Erythropoietin Effects on Skeletal Muscle Bioenergetics in the Rat

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Introduction:

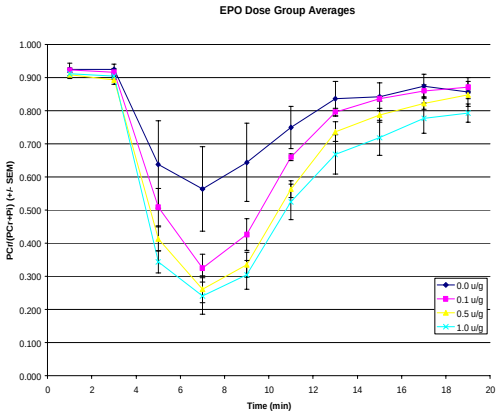
Erythropoietin (EPO) has been shown to be necessary for the growth and proliferation of erythroid cells, and has also been shown to act as a precursor to erythroid progenitor cells. EPO-receptor has been found on non-erythroid cells as well, including endothelial and nerve cells. EPO-receptor has also recently been shown to be expressed on skeletal muscle satellite cells. The presence of the EPO-receptor on differentiating skeletal muscle cells suggests actions beyond that of a hematopoetic agent. Furthermore, the illegal use of EPO as a performance enhancing drug in athletics provides evidence for its beneficial bioenergetic effects. Besides increasing muscle oxygenation through increased hematocrit levels, we tested the hypothesis that erythropoietin improves skeletal muscle bioenergetic performance and increases overall force production capacity in rats.

Methods:

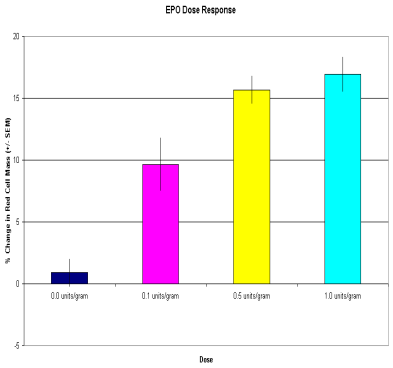
Adult F344 rats were treated with three doses of recombinant EPO: 0.1u/g ; 0.5u/g; 1.0u/g bodyweight (N=6, N=6, N=4 respectively) and compared to saline treated control animals (N=3). Recombinant EPO or saline was delivered subcutaneously by surgically implanted mini Alzet osmotic infusion pumps. Red blood cell mass was measured at days 0, 7, and 14. For electrically stimulated exercise experiments, rats were anesthetized with a 2% isofluorane oxygen mixture and electrodes were placed at the origin and insertion of the left quadriceps muscle. Muscle force was measured using a silk suture string tied around the left ankle and attached to a force transducer. The preload on the quadriceps muscle was standardized to 50g with the leg held at a 90 degree angle. Force was recorded using a PowerLab data acquisition system. Stimulus voltage was set to 120% of the voltage yielding maximal contraction force and ranged between 110-130 volts. Muscle bioenergetics were assessed at baseline, during and following electrically stimulated exercise by ^{31}P NMR spectroscopy performed on a Bruker Biospec 7T/30 NMR spectrometer at 14 days of treatment. ^{31}P spectra were obtained from the quadriceps muscle using a home-built single-tuned elliptical surface coil with one pulse sequence. Excitation was achieved with a 90 degree adiabatic pulse with width 256μs. Data were acquired with sweep width = 50ppm, 4096 complex data points, TR=2s, and 64 averages per spectrum. Two spectra were acquired at baseline, three during stimulation, and five during the recovery period. The ratio of PCr/(PCr + Pi) was compared across dose groups at various time points during the experiment. A total of 3 rats were excluded from the study (2 from the control group and 1 from the maximum dose group) due to an unusually low baseline PCr/(PCr+Pi). Recovery data were fitted to an exponential curve, yielding recovery time constants which were compared across EPO dose groups. Repeated measures ANOVA was applied to force and PCr/(PCr+Pi) data with significance set at $p < 0.05$.

Results:

EPO treated animals exhibited a greater decline in the PCr / (PCr+Pi) ratio during electrically stimulated exercise than saline treated controls ($p < 0.05$), with greater decline in PCr/(PCr+Pi) observed with higher EPO doses during late exercise and early recovery (Figure 1, Table). Red cell mass increased from day 0 to day 14 in a dose dependent manner, as expected (see right Figure 2). Recovery time constants did not differ significantly between groups, except that a trend towards delayed recovery was observed at the highest EPO dose. Force was not affected by EPO treatment.



[PCr/(PCr + Pi)] (mean doses)					
Time (min)	0.0 u/g	0.1 u/g	0.5 u/g	1.0 u/g	*p value*
1	0.924	0.922	0.908	0.911	0.650
3	0.924	0.915	0.893	0.904	0.435
5	0.638	0.509	0.413	0.343	0.047
7	0.564	0.325	0.262	0.241	0.533
9	0.644	0.426	0.335	0.304	0.010
11	0.750	0.660	0.563	0.525	0.004
13	0.836	0.796	0.736	0.668	0.052
15	0.842	0.836	0.786	0.718	0.052
17	0.874	0.859	0.822	0.777	0.142
19	0.856	0.870	0.847	0.793	0.133



Discussion:

Contrary to our hypothesis, EPO did not improve contractile force in rat skeletal muscle. Furthermore, EPO treatment resulted in a negative bioenergetic response. Specifically, the PCr/(PCr+Pi) ratio was lower at late stimulation and early recovery for groups treated with EPO. This detrimental effect increased with increasing the dose of EPO. The PCr/(PCr+Pi) ratio decline induced in a dose-response fashion by EPO, without change in contractile force, might suggest that EPO reduces skeletal muscle efficiency. Several explanations for this unexpected result can be entertained. Although the EPO doses used in this study were comparable to those used in early anemia treatment studies, it is possible that the RCM increase may have directly resulted in sludging and tissue ischemia, or increased oxidative stress. Alternatively, EPO treatment might have induced a switch in muscle fiber-type towards a bioenergetically less efficient phenotype. Finally, it remains possible that EPO treatment results in activation and proliferation of premature myocytes that are neither metabolically efficient nor capable of contributing to contractile force. Histological examinations to characterize cellular morphology, capillary densities and fiber type are currently underway.

References:

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