

# Erythropoietin Potentiates EDHF-Mediated Dilations in Cerebral Arteries

Poster # P19

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## Introduction

Recombinant human erythropoietin (EPO) is neuroprotective when given after hypoxic-ischemic and traumatic CNS injuries.

Previous data suggest EPO may influence cerebrovascular function.

Endothelium-derived hyperpolarizing factor (EDHF) is a major dilating mechanism in cerebral arteries that is endothelium-dependent, does not involve NO or the cyclooxygenase pathway, and is up-regulated after brain injury.

EPO may be neuroprotective by dilating cerebral arteries, or by potentiating EDHF-mediated dilations.

## Hypotheses

Erythropoietin receptor (EPO-R) is present in rat middle cerebral artery (MCA).

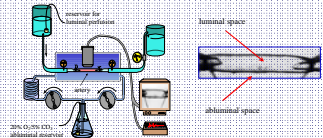
EPO can directly alter the diameter of rat MCA.

EPO can enhance EDHF-mediated dilations.

## Materials & Methods

**EPO-R.** RT-PCR and Western Blotting was performed for EPO-R on rat brain and MCAs.

**Vessel studies.** Isolated rat MCAs were mounted on glass micropipettes, pressurized to 85 mm Hg and luminally perfused at 150  $\mu$ L/min.



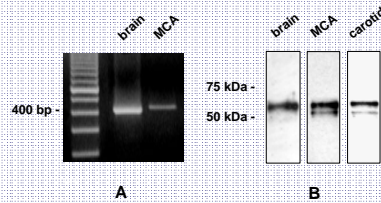
**Direct effects.** EPO was applied in increasing concentrations (0.001 – 10 U/mL) to the vascular smooth muscle (abluminal) or endothelial (luminal) surfaces of perfused MCAs at 15 min intervals.

**Indirect effects.** After treatment with EPO for 30 min *in vitro* or for 24 hours *in vivo*, endothelium-mediated dilations were elicited by applying UTP luminally. EDHF-mediated dilations were elicited by luminal UTP in the presence of L-NAME and indomethacin.

**EPO levels.** Serum EPO levels were measured by ELISA.

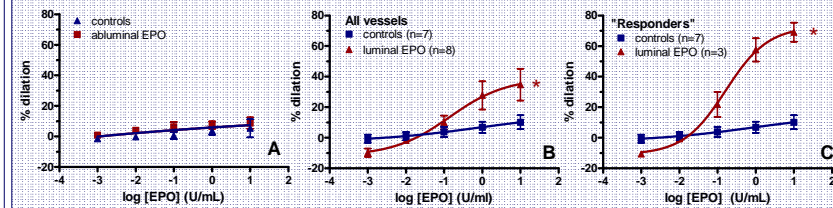
## Results

### I. EPO-R in rat MCA



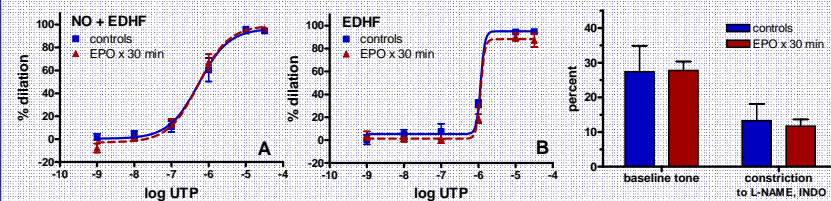
(A) Using RT-PCR, mRNA for EPO-R was identified in brain and MCA as an amplified sequence of ~ 400 bp. A 100 bp ladder is included for reference. (B) In Western blots for EPO-R, ~ 60 kDa bands were identified in rat brain, MCA, and carotid artery.

### II. Direct vasomotor effects of EPO



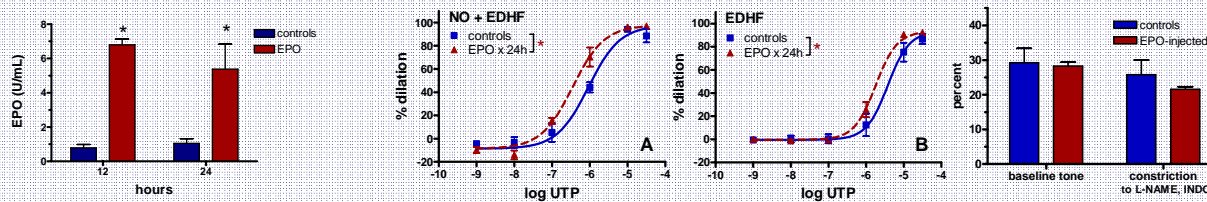
(A) Application of EPO to the vascular smooth muscle of rat MCAs did not alter diameter. (B) Application of EPO to the endothelium dilated vessels ( $p = 0.002$ ). (C) Of 8 MCAs from the EPO group in (B), 3 dilated markedly (shown,  $p < 0.0001$ ) while 5 did not (not shown). "Responders" did not differ from "non-responders" in initial myogenic tone or endothelial function, as confirmed by luminal application of  $10^{-5}$  M UTP at the end of each experiment. Means  $\pm$  SEMs are plotted.

### III. Effect of EPO x 30 min on endothelium-mediated dilations



Vessels were pre-treated luminally with EPO 1 U/mL for 30 min *in vitro*. (A) Endothelium-mediated dilations were not altered. (B) In the presence of L-NAME & indomethacin, EDHF-mediated dilations were not altered. (C) Tone after 30-min of EPO and constrictions due to L-NAME & indomethacin did not differ between groups. Means  $\pm$  SEMs are plotted.

### IV. Effect of EPO x 24 hrs on endothelium-mediated dilations



After injecting animals with 1,000 U/kg EPO s.c., serum EPO levels reached  $6.80 \pm 0.19$  U/mL at 12 hours (vs.  $0.79 \pm 0.12$  U/mL in controls,  $p < 0.001$ ) and  $5.38 \pm 0.84$  U/mL at 24 hours (vs.  $1.05 \pm 0.16$  in controls,  $p < 0.001$ ). Mean  $\pm$  SEM are plotted.

Animals were injected with 1,000 U/kg EPO or saline s.c. 24 hours prior. (A) Endothelium-mediated dilations were potentiated in the EPO group, as the  $EC_{50}$  decreased 2.6-fold ( $p = 0.008$ ). (B) EDHF-mediated dilations were also potentiated, as the  $EC_{50}$  decreased 2.1-fold ( $p = 0.001$ ). (C) Baseline tones and constrictions due to L-NAME & indomethacin did not differ between groups. Means  $\pm$  SEMs are plotted.

## Conclusions

1. EPO-R is present in rat MCA.
2. EPO can cause dilation of rat MCAs via the endothelium, though the response in variable. The reason for this heterogeneity unclear.
3. EPO administered for 24 hours *in vivo* potentiates endothelium-mediated, and specifically, EDHF-mediated dilations. EPO administered for 30 min *in vitro* does not potentiate either.

## Discussion

EPO could render cerebral arteries more responsive to endogenous dilators, or less responsive to constrictors, after injury. For instance, EDHF becomes up-regulated after brain injury while NO bioavailability is reduced; EPO could potentiate EDHF and NO in this context.

In our studies, direct and potentiated dilations caused by EPO occurred within an EPO concentration range observed physiologically and in previous neuroprotection studies. As EPO augments oxygen-carrying capacity, it seems befitting that EPO would also enhance dilations of cerebral arteries, promoting blood flow to vital, oxygen-sensitive organs such as the brain.

## Acknowledgements

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