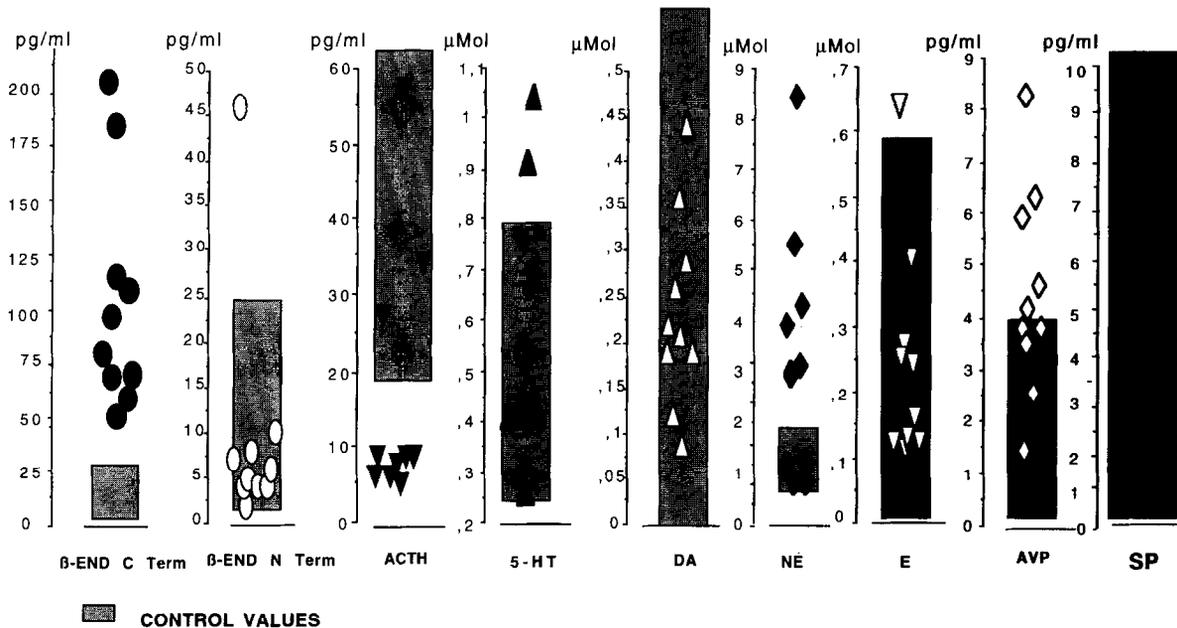


Fig. 3. Individual baseline neurochemical plasma measures in the whole population of subjects with autism (β -endorphin (β -END) C- and N-terminal, adrenocorticotrophic hormone (ACTH)), serotonin/5-hydroxytryptamine (5-HT), dopamine (DA), norepinephrine (NE), epinephrine (E), arginine-vasopressin (AVP) and substance P (SP). The range of control values from a normative group of children is indicated by the shaded histograms.

below normal (a mean of 16.8 pg/ml vs. a normal range between 20 and 80 pg/ml) with only three subjects falling within the normal range.

During the course of the trial, there were reliable reductions in three of the elevated chemistries — namely, C-terminal β -END ($F = 6.87$; $df = 2, 18$; $P < 0.01$), AVP ($F = 4.99$; $df = 2, 18$; $P < 0.02$), and 5-HT ($F = 11.1$; $df = 2, 18$; $P < 0.001$), with all 10 children exhibiting the β -END reductions, 9 out of 10 exhibiting 5-HT and AVP reductions, and 8 out of 10 exhibiting NE reductions, even though, because of extreme variability, the statistical test for NE was not reliable ($F = 1.78$; $df = 2, 18$; $P = 0.20$). These plasma chemistry normalizations appeared to be attributable to the NTX treatment (the largest effects were seen after NTX treatment), and in children receiving NTX first, the effects typically extended to the end of the PLC period. These extended effects were seen most clearly for AVP and 5-HT (but not for C-terminal β -END and NE). All of these effects were most

evident for the four strong responders (Fig. 4). Post hoc statistical contrasting of such selected subgroups is of questionable validity, but the reductions in the strong clinical responders were reliable at $P = 0.05$ for all chemistries except NE. ACTH was not normalized during the course of treatment, and was in fact numerically reduced from 34.8 to 19.7 to 38.5 pg/ml during the three phases of blood collection ($F = 2.17$; $df = 2, 18$; $P = 0.14$). It might also be noted that there was a reliable reduction of SP levels from 3.47 to 1.95 to 1.9 pg/ml ($F = 3.67$; $df = 2, 18$; $P < 0.05$), even though absolute levels in the autistic children remained within the normal control range. These reductions were essentially identical in strong and weak clinical responders.

To evaluate possible disorders in pro-opiomelanocortin (POMC) gene processing, the ratios of the two forms of β -END to ACTH were computed. This ratio is typically around 1.0 during the normal processing of POMC, which cor-

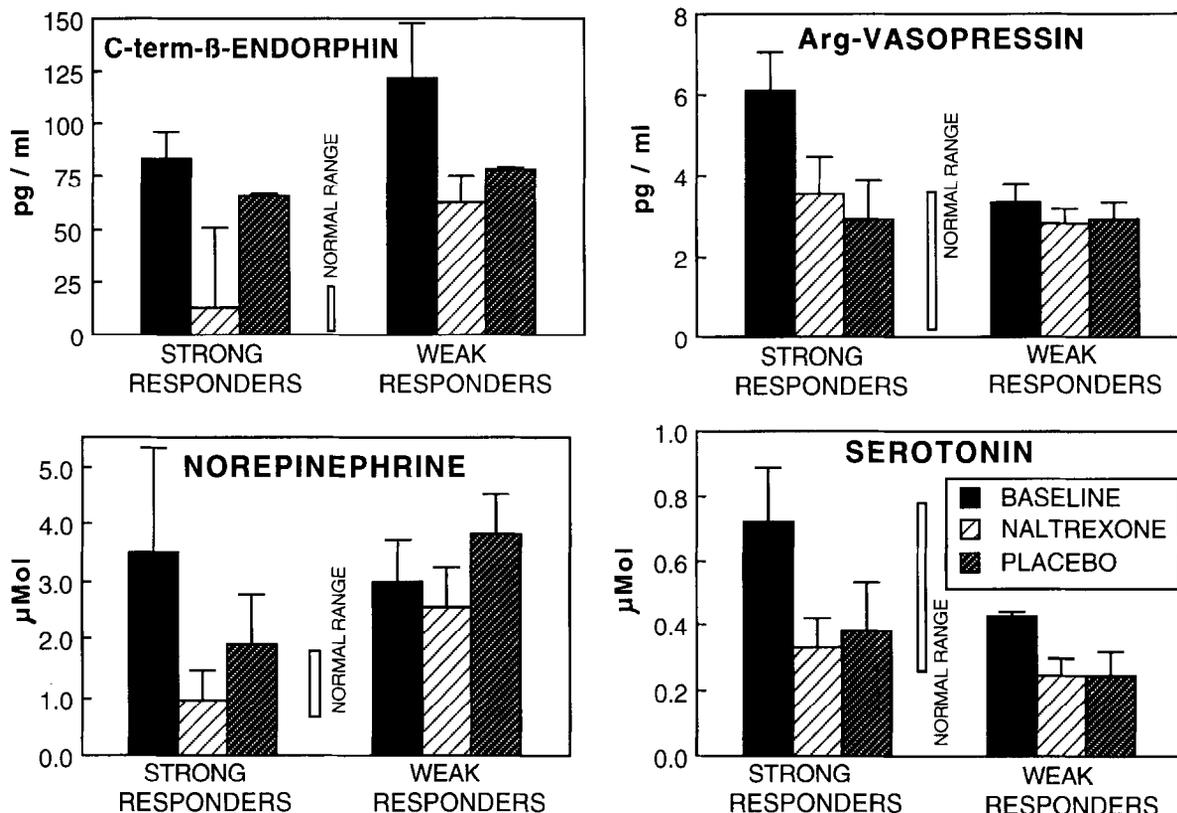


Fig. 4. Mean (\pm SE) plasma levels of the most informative chemistries (i.e., C-terminal β -endorphin, arginine-vasopressin, serotonin, and norepinephrine) during the baseline period and at the end of the counterbalanced naltrexone and placebo phases of the trial. The four best clinical responders are indicated as "strong responders" and the remaining six subjects as "weak responders." The range of control values is indicated.

responded well to the average *N*-terminal β -END/ACTH ratio during baseline of 0.96 and following NTX of 1.1. However, the *C*-terminal β -END/ACTH ratio during baseline was 18.0 and, following NTX, was significantly reduced to 7.6 ($P < 0.05$). This normalization was most evident in the four strong responders who went from a ratio of 9.5 to 0.9, while the remaining children dropped only from 13.9 to 8.1.

In a further attempt to relate baseline autistic symptoms to the various plasma chemistries, all baseline behavioral measures (the seven factors of the BSE and the nine factors of the CPRS) were cross-correlated with the baseline plasma chemistries and those which reached the $P < 0.05$ level are noted. Due to the high probability of

false-positives in this analysis (reflecting the number of comparisons), the results only provide a provisional source of hypotheses for future work. *C*-terminal β -END levels were correlated to the overall BSE score ($r = 0.66$); to sociability, communication, and feeding problem factors ($r_s = 0.55, 0.61, \text{ and } 0.60$, respectively); and to the anxiety factor of the CPRS ($r = 0.74$). ACTH was negatively related to the aggression/emotionality factor of the BSE ($r = -0.62$) and positively related to the depression factor of the CPRS ($r = 0.62$). SP was positively related to BSE communication and feeding problems factors ($r_s = 0.64 \text{ and } 0.68$, respectively) and the anxiety factor of the CPRS ($r = 0.80$). DA was positively related to whole scale BSE ($r = 0.57$) as well as to

its sociability factor ($r = 0.69$). NE was positively related to the depression factor of the CPRS ($r = 0.56$) and E to the compulsive factor of the CPRS ($r = 0.75$).

4. Discussion

The results reported provide further support for the involvement of the endogenous opioid system in autism and the potential efficacy of NTX in the treatment of some autistic subjects. Although this double-blind PLC study revealed only marginally greater therapeutic effects of NTX than PLC, in the context of a substantial decline of symptomatology during the entire course of the trial (a pattern also evident in the recent report of Campbell et al. [1993]), the results must be viewed in the context of several methodological concerns. A potential carry-over effect of NTX into the subsequent PLC period in the NTX-PLC children was evident. This trend was also evident in the post-treatment period. Such carry-over effects of NTX in the treatment of autism have been noted in several open trials (Barrett et al., 1989; Panksepp and Lensing, 1991; Lensing et al., 1992), and a recent SIB study has noted a trend toward increased NTX effectiveness as the study progressed (Thompson et al., 1994). However, the increasing benefits could also have been due to the demand characteristics associated with repeated testing, which would constitute a substantial PLC effect. We think this is unlikely to be the sole explanation since the objective plasma chemistries were similarly affected, especially in the strongest clinical responders. Furthermore, some measures, like the attention factor of the CPRS, affirmed that real clinical benefits were produced by NTX.

Our overall clinical results are certainly blurred by the fact that only a subset of autistic subjects showed a clearly beneficial response to NTX. Since the strong responders also exhibited the more striking normalization of plasma parameters, we believe there are critical constitutional differences among autistic children that determine whether NTX will produce clinical benefits. The heterogeneity of the autistic population is beginning to be generally accepted, and the present results suggest that we may eventually be able to

identify NTX responders by abnormal premedication plasma chemistry profiles. On the basis of the present trends, it would appear that future therapeutic trials with NTX should seek to categorize subjects on the basis of biochemical criteria as potential responders and nonresponders before the beginning of the trial — namely, subjects with elevated AVP and 5-HT levels seemed to be uniformly good responders in this study, and they also showed intermediate elevations of C-terminal β -END, while the initial elevations in NE did not seem to be indicative of responsivity. Although baseline levels of NE and C-terminal β -END did not discriminate well among responders, the normalization of these chemistries during NTX may be prognostic.

The present plasma chemistries replicate abnormal patterns reported previously (Leboyer et al., 1992, 1994), especially in the massive elevations of C-terminal β -END in most children, but also less striking elevations of AVP, 5-HT, and NE in some children as well as the new finding of reduced ACTH tiers in 70% of the children. The most compelling effect of NTX in the present study was the striking reductions of C-terminal β -END in all children, with the production of complete normalization in the best clinical responders. Whether higher doses of NTX could achieve comparable normalizations in the weak responders is unknown. The fact that the normalization of plasma profiles was sustained in many strong responders during subsequent PLC phases suggests that NTX can have long-lasting effects on underlying biochemical abnormalities. In some as yet unknown way, NTX appears to be inhibiting the excessive manufacture of circulating endorphin-like molecules as well as several other plasma factors. Whether this is a direct effect (e.g., on processing enzymes) or an indirect effect (e.g., on receptor-mediated regulatory feedback on genetic or other metabolic mechanisms) can only be resolved by further research.

It must be emphasized that we measured β -endorphin-like plasma activity with two different antibodies: one is directed at the carboxy terminal, as in our previous work (Leboyer et al., 1992, 1994), and the other, an antibody recognizing the amino N-terminal of β -endorphin, as in most pre-

vious studies (Weizman et al., 1988; Sandman et al., 1991). The disparity observed here between levels of *N*- and *C*-terminal β -END probably reflects the fact that our *C*-terminal antibody was detecting some presently unknown set of endorphin metabolites or other structurally related molecules that remain to be characterized. Elucidation of the exact substance(s) that is (are) detected in our assay should help clarify the biochemical abnormality that exists in many autistic children.

Given the fact that β -END and ACTH are released by the same POMC precursor molecule, the apparent dissociation of *C*-terminal β -END and ACTH may be consistent with a previous report by Sandman et al. (1991) that showed a discrepancy between β -END and cortisol plasma levels. It could also implicate other abnormalities, such as a deficient posttranscriptional or post-translational processing of POMC and/or an abnormality of the peptidases that inactivate endogenous opioids as previously described in ACTH-producing nonpituitary tumors (Vieau et al., 1989). It is especially noteworthy that the *C*-terminal β -END/ACTH ratio was normalized most clearly in strong responders, raising the possibility of a genetic regulatory effect by NTX. The suggestion of POMC dysregulation in autism, however, must be deemed highly provisional since *N*-terminal β -END activity was relatively normal in this as well as several other studies (see Herman, 1991). Indeed, Sandman et al. (1991) reported a slight reduction in the plasma levels of that peptide, a trend that was not clearly apparent in our children.

Of course, the present neuropeptide results must be viewed in the context of all the caveats that are routine in the use of immunological assay approaches to the measurement of neuropeptide levels. In particular, it should be stressed that discrepancies with other results could be explained either by the differences in sampling procedures and/or the immunological dosage. Indeed, we included the protease inhibitor aprotinin in our blood collection tubes to prevent cleavage of biologically active peptides by blood proteases. The absence of such an antiprotease could artificially lead to a decrease of opioid peptides. To

our knowledge, this precaution has never been used in previous work in the literature on peripheral measures of opioid peptides in autism. However, this is less of a problem for cerebrospinal fluid (CSF) measurements, since CSF contains less protease activity than blood, which may help explain some discrepancies between plasma and CSF results (Gillberg et al., 1985, 1990; Ross et al., 1987).

In sum, the types of methodological concerns raised by this study indicate that future controlled studies should incorporate extensive washout periods between successive treatment conditions and extensive habituation of subjects to all testing procedures before pharmacologic interventions. How long a washout period needs to be incorporated into future work remains uncertain, but in our estimation it needs to be more than a month if a month of NTX treatment has been employed. Of course, the carry-over effects that occur following NTX are to be expected if any type of beneficial psychosocial learning occurs during NTX therapy or if long-term neurochemical regulation takes place such as might occur in receptor sensitivities. Such carry-over effects would be of substantial clinical importance and need to be more clearly evaluated by future research. From the present pattern of results, we also anticipate that further analysis of plasma neuropeptide and biogenic amine changes in response to NTX may help identify the subset of children that will exhibit the best clinical responses to NTX. It also seems likely, however, that parents of autistic children are especially susceptible to generating PLC effects in DB studies such as these (also see Campbell et al., 1993) and that concern must be addressed in future work through the use of more objective and subtle socioemotional measures of therapeutic progress.

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