Plasma β-Endorphin Immunoreactivity in Schizophrenia

Abstract. *Plasma β-endorphin-like immunoreactivity was measured by a method that was equally sensitive to β-endorphin and [Leu⁵]-β-endorphin. Immunoreactivity in 98 schizophrenic patients did not differ greatly from that in 42 normal subjects. No immunoreactivity was detectable in dialyzates from first-time hemodialysis of eight nonpsychotic renal patients and nine schizophrenic patients. These results are not compatible with recent reports of extremely high concentrations of [Leu⁵]-β-endorphin in hemodialyzates from schizophrenic patients.*

Opiate alkaloids have profound effects on pain, mood, and behavior in man. Endogenous opioid peptides (endorphins) produce similar effects by their actions on the opiate receptors in the brain (*1*). Thus, speculation has arisen that dysfunction of the endorphin system could lead to behavioral disturbances that are characteristic of certain mental illnesses. In particular, it has been postulated that increased endorphin concentrations could be associated with the symptomatology of schizophrenia (*2*).

Indirect support for this hypothesis came from studies with the opiate antagonist naloxone. In an early single-blind experiment, Gunne *et al.* (*3*) found that four of six schizophrenic patients had fewer auditory hallucinations after a small intravenous naloxone dose. Watson *et al.* (*4*), in a double-blind crossover experiment, found that 10 mg of naloxone reduced hallucinations in seven of nine carefully selected schizophrenics. Similar results were obtained by Emrich *et al.* (*5*), but other studies in which lower naloxone dosages were used had negative results (*6*).

Evidence for an endorphin abnormality in schizophrenia was also obtained by Terenius *et al.* (*7*), who noted increased concentrations of an unidentified opioid substance in the cerebrospinal fluid of some schizophrenic patients.

Success has been claimed in treating schizophrenics by means of hemodialysis (*8*). The implication is that a substance present in blood, and removable by dialysis, may play a role in causing the psychiatric symptoms displayed in this illness. Some of these dialyzates were reported to contain very high concentrations of β-endorphin (Met⁵-β-endorphin) and of a previously unknown peptide, [Leu⁵]-β-endorphin, in which leucine is substituted for methionine at position 5 of the peptide (*9*). In patients undergoing dialysis for the first time, endorphin concentrations in dialyzates were reported to be greater than 1 n*M*. This concentration is hundreds of times greater than that now known to be present in normal human blood plasma (*10*). Since peptides in the 3000-dalton range are cleared poorly through dialysis membranes (*11*), the endorphin levels in plasma were presumably even higher than those reported for the dialyzates.

Sensitive radioimmunoassays for β-endorphin have been developed in this laboratory (*12*). The antiserum used here recognizes human Met⁵-β-endorphin and human [Leu⁵]-β-endorphin equally. The binding of radioactively labeled β-endorphin is inhibited 50 percent by 2 fmole (7 pg) of either peptide in an assay tube. The antiserum cross-reacts 30 percent with β-lipotropin; this has also been demonstrated in human plasma (*13*). The values we report for β-endorphin-like immunoreactivity represent a composite of contributions from circulating β-endorphin and β-lipotropin.

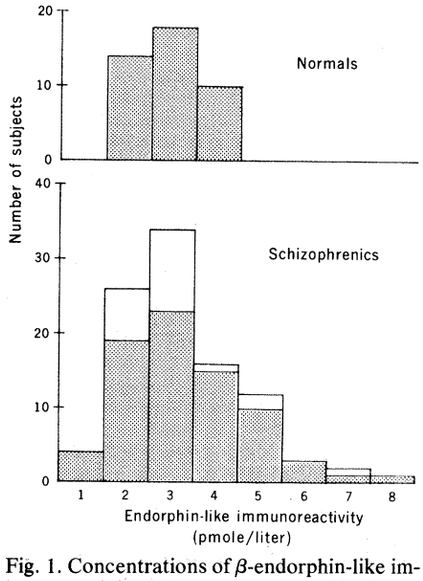

Fig. 1. Concentrations of β-endorphin-like immunoreactivity in blood plasma of 98 schizophrenic patients and 42 normal subjects. Concentrations are expressed in terms of human β-endorphin. Values reported are means of duplicate extractions, each of which was assayed in triplicate. Shaded bars represent data for determinations within the assay range. Open bars represent upper limits in samples in which the actual concentration of β-endorphin was below the sensitivity limit of the assay. Sensitivity limit is the concentration of human β-endorphin that inhibits radiolabeled peptide binding by 15 percent. Different sensitivity limits reflect availability of different volumes of plasma. Concentration estimates were obtained from least-squares lines on log concentration probit plots of inhibition of radioactively labeled human β-endorphin binding. Agreement between duplicates was within a factor of 2 for all data shown. Data from five samples were rejected and are not included because duplicates differed by a greater amount. Three were from patients, yielding concentrations of (in picomoles per liter) < 4.9, 10.1; < 4.9, 10.3; and < 4.9, 13.8. Two were from normal subjects, yielding 0.6, 1.4; and 2.7, 7.1.

We drew blood from 92 male and 6 female patients, 19 to 78 years of age (mean age, 37), who had been diagnosed as schizophrenic (paranoid, residual, undifferentiated, catatonic, or schizoaffective). Each diagnosis was confirmed by using Research Diagnostic Criteria (RDC) in a semistructured interview conducted by clinical investigators trained to make RDC diagnoses (*14*). Two of the patients had shown a therapeutic response to naloxone in a previous study (*4*). Most of the patients were maintained on their routine dosage of antipsychotic medication; however, 15 had received no neuroleptic medication for at least 2 weeks, and five had received no drugs for 13 days. Thirty-three male and nine female volunteers served as controls. They ranged in age from 19 to 59 years (mean age, 32) and had no history of mental illness. All blood samples were drawn between 8 a.m. and 10 a.m. Plasma was extracted and processed by using the method described in (*15*).

Histograms of the levels of β-endorphin-like immunoreactivity in the schizophrenic patients and normal controls are shown in Fig. 1. Values are not corrected for losses that occurred during extraction, but in other of our experiments the recovery of β-endorphin added to freshly drawn blood has been about 50 percent. All subjects had plasma levels of total immunoreactivity in the low picomolar range. The majority of patients had values within the range observed for the normal subjects, but about one quarter of them had somewhat higher values. No differences were observed among the several diagnostic categories or between patients who were still taking antipsychotic medications and those who were not (Table 1). In the two patients who had shown some reduction in schizophrenic symptomatology after treatment with naloxone (and who, therefore, would seem most likely to have increased endorphin concentrations), the plasma concentrations were 2.0 and 0.9 pmole/liter, well within the normal range.

Since stress stimulates release of β-endorphin-like immunoreactivity into the plasma in rats (*16*), we considered the possibility that subjects undergoing dialysis for the first time might display increased dialyzate concentrations of β-endorphin as a result of stress. Accordingly, dialyzates were collected from eight nonpsychotic patients with renal

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Table 1. Plasma concentrations of β-endorphin-like immunoreactivity by diagnostic category and medication status. Four patients in the group not currently on neuroleptic medication were receiving other drugs at the time of blood sampling: diazepam, choline chloride, thiamine, and penicillin (a different drug for each patient). Values below the detection limit of the immunoassay (see legend to Fig. 1) are treated as though they were equal to the detection limit. Consequently, some of the mean values are slight overestimates. N = number of subjects.

Subject	β-Endorphin-like immunoreactivity (pmole/liter)		
	N	Mean	Range
Schizophrenic patients	98	2.8	0.9 to 7.4
Diagnostic category			
Residual	26	2.8	1.3 to 5.2
Undifferentiated	16	2.5	1.3 to 6.0
Paranoid	27	2.7	0.9 to 6.8
Schizoaffective	28	3.0	0.9 to 7.4
Catatonic	1	4.6	
Medication status			
Currently receiving	78	2.8	0.9 to 7.4
No medication, ≤ 13 days	5	3.2	1.3 to 5.0
No medication, ≥ 14 days	15	2.6	0.9 to 4.0
Normal controls	42	2.4	1.2 to 3.8

failure who were undergoing their first hemodialysis. Levels of β-endorphin-like immunoreactivity were below the sensitivity limit of the assay in all cases, indicating a concentration of less than 9 pmole/liter. Similarly, no immunoreactivity could be found in dialyzates from nine schizophrenic patients undergoing hemodialysis for the first time (*17*).

We did not measure even one grossly increased plasma β-endorphin concentration among 98 schizophrenic patients. From the Poisson expectation (*18*), we conclude with 99 percent confidence that less than 6 percent of schizophrenics of the kind represented by our sample could have concentrations greater than the highest we measured (7.4 pmole/liter). Nor did we detect any β-endorphin in dialyzates from nine schizophrenic patients at a detection limit of 9 pmole/liter. Thus, our results are in striking disagreement with those of Palmour *et al.* (*9*), who reported concentrations nearly 1000 times higher in dialyzates than the highest we observed in plasma. It is possible, of course, that the patients studied by Palmour *et al.* were poorly representative of all schizophrenics.

The only evidence of a biologic source of [Leu^5]-β-endorphin comes from the same dialyzate samples in which grossly increased total endorphin concentrations were reported. Until these remarkable findings are confirmed, therefore, it remains questionable whether this β-endorphin variant occurs naturally. Recently, Lewis *et al.* (*19*), using gel filtration and high-pressure liquid chromatography, were also unable to detect human β-endorphin or [Leu^5]-β-endorphin in hemodialyzates from two schizophrenic patients at a detection limit of 30 pmole/liter. Likewise, Höllt *et al.* (*20*) did not find any remarkable differences in β-endorphin-like immunoreactivity in cerebrospinal fluid between schizophrenics and normal subjects.

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17. These dialyzates were obtained as part of a collaborative study with I. Cohen, S. Scheiber, T. D. Reisine, and H. I. Yamamura of the University of Arizona Health Sciences Center. We thank them for permission to report the immunoassay results here.
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