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Insulin-like growth factor 1-receptor (IGF-1R) in developing and adult human dorsal root ganglia

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Summary

Insulin-like growth factors (IGFs) promote neurite outgrowth in cultured sensory neurons that binding to IGF type 1 receptor (IGF-1R) and seem to be involved in the pathogenesis of some metabolic and toxic peripheral neuropathies coursing with altered sensitivity. However, the distribution of IGF-1R in the human sensory peripheral nervous system is unknown. In this study, we used light immunohistochemistry to analyse the occurrence and localization of IGF-1R in developing (6 to 22 weeks of estimated gestational age, e.g.a.) and adult (age range 25-41 years) human dorsal root ganglia (DRG). In human embryos (6-8 weeks e.g.a.) and young foetuses (9 weeks e.g.a.), most neurons (84%, 92%, and 83%, respectively) displayed IGF-1R immunoreactivity (IR). In older foetuses (12 and 22 weeks e.g.a.), the number of immunoreactive neurons decreased progressively (78 and 68%, respectively) reaching values similar to those observed in adults (64%). The subpopulation of adult primary sensory neurons showing IGF-1R IR covered the entire size range, but the neurons were mainly small. Furthermore, in adults all satellite glial cells and Schwann cells were immunoreactive. The present results suggest a role for IGF-1R in the differentiation and maturation of primary sensory neurons, and in the maintenance of a subset of them in adulthood, as well as in the control of peripheral glial cells (Schwann and satellite glial cells). These findings might serve as a basis for future studies in pathologic DRG, in which IGF-1R or its ligands may be involved.

Key Words: Dorsal root ganglia – Primary sensory neurons – Satellite glial cells – Insulin-like growth factor type 1 receptor – Human

Introduction

The development and maintenance of primary sensory neurons in mammalian dorsal root ganglia (DRG) are under the control of a series of molecules with neurotrophic properties, mainly the members of the neurotrophin family (Barbacid, 1995; Fariñas, 1999). In recent years, evidence has been accumulated that insulin-like growth factors, IGF-1 and IGF-2, also provide neurotrophic support for neurons and non-neuronal cells of the peripheral nervous system (Baker et al., 1993; Le Roith et al., 1995; Stewart and Rotwein, 1996; Feldman et al., 1997).

IGFs act on responsive cells through a transmembrane glycoprotein signalling-receptor called IGF type 1 receptor (IGF-1R; see Le Roith et al., 1995), a heterotetrameric protein (α_2-β_2) that shares a high degree of homology with the insulin receptor (White and Kahn, 1994; Ishii, 1995). The extracellular domain (α subunit) binds IGFs with low affinity, whereas the intracellular one has tyrosine kinase activity (Ullrich et al., 1986). IGF-1R controls a broad spectrum of actions during growth and differentiation of the nervous system in vertebrates (see De Pablo and de la Rosa, 1995), although its role in the development of the peripheral nervous system is still obscure (Jones and Clemmont, 1995). Despite this, it has been demonstrated that IGFs stimulate neurite outgrowth of cultured developing and adult sensory neurons (Recio-Pinto et al., 1986; Fernyhough et al., 1993), especially during regeneration of peripheral nerves (Ishii, 1995; Pu et al., 1995; Akahori and Horie, 1997), and induce myelin formation (Mozell and McMorris, 1991; Barres et al., 1992; Roth, 1995). Furthermore, recent studies have shown that IFG-1 is involved in the pathogenesis of diabetic neuropathy (see Ishii, 1995; Bitar et al., 1997), drug-induced

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peripheral neuropathy (Apfel et al., 1993; Contreras et al., 1997), in the promotion of myelin regeneration during experimental autoimmune encephalomyelitis (Yao et al., 1995; Cannella et al., 2000), and probably also in some other neurodegenerative disorders (De Keyser et al., 1994). Finally, IGFs have been used successfully in the experimental treatment of different neuropathies with sensory dysfunction (Barinaga, 1994; Schäztl, 1995). However, no data are available in humans during development or in adulthood about the expression of IGF-1R in DRG. This study was therefore designed to analyse the occurrence and distribution of IGF-1R in developing and adult human DRG using immunohistochemistry. The results could provide information about the role of the IGFs/IGF-1R complex in developing sensory neurons and are of potential clinical relevance since they might serve as a basis for future studies in peripheral neuropathies involving DRG neurons and/or sensory nerve fibres.

Materials and Methods

Treatment of tissues

Lumbar human DRG (n=14) were obtained during the removal of organs for transplantation from 8 adult men (age range 25-41 years) who had died in traffic accidents. The pieces were obtained in compliance with Spanish Laws for brain-dead subjects and organ transplantation (Law 30/79 and Royal Decree 426/80), and according to the recommendations published in the Declaration of Helsinki II. The pieces were fixed in 10% formaldehyde in 0.1M phosphate buffer saline (pH 7.4), dehydrated, paraffin-embedded and processed for immunohistochemistry.

DRG from seven human embryos were also studied. These were obtained through collaboration with obstetricians and were products of spontaneously (n=3) or artificially (n=4) interrupted pregnancies in compliance with Spanish Laws. The external features of embryos were examined under a binocular dissecting microscope, and the developmental stage was determined. The estimated gestational ages (e.g.a.) were 6 (n=2), 8 (n=2), 9, 12 and 22 weeks. Specimens were collected in ice-cold physiological solution and the lumbo-sacral region was dissected and fixed for 28 h with Bouin´s fixative. Finally, pieces were dehydrated and embedded in paraffin.

Immunohistochemistry

Sections from adult DRG and whole embryos were cut at 10 µm thick, mounted on gelatine-coated slides and processed for immunohistochemistry. Briefly, deparaffinized sections were washed in Tris-HCl buffer (0.01M, pH 7.5) containing 0.1% Triton X-100 (Sigma) and 0.1% bovine serum albumin. Thereafter sections were incubated overnight at 4° C in a humid chamber with a mouse monoclonal antibody (clone αIR3, Oncogene Science; Flier and Moses, 1985) diluted 5 µg/ml. This antibody maps within the α-subunit of IGF-1R. After washing, sections were incubated for 1 h at room temperature with peroxidase-labelled anti-mouse IgG (Ammersham, diluted 1:100), and finally the antigen-antibody reaction was visualized using 3-3´ diaminobenzidine (Sigma) as chromogen.

For control purposes, representative sections were processed in the same way using a non-immune mouse serum instead of the primary antibody or omitting incubation with the primary antibody. Under these conditions, no specific immunoreactivity was observed. As positive controls, some sections were incubated with mouse monoclonal antibodies against the 200 kDa neurofilament protein subunit (clone NE 14, Sigma, diluted 2 µg/ml) to label nerve cell bodies, or mouse monoclonal antibody against S-100 protein (clone 14E2E2, Boehringer- Mannheim, dilution 5 µg/ml) to label satellite glial and Schwann cells.

Quantitative analysis

The percentages of DRG nerve cell bodies showing IGF-1R IR, and the intensity of immunostaining were evaluated in paraffin-embedded sections using an image analysis system (MIP system, Servicio de Analysis de Imagines, Universidad de Oviedo), as reported previously (Vega et al., 1994). In adult DRG the relationship between neuron cell body-size and the intensity of immunostaining was also evaluated. The total number (100%) of nerve cell bodies was determined by counting neurons displaying IR for 200 kDa neurofilament proteins. Three sections per ganglion (100 µm apart to avoid counting the same neuron twice) were studied, evaluating three randomly-selected fields per section. The total number of measured neurons is shown in table 1. To evaluate neuron size, the mean diameter of neuronal profiles was measured in neurons showing apparent nuclei. Neurons were divided into 3 classes, referred to here as small (≤ 20 µm), intermediate (21-50 µm) and large (≥ 51 µm). The intensity or immunostaining was measured in arbitrary units of grey-level ranging from 1 (black) to 256 (white). On the basis of this criterion, neurons were divided into four groups (64 arbitrary units of grey level each) referred to in the text as strong, high, intermediate and low intensity of immunostaining. Nerve cell bodies showing values higher than 237 (which was the mean value of the background measured in control sections) were considered as unreactive. The results obtained from embryos and foetuses are expressed as percentages of

labelled neuronal profiles according to the intensity of immunostaining; while in adults the immunoreactive neurons were divided up on the basis of their size and the intensity of the immunoreactivity (see for details Vega et al., 1994).

Results

Identification of developing DRG, especially in embryos and young foetuses, was accomplished on the basis of their localization and IR for the 200kDa neurofilament protein (Fig. 1A). Light immunohistochemistry revealed specific immunolabelling for IGF-1R in both developing and adult human DRG. At 6 and 8 weeks e.g.a., the percentages of immunoreactive nerve cell profiles was 84% and 92%, respectively (Fig. 1 B and C; Table 1), with an intensity of immunostaining considered as strong or high (Table 1). At 9 weeks e.g.a., the percentage of immunolabelled nerve cell-body profiles was similar to that of the embryonic period although overall the intensity of immunostaining decreased (Table 1). At 12 weeks e.g.a., a reduction of about 5-10% in the percentage of neurons expressing IGF-1R with respect to the early developmental stages (Table 1), as well as in the intensity of immunostaining (Fig. 1D; Table 1) was observed. Finally, by 22 weeks e.g.a., 68 % of nerve cell bodies expressed IR, the intensity of IR being similar to that found in 12 weeks old foetuses (Fig.1E). It was not possible to confirm the presence of IGF-1R IR in satellite glial cells of DRG at all embryonic and foetal times analysed.

IGF-1R IR in adult DRG was distributed in both neurons and satellite glial cells, as well as in the Schwann cells ensheathing the intraganglionic axons (Figs. 2A and 2B). The cytoplasmic immunostaining in neurons showed a very faint

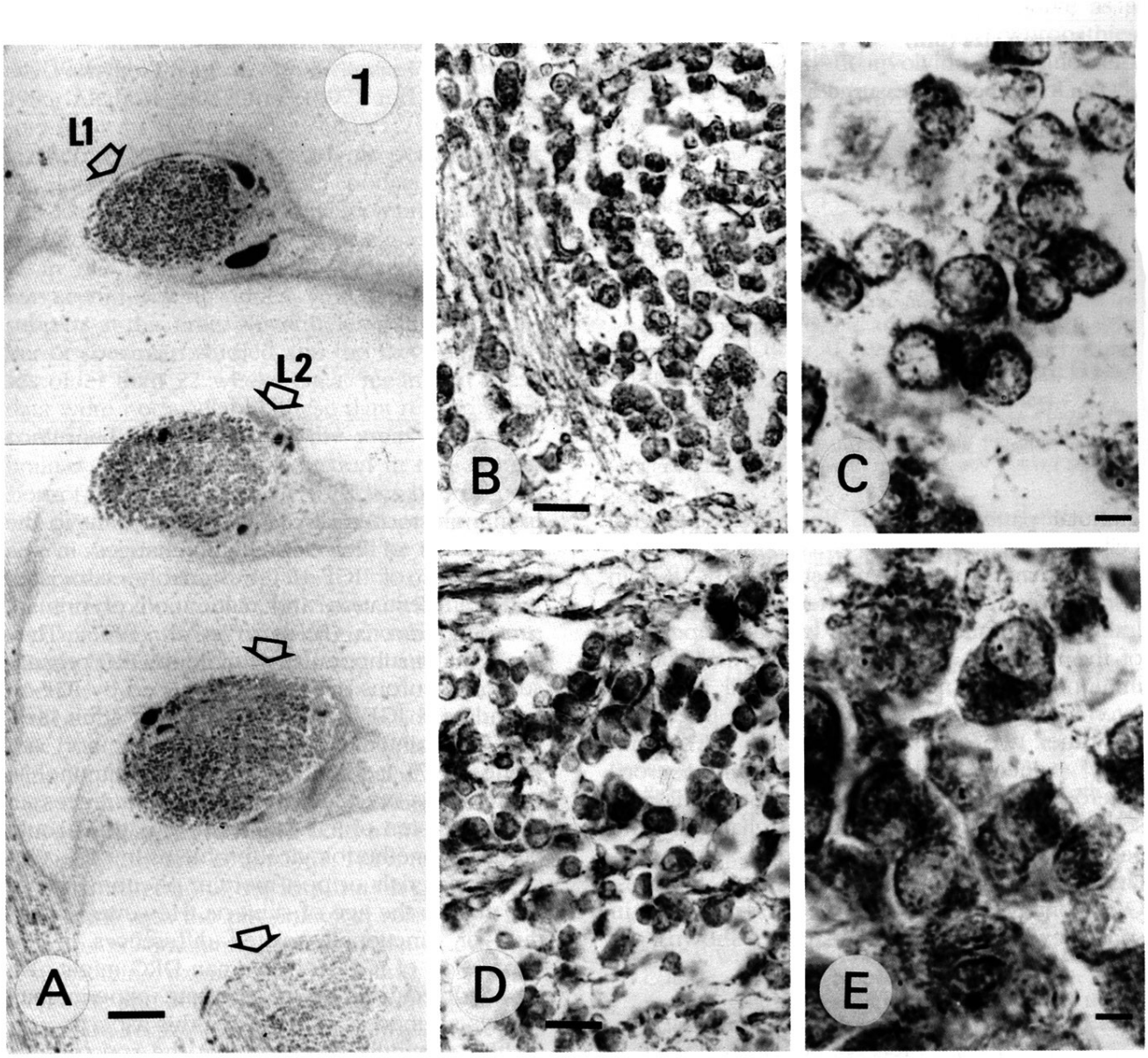

Fig. 1.- Localization of DRG in an embryo of 8 weeks of estimated gestational age using a monoclonal antibody against the 200 kDa neurofilament protein (**A**; scale bar =50 μm). Light immunohistochemistry demonstrated IGF-1R 1R in most of the nerve cell profiles of human embryos (**B**: 6 weeks e.g.a.; scale bar = 40 μm. **C**: 8 weeks e.g.a.; scale bar = 8 μm) and a time-dependent reduction in the percentage of immunoreactive neurons by 12 weeks e.g.a. (**D**; scale bar = 40 μm) to 22 weeks e.g.a. (**E**; scale bar = 8 μm).

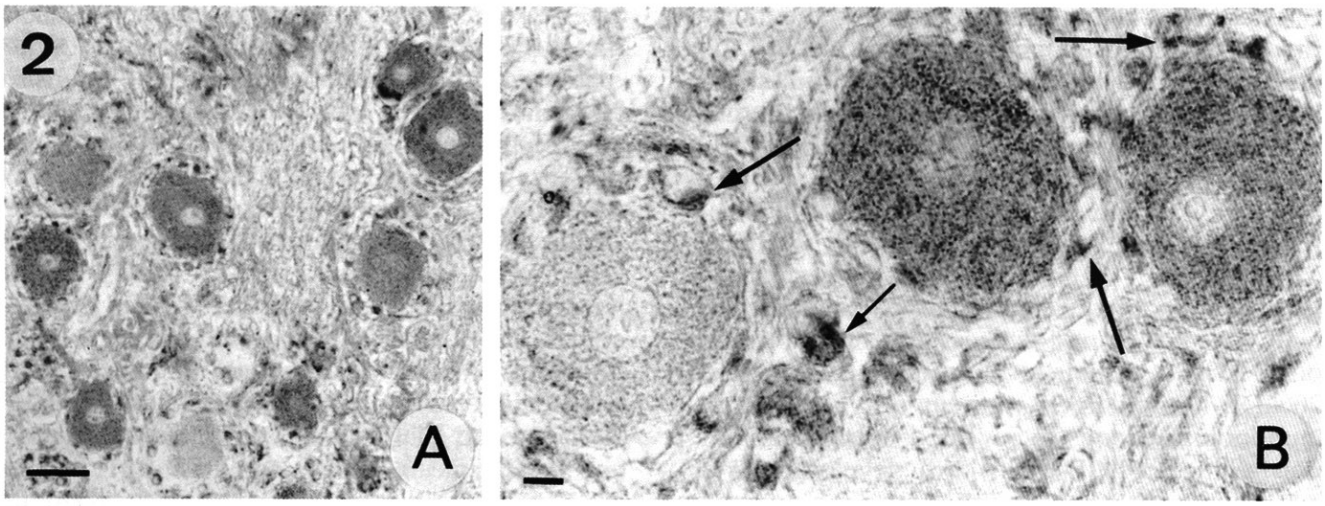

Fig. 2.- Immunohistochemical localization of IGFr-I in adult human DRG. Specific immunoreactivity was localized in a neuronal subpopulation (64%) as well as in the satellite glial cells (arrows in B). Scale bar = 40 μm in A; 5 μm in B.

Table 1.- Percentage of primary sensory neurons displaying IGF- 1R immunoreactivity divided on the basis of their intensity of immunostaining.

Neuron size (μm) n = number of measured neurons	% of IR Neurons	% of no IR Neurons	Intensity of IR S	H	I	L
Embryos						
6 weeks e.g.a. (n = 402)	84	16	38	46	—	—
8 weeks e.g.a. (n = 417)	92	8	53	28	11	—
Foetuses						
9 weeks e.g.a. (n = 483)	83	17	19	41	17	6
12 weeks e.g.a. (n = 532)	78	22	12	38	21	7
22 weeks e.g.a. (n = 437)	68	32	18	37	10	3
Adults (n = 390)	64	36				
Small	41	12	19	12	10	—
Intermediate	16	22	5	9	2	—
Large	7	2	3	2	1	1

S: strong; H: high; I: intermediate; L: low.

granular pattern, whereas that of satellite glial cells and Schwann cells was rather homogeneous. Image analysis demonstrated IGF-1R IR in approximately 64% of the neuron profiles, the intensity of immunostaining being independent of the neuronal size. Nevertheless, most of the immunolabelled neurons were small (41%) or intermediate (16%) in size (Table 1). Regarding the other two antibodies assessed, NE14 antibody stained developing and adult neurons heterogeneously, while S-100 protein IR was selectively found in the satellite glial cells and Schwann cells of adult DRG and in a subpopulation of glial cells in embryos and foetuses. Occasionally, S100 protein IR was observed in some developing neurons (data not shown).

Discussion

The present study was undertaken to analyse the occurrence and distribution of IGF-1R in human DRG during development and adulthood using immunohistochemistry. The results point to the occurrence of developmental changes in the expression of IGF-1R presumably associated with differentiation and maturation of primary sensory neurons (Feldman et al., 1997). They show that a subpopulation of adult DRG primary sensory neurons might be influenced by IGF-1R ligands, i.e. IGF-1 and IGF-2, during adult lifespan for maintenance and survival (Akahori and Horie, 1997). It should be noted that immunohistochemistry revealed individual differences in the expression of IGF-1R. These variations could be due to methodological problems in collection of the materials or post-mortem phenomena rather than to the age of subjects. However, individual or functionally-related differences in the expression of IGF-1R by human DRG might also be involved, and more accurate experimental studies are necessary to clarify this aspect.

It is generally accepted that the spectrum of biological actions of growth factors is determined by the site and extent of the expression of the corresponding receptors. Therefore, the

occurrence of IGF-1R in a cell could consequently be regarded as indirect evidence for a role of its ligands in such cells. The presence and distribution of IGF-1R in the central nervous system of vertebrates, including man (Adem et al., 1989; Bondy et al., 1992; De Pablo and de la Rosa, 1995; Le Roith et al., 1993; Pomerance et al., 1988), and its developmental regulation (for ref., see De Pablo and de la Rosa, 1995; Le Roith et al., 1993) have been previously reported. Conversely, only scarce information has been made available about the presence of these receptors in the peripheral nervous system (Karagiannis et al., 1997; Feldman et al., 1997; Akahori and Horie, 1997). The present data offer the first report concerning the distribution of IGF-1R in developing and adult human DRG, although their presence at mRNA level has been recently demonstrated in developing chicken DRG (Holzenberger et al., 1996). Nevertheless, in vitro studies lend support to our results because IGFs are able to stimulate neurite outgrowth in cultured rat (Fernyhough et al., 1993; Recio-Pinto et al., 1986; Akahori and Horie, 1997) and avian sensory neurons (León et al., 1995).

By 6 to 12 weeks e.g.a. a large percentage of developing DRG nerve cell bodies displayed IGF-1R IR. Then, when development has progressed from 12 to 22 weeks of e.g.a., a decrease in the number of immunoreactive neurons was seen, reaching values close to those of adult subjects at this time. Nevertheless, the low number of specimen studied and the fact that foetuses older than 22 weeks e.g.a. mean that these data were not available, these data remain to be confirmed, although in some areas of the rat brain a developmental reduction in the expression of the α subunit of IGF-1R has been reported (Pons et al., 1991). Taken together, these results suggest that IGF-1R would be involved in the proliferation and differentiation of DGR and these receptors could participate in the survival and maintenance of a neuronal subpopulation during later developmental stages and in adulthood.

The distribution of IGF-IR in adult human DRG, i.e., neurons, satellite glial cells and intraganglionic Schwann cells, is in good agreement with those found in cultures where high levels of IGF-1R mRNA were found both in neuronal and glial cells (Shemer et al., 1987; 1989; Syroid et al., 1999). Furthermore, the localization of IGF-IR is consistent with the physiological role of IGFs in neurons in stimulating the induction of cytoskeletal proteins, axonic outgrowth and, in satellite glial cells and Schwann cells, in inducing myelin formation (Barres et al., 1992; Fernyhoug et al., 1993; Mozell and McMorris, 1991; Pu et al., 1995; Roth et al., 1995; Svenningsen and Kanje, 1996). Nevertheless, the function of IGF-1R and their ligands in the developing and adult peripheral sensory neurons and glial cells remains largely unknown (see Syroid et al., 1999).

The IGF system (Jones and Clemmonds, 1995) has been implicated in the pathogenesis of some neuropathological disorders (Contreras et al., 1997; Bitar et al., 1997; Cannella et al., 2000), especially diabetic neuropathy (Ishii, 1995; Schäztl, 1995; Yao et al., 1995). The present findings strongly suggest that in addition to the nerve fibres, the cell bodies and satellite glial cells of DGR, could be also be affected in these diseases because they express IGF-1R (Ishii, 1995). In our study most of the neurons displaying IGF-1R IR were small and intermediate in size. Such neurons are the origin of a broad spectrum of nerve fibres, especially C and Aβδ (Perl, 1992), which are those mainly affected in human diabetic neuropathy (Apfel et al., 1994). Although further studies are necessary to clarify the function of the IGF system in the peripheral nervous system present data might serve as a basis to study peripheral sensory neuropathies related to IGFs/IGF-IR involving DRG and sensory nerve fibres (Barinaga, 1994; Schäztl, 1995; Doré et al., 1997).

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