Reductions in plasma prolactin during acute erythropoietin administration

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Abstract

OBJECTIVES: The presence of erythropoietin (EPO) and its receptors in several central nervous system regions indicates additional functions beside its hematopoietic role. Preclinical data suggest that it may slow down the process of neuronal loss, and that EPO may cause dopamine release, and thus influence hormone release, especially prolactin. This possibility has not yet been studied in humans.

METHODS: During a clinical trial on possible protective effects in patients with amyotrophic lateral sclerosis (ALS), we studied the acute effects of EPO administration on prolactin, the release of which is under tonic inhibition of hypothalamic-pituitary dopaminergic activity. Prolactin as well as EPO levels were estimated in blood samples taken every 30 min over 2 hours after administration of 3000 IU EPO i.v. in seven male and four female patients with ALS.

RESULTS: The baseline PRL levels of the 11 patients were all within normal range (4.5–10.5 ng/ml). EPO administration caused a significant reduction in prolactin levels, maximal at 60 min after administration. Reductions in PRL were not related to EPO dose (IU per kg body weight), or to duration of illness.

CONCLUSIONS: The findings indicate that EPO promotes dopamine release in humans, and is consistent with preclinical data showing that EPO releases dopamine from rat striatal slices. Previous reports showed that dopaminergic neurons express EPO receptors, which exert a facilitating action on dopamine release, and the present data indicate that this may hold true in humans.

Abbreviations:
EPO – erythropoietin
PRL – prolactin
ALS – amyotrophic lateral sclerosis

Introduction

In addition to its hematopoietic role through its action on erythroid precursor cells, erythropoietin (EPO) seems to exert additional physiological actions. The substance, as well as its receptor, has been found in mouse brain, localized in areas as cerebral cortex, hippocampus, and midbrain [1], where EPO mRNA increases under hypoxic conditions. EPO gene expression was detected also in monkey brain areas, as well as in biopsies from human hippocampus, amygdala, temporal cortex, and astrocytes [2]. Cultured hippocampal and cerebral cortical neurons that express the EPO receptor are protected from glutamate neurotoxicity by EPO [3]. There is also evidence that EPO protects neuro-

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nal cells from ischemic damage [4], preventing neuronal apoptosis [5]. Iwasaki et al. [6] showed protective effects of EPO against axotomized motor neuron death, and suggested potential use in treating diseases involving degeneration and death of motor neurons. In a recent report [7], EPO administration was found to protect dopaminergic neurons in substantia nigra and ventral tegmental area in neonatal rats subjected to hypoxia-ischemia.

There are several reports regarding EPO actions on the brain dopaminergic system. This specific interest is related to the mechanisms underlying possible neuroprotective effects of EPO, as well as to the understanding of the mechanisms that alter hormone levels, especially of the hypothalamus – pituitary axis, that occur in patients given EPO to treat anemia (mainly dialyzed patients), since prolactin release is under tonic inhibition by dopamine. Hyperprolactinemia is common in chronic renal failure [8], and treatment of anemia with EPO normalizes elevated PRL levels [9–11].

Koshimura et al. [12] showed that EPO increases dopamine release in differentiated PC12 cells, a model of neuronal cell, which possess EPO receptors, while Kawakami et al. [13] reported a suppression of calcium-induced dopamine release from PC12 cells. Incubation of rat striatal slices with EPO increases dopamine release in a dose-dependent manner [14]. Csete et al. [15] reported that EPO receptors are expressed in adult rat dopaminergic neurons, and suggested that EPO functions as a neurotrophic factor promoting proliferation, differentiation, and protection.

In the context of the above-mentioned research on neuroprotective effects of EPO, several preclinical studies suggest that EPO may have therapeutic potential for neuronal loss prevention in stroke, cerebral trauma, multiple sclerosis, and neurodegenerative disorders (for review see [16]), and a beneficial effect on stroke patients has been reported [17]. The report of Iwasaki et al. [6] on motor neuron survival in rats, suggests that the substance may have a beneficial effect in patients with amyotrophic lateral sclerosis (ALS) by slowing down the process of neuronal loss. We thus initiated a clinical trial in which we administered EPO in patients with ALS. In addition to the evaluation of its possible neuroprotective effect, we were interested to select information about its mechanisms of action in the central nervous system. Here we report the effects of the acute administration of EPO on plasma prolactin as a measure of alterations in the hypothalamic-pituitary dopaminergic system, as it is well established that dopamine is the main regulator of prolactin release, exerting a tonic inhibitory effect on the pituitary lactotrophs.

Subjects and Methods

Eleven patients with diagnosis probable or definite amyotrophic lateral sclerosis (El Escorial criteria) were studied, seven males (age range 58 to 71 years), and four females (age range 45 to 71 years). Mean age was 60.6 (SD=8.2) years, and mean duration of illness 18 (SD=13) months (range 6 to 36 months). They were all under treatment with riluzole in a stable dose for at least one month.

3000 IU EPO were administered i.v., and blood samples of 5 ml were taken at times 0, 30, 60, 90, and 120 minutes, and blood pressure and heart rate were recorded. Plasma was separated by centrifugation and stored at –30° C until estimations.

Radioimmunoassay kits of BioChem Immuno-Systems, Italy, were used for the estimation of PRL. In addition, we estimated in the same samples the levels of erythropoietin, using the radioimmunoassay kit of Diagnostic Systems Laboratories (Webster, Texas, USA). EPO concentrations are expressed in mIU/ml plasma. The intra- and inter-assay coefficients of variation were less than 5% for both estimations.

For the statistical evaluation of the data we used repeated measures analysis of variance with age and sex as covariates, followed by post-hoc comparisons. Linear regression analysis was used in searching correlations between variables.

Results

The baseline plasma PRL levels of the patients were all well within the normal range (mean±SD 6.04±1.66, range 4.49–10.5 ng/ml).

Since the responses of PRL may differ between males and females, we first compared the PRL responses to EPO between these subgroups, using repeated measures ANOVA. A significant time effect was found (df=4,36, F=5.45, p=0.016), while sex effect and sex-time interaction were non-significant (Table). Having excluded a significant sex effect, we proceeded to the evaluation of EPO effects on PRL for the whole group of 11 patients. The mean values of PRL during EPO administration in the male and female subgroups, as well as in the whole group, are shown in the Figure.

The mean values (±SD) of the levels of EPO and PRL measured in the plasma of all 11 patients, as well as mean arterial pressure and heart rate, are shown in the Table. Statistical evaluation of the PRL data showed a significant time effect, with maximal reductions in PRL levels at 60 min after EPO administration (Table). EPO plasma concentrations rose from a mean of 8.8±1.7 mU/ml to 217±59 in the 30 min samples, and remained high during the two hours sampling. From the regression line of the diminishing mean values of EPO from 30 min to 120 min (four points, r=–0.9753, y=218–0.404x), we calculated a half-life for EPO of 4.5 hours, which is in accordance with the reported values after i.v. administration of 4–9 hours [18].

Baseline PRL levels were marginally positively related to age (r=0.5596, p=0.07), and not related to duration of illness (r=0.1377, n.s.) or to body mass index. The reductions in plasma PRL were not related to the EPO dose expressed in IU per kg body weight (r=0.0434, n.s.), or to age (r=0.4493, p=0.17) and duration of illness

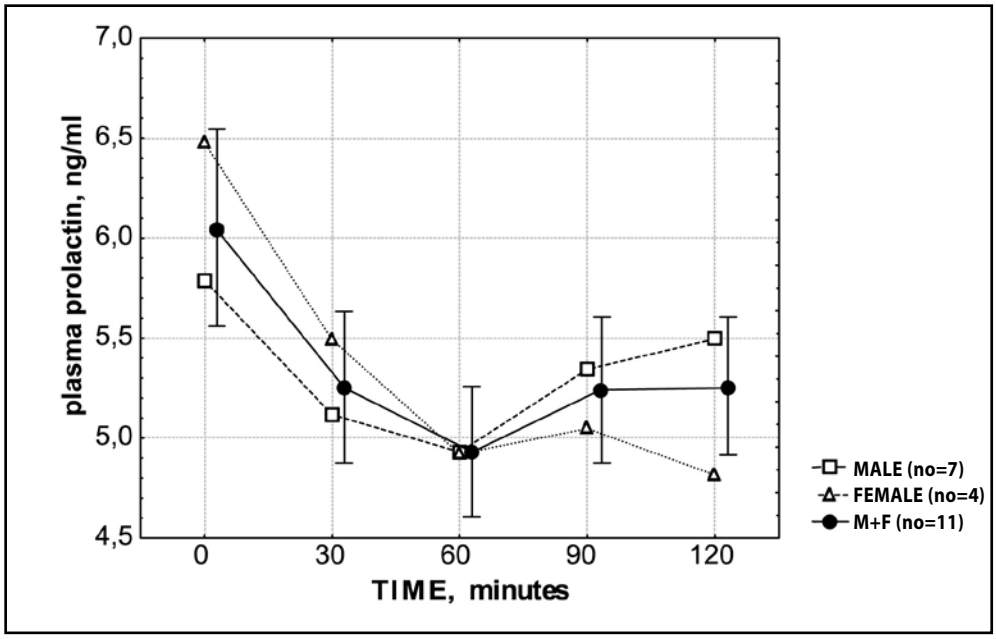

Figure. Plasma prolactin levels (mean±SEM) of 11 patients with amyotrophic lateral sclerosis after administration of 3000 IU erythropoietin intravenously. The responses of the male and female subgroups are shown separately.

Table. Plasma levels of erythropoietin and prolactin (mean±SD) in 11 patients with ALS after the administration of 3000 IU EPO i.v. Statistical evaluation by analysis of variance for repeated measures. The changes in mean arterial blood pressure (MAP) and heart rate (HR) are also given.

Time	EPO	MAP	HR	PRL
Baseline	8.8±1.7	95.7±7.5	78.9±9.8	6.04±1.66
30	217±59	101.7±7.8	86.1±15.2	5.25±1.26
60	207±43	99.3±6.7	82.3±12.6	4.92±1.12
90	190±42	96.6±6.5	83.1±13.5	5.23±1.24
120 min	186±42	96.2±7.9	79.6±12.4	5.34±1.18
F		3.58	2.32	4.35
p		0.014	0.07	0.005
Post-hoc comparisons (Tukey-test)				
0 versus 30		0.023	0.07	0.057
60		0.35	0.72	0.003
90		0.99	0.53	0.049
120 min		0.99	0.99	0.055

(r=0.4101, p=0.21). Baseline EPO levels gave a negative correlation to duration of illness, which though did not reach significance (r=–0.5184, p=0.102).

Discussion

Acute i.v. administration of EPO in patients with ALS caused a significant reduction in plasma PRL levels, maximal at +30 min. The reduction was sustained during the two hours sampling, where the EPO concentrations remain high. Since there are no indications for any disturbances of the hypothalamus – pituitary axis in ALS, we can accept that this action is not restricted to this patient group. These data provide an indication for an action of EPO on the dopaminergic pituitary input, stimulating the release of dopamine, and are in accordance with the increases in dopamine release by EPO reported by Koshimura et al. [12] who worked with PC12 cells which possess EPO receptors, and the increases in dopamine release from rat striatal slices after incubation with EPO [14]. Since dopaminergic neurons express EPO receptors [15], it seems likely that EPO exerts a facilitating action on dopamine release by acting on these receptors.

The acute effect of EPO on plasma PRL levels is line with the reduction in PRL found in dialysed patients after long-term EPO administration to treat anemia, as reported in the studies cited above. It has to be mentioned though, that in this study, we did not observe a

decrease in baseline plasma PRL levels after one or two months weekly EPO administration in 10 patients (mean PRL values 7.1±2.5 at baseline, 8.1±2.9 after one month, and 7.7±2.0 ng/ml after two months). Our patients had all baseline PRL levels within the normal range, while hyperprolactinemia is common in patients under hemodialysis. On the other hand, PRL seems to interfere with the hematopoietic process by increasing the number of EPO-responsive hemopoietic precursors [19], and hyperprolactinemia in patients with reduced EPO production is regarded as a compensatory mechanism.

The relation between EPO and PRL/dopamine deserves further study. The elevated PRL levels in psychotic patients treated with neuroleptics may have implications on the hematopoietic process, while EPO may affect central dopaminergic activity. In this respect, it is of interest that EPO is proposed as a candidate compound for neuroprotection in schizophrenia [20].

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