VISUALIZATION OF IGF-2 RECEPTORS IN RAT BRAIN

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The notion that trophic factors are present in the brain and serve to maintain neurons, promote target tissue innervation, and stimulate synaptogenesis has been a well-accepted theory for some time. The transport of trophic factors to neuronal endings has been a favorite explanation for the axonal flow of materials in neurons which was first discovered by Weiss in 1948. More recently, reports of trophic effects of NGF on lesioned CNS neurons and evidence suggesting that insulin and the somatomedins are present in the CNS encouraged us to explore the localization of their receptors. In the experiments described here, we used iodinated human insulin-like growth factor-2 and autoradiography to visualize the receptors for this peptide in rat brain.

Human IGF-2 (hIGF-2) was prepared through recombinant DNA technology. The cloned material was expressed as a fused gene product with the tryp LE4 operon in E. coli. After purification, the amino acid sequence, disulfide bond formation, and secondary structure were confirmed by amino acid analyses, N-terminal amino acid sequencing, and fast atom bombardment of fragments prepared by pepsin digestion. Biological activity and potency were evaluated using a competitive serum protein binding assay and evaluation of the potency to stimulate amino acid transport in human fibroblasts. In that assay, hIGF-2 had an ED50 of 2-3 ng/ml and was roughly equipotent with human IGF-1. Human insulin was approximately 10-fold less potent than either of the somatomedins in this assay.

Brains were removed from freshly decapitated rats and rapidly frozen in liquid 4% carboxymethyl cellulose by immersion in isopentane cooled with an acetone dry-ice bath. Serial sections of these frozen rat brains were thaw-mounted onto slides and stored under vacuum at 4°C overnight. The dehydrated sections were either stained with thionine or incubated with 125I-IGF-2 (10 pM) for 16 hrs at 4°C. The specificity of IGF-2 binding was assessed by incubating adjacent sections with labeled IGF-2 in the presence of human insulin (80 nM) or hIGF-2 (80 nM). The assays were terminated by three brief washes in ice-cold buffer. After drying, the slices were exposed to X-ray film for 4-7 days at 4°C.

Autoradiographs of IGF-2 receptors showed that these sites were present in many brain regions but were also discretely localized. The most striking observation was the presence of receptors in hippocampal CA1-CA4 regions and the dentate gyrus (Fig. 1A). Labeling in the CA3 and CA4 areas was consistently heavier than in the CA1 and CA2 regions and was present over neuronal elements. Other labeled areas included the entorhinal cortex, which projects to the hippocampus, and the deep layers of the cortex. Incubation in the presence of...
Figure 1A. Autoradiograph of dorsal hippocampus of rat brain labeled with $^{125}$I-hIGF-2.

Figure 1B. Autoradiograph of dorsal hippocampus of rat brain in the presence of excess unlabeled hIGF-2.

Figure 2A. Autoradiograph of cerebellum of rat brain labeled with $^{125}$I-hIGF-2.