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Effect of recombinant human erythropoietin on gamma-aminobutyric acid (GABA) tissues concentration in uremic rats

The beneficial effect of rHuEPO is partly limited by frequently observed hypertension leading in some part of patients to hypertensive encephalopathy and seizures. Data from uncontrolled multicenter trials (based on a report from National Kidney Foundation analyzing the incidence of patients receiving rHuEPO have shown the mean percentage of patients with seizures to be 3%, with a range of 0% to 13% (Clinical Practice Guidelines by National Kidney Foundation). These studies did not report the presence or absence of seizure history prior to the use of rHuEPO. Cengiz et al. reported medical history of 6 hemodialysis patients with development of epileptic seizures successfully treated by anti-epileptic therapy (3). Several observations demonstrate successful using of centrally acting gamma-aminobutyric acid (GABA) agonists (Vigabatrin) in the treatment of patients with refractory non-erythropoietin dependent epilepsy (2). The potential role of GABA system in the etiology of seizures in end stage renal failure in human and in animal model is successfully investigated (10, 11, 12). It was postulated that decreased GABA concentration in the brain tissues had an influence on seizures activity (10).

In the present study we have measured GABA concentration of brain, kidney, and heart tissues in male adult Wistar rats after rHuEPO administration in the non-uremic and uremic (after 5/6 nephrectomy) rats.

METHODS

Sixty Wistar adult male rats were divided into two groups: non-uremic and uremic in our study. The rats' weight ranged from 270 to 310 g. In each group ten animals were selected as a control group, the next ten were given rHuEPO (Janssen-Cilag AG, Ch) three times a week in the doses of 300 U/Kg body weight i.p. during six weeks' period. The last group of rats (N=10) was given rHuEPO three times a week in the doses of 600 U/Kg body weight i.p. during six weeks' period. All groups of rats were provided unlimited access to water and food.

5/6 nephrectomies for induction of chronic renal failure. The rats were anaesthetized with chloral hydrate supplemented as needed. The abdomen was opened through a middling incision, and the right kidney was removed after ligation of three branches of the left renal artery (9). The animals were kept for two months up to the time of the first rHuEpo injection.

GABA measurement protocol. The animals were killed by decapitation, and whole brain, kidney and heart were rapidly removed and dissected in liquid nitrogen for 6 sec. The GABA tissues content was measured by spectrofluorometric methods reported by Lowe (8) in Sutton and Simmonds' modification (13), as described by Kleinrok and Turski (5).

It was reported that during decapitation and dissection no increase of GABA in the tissue could be observed (11, 12).

The GABA results were calculated in μg /g of wet tissue.

Statistical analysis. Data are presented as mean±standard error deviation (SED). Differences between groups were compared using the non-parametric Mann-Whitney U-test and the probability (P) <0.05 was assumed to reject the null hypothesis.

RESULTS

The mean hematocrit made up 37.2.±2.4% in control group, 41.4±3% in the rats treated with 300 U/kg of b.w. rHuEPO, and 43.6±2.8% (600 U/kg b.w. of rHuEPO) in non-uremic animals. The mean hematocrit in the uremic rats increased from 34.6±1.5 to 40.3±1.3% (300 U/kg of b.w. rHuEPO), and respectively to 49.3±1.4% (600 U/kg of b.w.rHuEPO). There has been observed a mild increase of the urea and creatinine level after 6 weeks of rHuEPO treatment in uremic rats. (Table 1). The potassium, sodium, and ionized calcium values did not change during the rHuEPO therapy both in non-uremic, and uremic rats (Table 1).

Table 1. Effect of rHuEpo treatment on biochemical parameters in the blood of Wistar male rats (the mean value ± SEM); * p<0.05, **p<0.01, control versus 300 U/kg of rHuEpoin non-uremic rats, ° p<0.05,°° p<0.01, control versus 600 U/kg of rHuEpoin non-uremic rats, ▲ p<0.05,▲▲ p<0.01, control versus 300 U/kg of rHuEpoin uremic rats, ▼ p<0.05,▼▼ p<0.01, control versus 600 U/kg of rHuEpoin uremic rats

Plasma parameters	Non-uremic rats			Uremic rats		
	control (N=10)	300 U/kg rHuEPO i.p. (N=10)	600 U/kg rHuEPO i.p. (N=10)	control (N=10)	300 U/kg rHuEPO i.p. (N=10)	600 U/kg rHuEPO i.p. (N=10)
Hematocrit (%)	37.2±2.4	41.4±3*	43.6±2.8°°	34.6±1.5	40.3±1.3▲▲	49.3±1.4▼▼
Hemoglobin (g/dL)	13.9±0.8	16.3±0.8*	15.3±1.4°°	12.1±0.3	15.0±1.04▲▲	18.5±0.6▼▼
MCHC (g/dL)	37.4±1	39.5±1.1*	35.0±0.6°	37.2±0.6	39.2±0.9▲▲	36.5±0.1
MCH (pg)	20.3±0.1	22.5±0.7**	20.9±0.8	19.1±0.9	21.4±1.0▲▲	19.9±0.8
MCV (fL)	54.4±3.4	56.9±2.5	59.1±2.2°°	51.3±2.1	54.4±2.3	53.3±2.5▼▼
Urea (mg/dL)	31.4±2.3	36.5±11	32.0±1.4	195±17	254±18▲▲	261±54▼▼
Creatinine (mg/dL)	0.47±0.04	0.61±0.1	0.54±0.07	3.15±0.09	4.3±0.37▲▲	4.0±0.7▼▼
Sodium (mEq/l)	143.4±2.9	142.7±2.5	144.8±.2.6	144.4±1.6	144±5.1	142±2.8
Potassium (mEq/l)	6.1±0.4	6.2±0.4	5.6±0.6	6.8±0.3	7.6±0.9	6.7±0.7
Calcium (mmol/L)	1.43±0.02	1.38±0.05	1.39±0.08	1.42±0.03	1.24±0.12	1.4±0.1

The brain, kidney and heart tissues GABA concentration did not change during rHuEPO therapy in the groups of non-uremic rats. In the group of uremic rats kidney, and heart tissues GABA concentration statistically significantly decreased after giving the rats the rHuEPO 300 U/kg b.w. (p<0.01), and also after the dose of 600 U/kg b.w. of rHuEPO (p<0.01) (Table 2). The brain GABA concentration decreased (p<0.05) in uremic rats treated for 6 weeks with 600 U/kg b.w. of rHuEPO (Table. 2).

Table 2. Effect of rHuEpo treatment on GABA brain, heart and kidney tissues concentration (the mean value ± SEM); *-p<0.05,**-p<0.01, control versus 300 U/kg of rHuEpoin non-uremic rats, °-p<0.05,°°-p<0.01, control versus 600 U/kg of rHuEpoin non-uremic rats, ▲-p<0.05,▲▲-p<0.01, control versus 300 U/kg of rHuEpoin uremic rats, ▼-p<0.05,▼▼-p<0.01, control versus 600 U/kg of rHuEpoin uremic rats

GABA tissue concentration (μg/g tissue)	Non-uremic rats			Uremic rats		
	control (N=10)	300 U/kg rHuEPO i.p. (N=10)	600 U/kg rHuEPO i.p. (N=10)	control (N=10)	300 U/kg rHuEPO i.p. (N=10)	600 U/kg rHuEPO i.p. (N=10)
Brain	605.2±16.9	540.5±24.8	605.9±24.8	580.7±39.4	558.4±14.1	544.7±41.9▼
Heart	103.9±11.8	106.6±6.3	80.2±5.1	59.7±6.3	58.5±11.5▲	67.2±6.9▼
Kidney	88.3±2.9	86.1±12.9	99.3±3.0°	68.1±7.7	44.5±14.5▲▲	23.1±7.9▼▼

DISCUSSION

In patients treated with rHuEPO, a major side-effect is hypertension (6), but hypertensive encephalopathy and seizures have been observed in about 3% of the treated patients (1). The mechanism which causes seizures is still not clear. Several studies reported elevation of brain GABA concentration as a predictor of seizures activity in animals model (10, 11). Cengiz et al. reported medical history of six hemodialysis patients with development of epileptic seizures successfully treated by anti-epileptic therapy (3). GABA is considered to be the major inhibitory neurotransmitter in the brain and loss of GABA inhibition has been clearly implied in epileptogenesis. For this reason several anticonvulsant drugs, such as vigbatrin, tiagabine, gabapentine or toporimate, with a mechanism of action considered to be primarily via an effect on GABA, have been licensed (4), also in hemodialysis population (2).

In the present study in the group of uremic rats kidney, and heart tissues GABA concentration statistically significantly decreased after the rHuEPO dose of 300 U/kg b.w ($p<0.01$), and also after the dose of 600 U/kg b.w. of rHuEPO ($p<0.01$). The brain GABA concentration decreased ($p<0.05$) in uremic rats treated for weeks with 600 U/kg b.w. of rHuEPO i.p. At the same time we observed a higher level of creatinine level in uremic rats under rHuEPO treatment (control – 3.15mg/dL versus 4.3 mg/dL (300 U/kg of rHuEPO) and 4.0 (600 U/kg of rHuEPO).

In our preliminary data we observed a significant increase of different neurotransmitters, such as noradrenaline concentration in the hypothalamus, and in the blood of normotensive uremic rats after rHuEPO treatment (7), and this observation has been confirmed in humans (6).

In conclusion, we demonstrated a significant decrease of GABA brain, kidney and heart tissues concentration after six weeks of rHuEPO therapy in uremic adult Wistar rats (after 5/6 nephrectomy). This may suggest that the observed decreased GABA-ergic activity after rHuEPO therapy may increase seizures activity, however direct effect of experimental uremia should be discussed.

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SUMMARY

Chronic treatment of anemia with erythropoietin (rHuEpo) in humans and animals is asscociated with important complications, such as hypertension and seizures. The mechanism responsible for occurring hypertensive encephalopathy and seizures is still not clear. There are ample physiologic and behavioural data to suggest that gamma-aminobutyric acid (GABA) play an important role in the regulation of "seizures activity" in both human and animal brain. The aim of our study was to evaluate the effect of rHuEpo intraperitoneal administration on the brain, heart, and kidney GABA tissues level in 30 non-uremic, and 30 uremic (after 5/6 nephrectomy) Wistar male rats. The rHuEpo has been injected intraperitoneally in the dose of 300 U/kg and 600 U/kg b.w. during six weeks. The significant decrease in brain ($p<0.05$), kidney ($p<0.01$) and heart ($p<0.01$) tissues of GABA contents has been observed in uremic rats after six weeks of 600 U/kg b.w. of rHuEpo therapy. However, during observing period mild deterioration of renal function during rHuEPO therapy has been reported in uremic animals. We concluded that the observed decrease of GABA brain concentration may contribute to higher seizures activity during rHuEPO therapy.

Wpływ ludzkiej rekombinowanej erytropoetyny na tkankowe stężenie kwasu gamma amino-masłowego u szczurów z eksperymentalną mocznicą

Leczenie niedokrwistości u ludzi i zwierząt za pomocą ludzkiej rekombinowanej erytropoetyny (rHuEpo) indukuje istotne powikłania w postaci nadciśnienia oraz drgawek. Patomechanizm powstania encefalopatii nadciśnieniowej i drgawek jest nie do końca jasny. Badania eksperymentalne na zwierzętach oraz u ludzi wskazują na istotną rolę kwasu gamma amino-masłowego (GABA) w regulacji „aktywności drgawkowej" w obrębie struktur mózgu. Celem niniej-szej pracy eksperymentalnej było zbadanie wpływu działania podawanej dootrzewnowo rHuEpo na stężenie GABA w homogenacie tkankowym mózgu, serca oraz nerek u 30 szczurów bez mocznicy oraz u 30 szczurów z eksperymentalną mocznicą (po 5/6 nefrektomii). W eksperymencie brały udział osobniki płci męskiej szczepu Wistar. Preparat rHuEpo podawano

dootrzewnowo w dawce 300 U/kg oraz 600 U/kg masy ciała w ciągu 6 miesięcy. Stwierdzono statystycznie istotne obniżenie stężenia GABA w homogenatach mózgu ($p<0.05$), nerkach ($p<0.01$) oraz serca ($p<0.01$) u szczurów z eksperymentalną mocznicą po 6 tygodniach podawania rHuEpo w dawce 600 U/kg masy ciała. W ciągu badanego okresu stwierdzono ponadto istotne pogorszenie funkcji nerek u szczurów mocznicowych. Konkluzją jest stwierdzenie, że wykazane obniżenie poziomu GABA w mózgu szczurów z eksperymentalną mocznicą powoduje wzrost aktywności drgawkowej po podaniu preparatu ludzkiej rekombinowanej erytropoetyny (rHuEPO).