Effects of rHuEPO on Q-EEG and event-related potentials in chronic renal failure

TERESA SAGALES, VICTOR GIMENO, M. JESUS PLANELLA, NURIA RAGUER, and JORGE BARTOLOME

Servei de Neurofisiologia Clinica and Servei de Nefrologia, Hospital General Universitari Vall d’Hebron, Barcelona, Spain

Effects of rHuEPO on Q-EEG and event-related potentials in chronic renal failure. Quantitative electroencephalography is a powerful tool to evaluate brain function, and preliminary data have shown its usefulness in the evaluation of patients with chronic renal failure (CRF). In this study, baseline values of different quantitative EEG variables, as well as data from the P300 component of the visual event-related potential, in 43 patients with chronic renal failure, were compared with those of a group of healthy subjects and with the results obtained after 3, 6, 9 and 12 months of treatment of these patients with rHuEPO. Baseline total power was much lower in patients with CRF than in healthy subjects, and the distribution of power among the frequency bands was also abnormal. rHuEPO promptly normalized total power and progressively improved power distribution, although full normality was not achieved. Mean dominant frequencies in brain areas were abnormal in patients with CRF, and progressive improvement was seen along the study. The latency of P300, which was increased before treatment, decreased in all subjects, but normal values were not reached. The same applies to the hypomanic and psychopathic scores of psychological tests. Altogether, brain dysfunction of CRF seems to substantially improve by treatment of the anemia with rHuEPO.

Anemia is a common complication of chronic renal failure (CRF) and can be detected in over 90% of patients with this condition [1]. Although there are many contributing factors to this complication, the most important cause is a decrease in erythropoietin production by the failing kidney [1–4]. On the other hand, a uremic syndrome, mainly manifested as a neurological and neurobehavioral dysfunction, can be quantified by neuropsychological and electrophysiological studies [5–9]. Chronic hemodialysis corrects the uremia of CRF, but careful measurements show that it only affords a partial reversibility of the neurobehavioral dysfunction [9–12]. This suggests that uremic toxins are probably not the sole cause of the altered brain function, and it has been suggested that anemia could play a substantial role. Availability of recombinant human erythropoietin (rHuEPO) has offered a tool to test this hypothesis, and reports have already been published showing an improvement in cognitive function, assessed by psychological tests and the P300 component of the event-related potential, in CRF patients treated with erythropoietin [13–15].

Central nervous system alterations in CRF are not necessarily limited to cognitive function. Quantitative EEG is a powerful tool to evaluate brain dysfunction [16–18] and has elicited differences between normal subjects and patients with CRF that can partially be corrected by peritoneal dialysis or hemodialysis [20–22]. In this context, it seemed of interest to evaluate the effects of treatment with rHuEPO on brain function in patients with CRF, using quantitative EEG and brain mapping, together with other electrophysiological and neuropsychological measurements.

Methods

Subjects

A group of 43 patients (17 males, 26 females; age range: 22 to 74, median 66 years) with CRF currently on center hemodialysis at least for the last six months has been studied. The original diagnoses were: interstitial nephropathy (N = 7), nephrosclerosis (N = 7), diabetic nephropathy (N = 8), chronic glomerulonephritis (N = 5), chronic pyelonephritis (N = 5), focal glomerulosclerosis (N = 2), and toxic nephropathy (N = 2). CRF was not typified in seven cases.

All patients had been under hemodialysis treatment thrice weekly during the six month period prior to initiation of the study. All them had been previously polytransfused. Mean duration of the hemodialysis treatment since its onset was 52 months. Mean basal hemoglobin values were 70 ± 5 g/liter. None of them had primary central nervous system disease or uncontrolled arterial hypertension. Throughout the study, the hemodialysis program was kept unchanged, that is, all patients were submitted to dialysis thrice weekly. Hemodialysis was performed with a hollow-fiber cuprophane dialyzer of 1 to 1.3 m². Blood pump speed was 200 to 300 ml/min, and the flow of the dialysis fluid was kept constant at 500 ml/min. Length of the dialysis session was three to four hours. Blood urea nitrogen was 183.0 ± 22.0 mg/100 ml at the beginning of the study and 168.0 ± 27.0 mg/100 ml 12 months thereafter. Corresponding values of serum creatinine were 12.3 ± 1.9 and 12.1 ± 1.3 mg/100 ml.

rHuEPO was also administered thrice weekly, upon termination of each dialysis session, with an initial dose of 40 U/kg until a hemoglobin concentration of 100 g/liter was reached. This happened shortly before completion of the first trimester of treatment in all cases. Mean maintainance dose was 37.9 U/kg.
thrice weekly. No blood transfusions were administered to any of the patients during the study, and none of them suffered any intercurrent illness that would affect the central nervous system. A day in between hemodialysis treatment was selected to perform all the explorations.

A group of eight healthy volunteers (age range: 31 to 70, median 65 years) was used as a baseline control.

Quantitative EEG and brain mapping

Patients were evaluated before rHuEPO treatment (T0) and after 3 (T3), 6 (T6), 9 (T9) and 12 (T12) months of continuous treatment.

An electrodes cap was used for EEG recording, with the 10–20 International System placement. Linked-ear electrodes were used as reference. An 18 channel Nicolet EEG IA97 and a Nicolet Brainlab were used for the recording. The pass band of the amplifier was set between 0.5 and 30 Hz. Twenty artifact-free epochs of four second duration were recorded and analyzed. That means a total of 80 seconds of cerebral electrical activity free from artifacts. A computerized artifact rejection mechanism, together with visual control, was used in the selection of the epochs. Eye blinks, muscle activity, vascular pulses and electrode artifacts were detected and rejected, as well as periods of drowsiness. Screening was done by two authors thoroughly experienced in routine electroencephalography and evoked potentials.

The EEG is a complex waveform whose amplitude and frequency varies with time, but quantitative data can be extracted from it. The “power” of the signal, measured in μV² is the square of the signal effective value [23], and a typical approach is to obtain the Power Spectral Density (PSD) of the EEG signal. The PSD gives the distribution of the EEG total power among the frequencies. In this study, total EEG power and power in each of the classical frequency bands differentiated in EEG recordings, that is, delta (0.5 to 3.75 Hz), theta (4 to 7.75 Hz), alpha (8 to 11.75 Hz), beta-1 (12 to 15.75 Hz), beta-2 (16 to 19.75 Hz) and beta-3 (20 to 24 Hz) were calculated. Mean dominant frequency (MDF), extracted from the PSD function and measured in Hz was also calculated. Power in the different bands and MDF were calculated for five cerebral areas: frontal, temporal, central, parietal and occipital.

Brain mapping [24] is a medical imaging technique based on computerized EEG that shows, in the form of a two-dimensional color distribution map, the brain activity in the different scalp areas. Power in the different EEG frequency bands is typically represented.

EEG recordings are customarily obtained in resting subjects with their eyes closed. Recordings with eyes open are markedly different, and with the advent of computerized EEG this difference is routinely used as an indication of central nervous system reactivity. In this study, brain maps were obtained in all cases under two different conditions: (a) awake, eyes closed. During the recording the subjects were resting with closed eyes, and (b) awake, eyes open. During the recording the subjects were in a resting position, but they were asked to look at a central point placed at a distance of three meters.

Table 1. Total EEG power in control subjects and in patients with CRF before (T0) and after 3 (T3), 6 (T6) 9 (T9) and 12 (T12) months of treatment with rHuEPO

<table>
<thead>
<tr>
<th></th>
<th>Eyes closed</th>
<th>Eyes open</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1620.0 ± 530.0</td>
<td>1117.9 ± 269.0</td>
</tr>
<tr>
<td>CRF T0</td>
<td>725.5 ± 490.0a</td>
<td>551.3 ± 339.7a</td>
</tr>
<tr>
<td>T3</td>
<td>3486.5 ± 1280.0b,c</td>
<td>2144.8 ± 798.0b,c</td>
</tr>
<tr>
<td>T6</td>
<td>2122.1 ± 1275.9b,c</td>
<td>1495.2 ± 950.0b,c</td>
</tr>
<tr>
<td>T9</td>
<td>2253.8 ± 1045.0b,c</td>
<td>1252.7 ± 894.6b,c</td>
</tr>
<tr>
<td>T12</td>
<td>1145.0 ± 499.3c</td>
<td>751.9 ± 282.0c</td>
</tr>
</tbody>
</table>

Figures between parenthesis indicate the number of patients completing the test at each particular period. Values are mean ± SD.

a P < 0.05 for the difference when compared with the control group
b P < 0.05 for the difference when compared with baseline values (T0)

Event-related potentials

Event-related potentials (ERP) were also recorded before initiation of rHuEPO treatment and 12 months thereafter in 10 of the 43 patients.

A visual “oddball” detection task was used for the collection of ERP. A black and white chessboard pattern stimulus was used as frequent stimulus (non-target stimulus), randomly replaced occasionally (18% of stimuli) by a red and black chessboard pattern stimulus (target stimulus). Subjects were instructed to ignore the first ones and keep an internal addition of the target stimuli. Trial was finished after a total of 22 artifact-free responses to target stimuli. Frequency band width was set between 0.5 and 30 Hz.

The P300 wave of the ERP was identified as the large positive wave appearing 280 msec or later after the onset of the stimulus, that was present in responses to target stimuli and absent, or greatly decreased, in responses to non-target stimuli. The Pz channel was chosen to measure the latency and amplitude of the different waves of the ERP.

Neuropsychological assessment

The MINIMULT and WAIS test (digits, dices, similarities and unfinished figures) were performed in 18 of the 43 patients, before and after 12 months of rHuEPO treatment.

Statistical analysis

Student’s t-tests for nonpaired data and for paired data were used in the statistical analysis of the EEG and P300 data. Nonparametric Wilcoxon’s test for paired data was used in the statistical analysis of neuropsychological data [25].

Results

Brain mapping

Power values and brain maps for the control group and in patients with CRF, before and after different treatment periods with rHuEPO, are presented in Table 1 and Figure 1. As shown there, baseline total power in patients with CRF was much lower than in normal subjects (1620.0 ± 530.0 vs. 725.5 ± 490.0 μV², P < 0.05, in recordings with eyes closed, and 1117.9 ± 269.0 vs. 551.3 ± 339.7 μV², P < 0.05, in recordings with the eyes open). Another differential feature between the two groups
was the distribution of power among the frequency bands. As expected, eyes closed recordings in normal subjects revealed a dominancy of alpha power, with peak values in occipital areas, that was not seen in patients. Also, the increase in delta activity, elicited by recording with the eyes open, was much more marked in patients than in normal subjects.

Continuous treatment with rHuEPO promptly normalized total power in the brain. Values after three months were much greater than baseline and not statistically different from those in the control group, in both recording conditions, and the same was observed in later evaluations. Progressive changes toward normality were detected in the maps recording power distribution in the brain after 3, 6, 9 and 12 months of treatment, but a completely normal distribution was not achieved. Analysis of data from the eight patients that were tested at all five time points yielded similar results, that is, statistically different values from baseline recordings were already observed after three months of treatment and this change persisted in later evaluations. Even in this limited and more homogeneous group, power tended to be greater at T3, with a progressive decrease at T6, T9 and T12. This trend was similar in different age or length of disease groups analyzed.

Figure 2 illustrates the statistical significance of the changes in eyes closed/eyes open reactivity along the study in patients.
treated with rHuEPO. Again, progression toward normality is evident.

Mean dominant frequency (MDF) in different brain areas in sequential recordings along the study is presented in Figure 3. With eyes closed, the typical preponderance of higher frequencies in the occipital areas was elicited in normal volunteers, whereas a rather uniform distribution of the different frequencies among the different areas was seen in patients with CRF. Again, treatment with rHuEPO promptly normalized the frequency distribution, and the effect was maintained throughout the study.

**P300**

Patients with CRF exhibited a P300 amplitude similar to that observed in healthy subjects (10.1 ± 4.5 vs. 11.3 ± 1.9 μV), but an increased latency of this wave (376.1 ± 15.5 vs. 441.1 ± 26.5 msec, \( P < 0.05 \)) was detected.

No consistent changes were noted in the P300 wave amplitude before and after 12 months of rHuEPO treatment, whereas a decrease of P300 latency was observed in all patients. The mean P300 latency of 441.1 ± 26.5 msec was reduced to 418.3 ± 24.7 msec after 12 months (Table 2).

**Psychological tests**

Tests performed on 18 patients before and after 12 months of continuous treatment with rHuEPO revealed statistically significant changes in psychopathic and hypomanic scores of MINIMULT. In the clinical psychopathic scores, the initial values of 53.2 ± 3.4 decreased to 48.4 ± 6.5 \( (P < 0.05) \), whereas the baseline hypomanic score of 46 ± 6.7 decreased to 41 ± 5.5 \( (P < 0.05) \). WAIS scores were not modified by rHuEPO treatment.

**Discussion**

Our results clearly show that quantitative EEG and brain mapping are powerful tools to evaluate the central nervous system dysfunction of chronic renal failure. Power spectral density relates amplitude and frequency of the electrical waves originating from cerebral activity. This integrated variable was clearly depressed in our patients with CRF when compared with a control group of healthy volunteers of the same age range. Furthermore, when power for the different frequency bands was evaluated in the different brain areas currently considered in EEG evaluations (frontal, central, temporal and...
Focus on the frequencies of brain electrical activity also differentiated patients with CRF from healthy subjects. In the control group, clear cut differences in the mean dominant frequency were observed among different brain regions. Lower frequencies were dominant in the frontal areas, while progressively higher frequencies dominated in the central, temporal and occipital areas. This pattern was completely lost in patients with CRF, in which no substantial differences in MDF were elicited among brain areas.

Treatment of the anemia in these patients by continuous administration of rHuEPO for 12 months resulted in a prompt improvement of the above-mentioned abnormalities. Three months after initiation of treatment, when hemoglobin concentrations were equal or above 100 g/liter in all cases, total power was more than twice that of baseline recordings and had reached a mean value not statistically different from that of the control group. Normal power values were recorded from then on.

Distribution of power for the different frequency bands also improved sensitive to treatment with rHuEPO, but in this case the effects took longer to establish. After three months of treatment, the brain maps were still quite abnormal, although alpha activity was increased in occipital areas. Power distribution was not much improved after six months of treatment, and even after 12 months some abnormalities persisted in the brain maps. Thus, although the distribution of alpha and beta activities could be considered roughly normal, there was still an excess of slow activities throughout the brain.

It is worth commenting on the slightly different results yielded by evaluation of total power and of brain mapping. Power quantification indicates that a therapeutic response to rHuEPO is seen after three months of treatment, when values are statistically different from baseline but not from those obtained in normal subjects. After this point, however, numerical values seem to decrease and the impression left is that there is some kind of "escape" to treatment. On the other hand, brain maps prove more sensitive. Maps at T3 are different from those in healthy subjects and progressive changes are seen at later points, normality being approached, but not fully achieved. Interestingly, brain maps also offer a clue for the interpretation of the apparent escape effect seen in total power analysis. Inasmuch as EEG power reflects amplitude (square) versus frequency of the electrical signal of the brain, an increase of delta activity, as detected at T3 and T6, would result in greater total power values than would be expected if lower frequencies predominated.

The effects of erythropoietin treatment were also reflected in the values of the mean dominant frequency in different brain areas. The increase in alpha activity in the occipital areas after three months of treatment led to a partial normalization of the distribution of MDFs at that time, but distributions closely resembling those observed in healthy subjects were not observed until after 9 or, to a greater extent, 12 months of rHuEPO treatment.

The marked improvement of brain function, as elicited by quantitative EEG and brain mapping, after continuous rHuEPO treatment.
treatment for one year, parallels the effects observed in another electrophysiological measurement, namely the latency of P300 wave of the event-related potential. The abnormal latency of this wave in patients with CRF when compared with a group of healthy volunteers was clearly diminished after 12 months. This change was observed in all subjects, which strongly suggests a treatment effect, but mean values did not reach normality. Similar considerations apply to the results of psychological tests, which elicited an improvement, but not full normalization, of the psychopathic and hypomanic scores.

Our baseline evaluation of patients with CRF by means of quantitative EEG yields results which are in agreement with those of other studies [19–21], and the same applies to the studies using P300 as an indicator of brain dysfunction in these patients [9, 13–15]. Previous studies of quantitative EEG in CRF have only focused on the effects of peritoneal dialysis or hemodialysis, but none has considered the possible effects of improvement of the anemia. In the case of P300, several studies have dealt with the effects of rhEPO treatment for different periods of time up to one year [13–15]. In a study of the effects of a six month treatment, Grimm et al. [13] also observed a partial normalization of P300 latency, with moderate but not statistically significant effects on P300 amplitude induced by rhEPO. It should be mentioned that the event-related potential in that study was elicited by means of acoustic stimuli, whereas in our case the stimuli were visual. This is obviously an important difference. Acoustic stimuli were also used in the studies of Brown et al. [14] and of Marsh et al. [15], which reported no effects of rhEPO treatment on P300 latencies. However, in both cases there are indications, acknowledged by the authors, that the patients studied were less ill than those of Grimm et al and, as judged by the published data, than ours.

The lack of full normalization of several electrophysiological variables (power distribution, P300 latency) in spite of marked improvement of the anemia after a 12 month treatment with rhEPO, when hematocrit levels were above 30 in all cases, suggests that factors other than anemia also account for the neurobehavioral dysfunction of patients with CRF. Whatever the role of hormonal or other factors [22, 25–27] in uremic encephalopathy, it seems evident that decreased oxygen delivery to the brain due to anemia is an important determinant of the electrophysiological abnormalities detected in patients with chronic renal failure, and that treatment of the anemia with rhEPO results in a substantial reversal of these abnormalities, as well as an improvement in the scores of psychopathic and hypomanic traits in psychological tests.

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Reprint requests to Dr. Teresa Sagalés, Servei de Neurofisiologia Clínica, Hospital General Universitari Vall d’Hebron, 08035 Barcelona, Spain.

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