COMPLEMENTARY ROLES OF THE INSULIN FAMILY OF FACTORS AND RECEPTORS IN EARLY DEVELOPMENT AND NEUROGENESIS.

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Insulin and its related insulin-like growth factor I and II (IGFs) are multifunctional polypeptidic factors signalling through membrane tyrosine kinase receptors, the insulin receptor and the IGF-I receptor. While in vertebrates the IGFs are locally synthesized in multiple tissues during development, acting in paracrine fashion, insulin is highly expressed in the differentiated pancreas and serves a classic endocrine role as metabolic regulator.

We have proposed that in early development insulin and/or its precursor proinsulin acts as well in autocrine/paracrine form to stimulate proliferation and differentiation, either directly or indirectly through the support of cell survival. In the chick embryo (E), the temporal expression of preproinsulin mRNA in two model systems, the neurulating embryo and the neuroretina, is complementary to the expression of IGF-I mRNA. In contrast to the expression of the mRNAs for the factors, highly developmentally regulated, both the insulin receptor and the IGF-I receptor mRNAs are expressed at all ages in similar levels.

There is prepancreatic expression of preproinsulin mRNA in the chick embryo during gastrulation (E0.5) and neurulation (E1-E2) until pancreatic organogenesis (E2.5). IGF-I mRNA expression, however, is not detectable by either RT-PCR (Figure 1) or in situ hybridization until E2-E3. At least the 10 somites embryo (E1.5) in culture is able to translate the preproinsulin mRNA into a secreted product recognized by anti-insulin antibodies (which also crossreact with chicken proinsulin). When insulin is added to this cultured whole embryo, DNA synthesis is stimulated and cell death by apoptosis is reduced. To further characterize the role of autocrine/paracrine acting insulin on cell survival and/or proliferation, we have used an antisense oligonucleotide approach for interference experiments. The addition of preproinsulin antisense oligonucleotides, but not that of sense or random oligonucleotides, to E1.5 in culture, increased the percent of cells that undergo apoptosis, detected by DNA fragmentation revealed by DAPI-staining and TUNEL labelling. This effect is reversed by the co-addition of exogenous chicken insulin. Similarly, antisense oligonucleotides designed for the chicken insulin receptor, applied in ovo, increased the naturally occuring cell death that affects the 10-15 somites embryo. This early embryonic apoptosis is now being characterized in regional and cell subtype specific patterns.

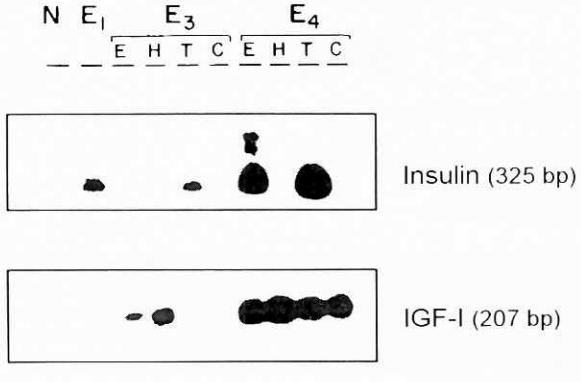

Figure 1. Analysis of preproinsulin and IGF-I gene expression by Southern hybridization of RT-PCR products. Total RNA from whole embryos (E1) and from sectioned E3 and E4, head (H), trunk (T) and caudal regions(C) were used for reverse transcription and 40 cycles amplification with specific chicken primers. Hybridization was performed with nested labelled oligonucleotides corresponding to insulin and IGF-I sequences. N= no RNA template. (Reproduced from Pérez-Villamil et al., 1994)

In the neuroretina, expression of preproinsulin mRNA also precedes expression of IGF-I mRNA (Figure 2). In the largely proliferative retina at E4-E6, there is predominant presence of preproinsulin mRNA. In the mostly differentiative retina at E9 and later, predominates the expression of IGF-I mRNA. At stages of synaptic maturation, E17-E20, there is a second wave of preproinsulin mRNA expression in neuroretina.

Characteristically, as in the neurulating embryo, mRNA expression for both the insulin receptor and the IGF-I receptor is widespread and shows little temporal regulation. Interestingly, however, there is high regulation of receptors at the protein level. In E6 retina, ligand-binding and cross-linking experiments revealed two types of receptors, one type with high affinity and specificities typical of an IGF-I receptor, and an atypical high affinity - low discriminating receptor with near equal affinity for insulin and IGF-I, and slightly less for proinsulin. This atypical receptor is no longer present in E12 neuroretina, when a population of more classic insulin receptors coexists with a population of IGF-I receptors. We characterized by HPLC and radioimmunoassay the relative content of peptides of the family in vitreous humor, in contact with the neuroretina. In E6-E8, there is a significant amount of a polypeptide with the characteristics of proinsulin which, at least in part, is the product of neuroretina biosynthesis and secretion. At much lower concentrations, there is also IGF-I in vitreous humor, which in E6 may be principally the product of the mRNA found in pigmented retina (including the cilliary processes) (Figure 2). Thus, this IGF-I could indeed be activating the neuroretina IGF-I typical receptor as well as the atypical receptor, while the latter could be activated in addition by locally synthesized proinsulin/insulin.

In organoculture of E5-E6 neuroretina, all proinsulin, insulin and IGF-I at 10^{-8} M estimulate similarly DNA and protein synthesis. Proinsulin is slightly less potent than insulin and IGF-I in promoting neural differentiation. These effects on proliferation and differentiation may be in part the consequence of a cell survival action of the peptides. Insulin indeed decreases the percent of apoptotic cells caused by deprivation of factors when the retina is placed in organotypic culture in defined medium. Detailed ongoing studies on the effect of insulin and IGF-I on the cell cycle and subpopulations of neural precursors should help elucidate the role of the factors of this family in the cellular processes involved in early neurogenesis. Eventually, it may become possible to understand their developmental complementary expression and regulation.

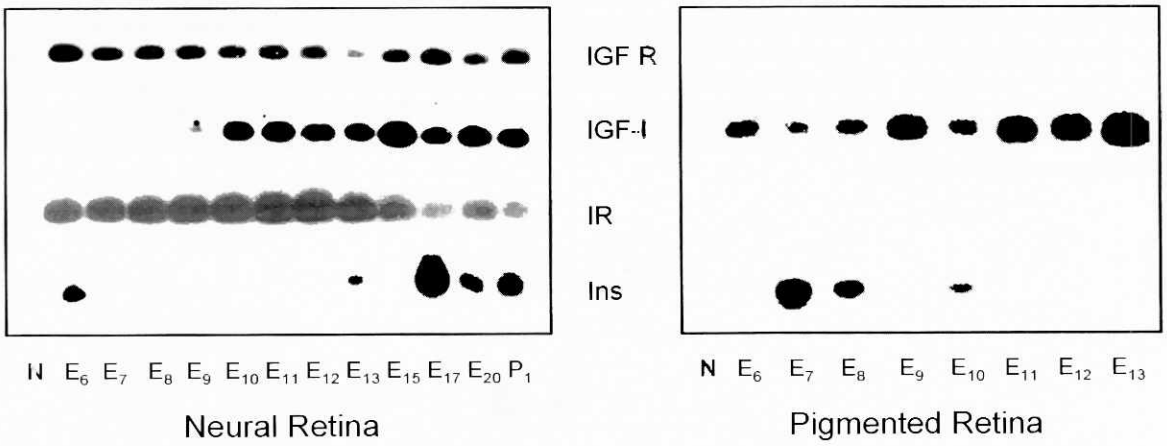

Figure 2: Analysis of gene expression by Southern hybridization of RT-PCR products from developing retina. Total RNA from neural retina of the embryonic (E) and postnatal (P) ages indicated (in days) were used for reverse transcription and 35 to 40 cycles (depending on the gene) amplification with specific chicken primers for IGF-I receptor (IGFR, 197 bp), IGF-I (215 bp), insulin receptor (IR, 244bp) and insulin (Ins, 325bp). Total RNA from pigmented retina of the embryonic ages indicated was similarly used in a RT-PCR for IGF-I and insulin. Hybridization was performed with nested labelled oligonucleotides correspondiing to each gene.

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