ERYTHROPOIETIN INHIBTS THE RELEASE, BUT NOT SYNTHESIS, OF CORTICOTROPIN-RELEASING HORMONE FROM RAT HYPOTHALAMIC EXPLANTS *IN VITRO*

Tringali Giuseppe, Lisi Lucia, Pozzoli Giacomo, Navarra Pierluigi

Institute of Pharmacology, Catholic University Medical School, Rome, Italy

Recent evidence indicates that erythropoietin (Epo) exerts a number of biological actions within the central nervous system (CNS) that are not related to its known effect on erythropoiesis; receptors mediating CNS effects might belong to a different subgroup compared to those mediating erythropoietic activity at the peripheral level. Epo has been identified as neurotrophic and neuroprotective agent in several in vivo and in vitro experimental models of brain damage. In particular, Epo and Epo receptors are expressed in neural tissues, and they undergo upregulation during hypoxia. The latter also activates Hypothalamic-Pituitary-Adrenal axis and stimulates corticotropin-releasing hormone (CRH) release and CRH mRNA expression in rat hypothalamus. Therefore in this study we used rat hypothalamic explants as an in vitro model to investigate whether EPO can directly modulate CRH release.

The effect on CRH production and release were evaluated by radioimmunoassay (RIA) measurement of the peptide in the incubation medium as well as in the tissue, and by RNA analysis of CRH gene expression by RNase protection assay. EPO activity was investigated in short-term (1-3h) experiments under basal conditions or after stimulation with a depolarizing solution (56 mM KCl) or with veratridine (10 μM); the latter depolarizes target cells primarily by activating sodium channels. Furthermore, CRH mRNA expression was investigated in hypothalamic explants exposed to EPO alone or EPO in the presence of 56 mM KCl or veratridine. EPO induced a reduction in CRH basal release that was statistically significant at 1 and 10 μM. EPO was also able to inhibit KCl as well as veratridine-stimulated CRH release. Interestingly, the decreases in released CRH was accompanied by parallel increases in intracellular CRH content, both changes occurring in a statistically significant difference compared to controls. EPO was also able to reverse the decrease in intracellular CRH content induced by depolarizing solutions. Further experiments showed that EPO does not modify CRH gene expression in hypothalamic explants, either in the absence or in the presence of KCl or veratridine. In this paradigm, KCl and veratridine *per se* failed to modify CRH gene expression. In conclusion, our findings show that EPO reduces both basal and stimulated CRH release from rat hypothalamic explants without affecting CRH gene expression, seemingly by interfering with ion-mediated secretion mechanisms.