p75 and TRK ONCOPROTEINS EXPRESSION IS DEVELOPMENTALLY REGULATED IN THE INNER EAR OF HUMAN EMBRYOS.

Esther VAZQUEZ [1], Isabel SAN JOSÉ [1], Javier NAVES [2], Jose Antonio VEGA [2] and Juan REPRESA[1]
[1] *Instituto de Biología y Genética Molecular Universidad de Valladolid-C.S.I.C,(Departamentos de Anatomía y Fisiología y Bioquímica), C/Ramón y Cajal 7, Valladolid 47005, Spain.* [2] *Departamento de Morfologia y Biologia Celular, C/Julian Clavería. Universidad de Oviedo, Oviedo, Spain.*

Embryonic development of human inner ear starts with the formation of the otic placode which forms the otic vesicle and the neurons of the cochleovestibular ganglion (CVG). The otic vesicle subsequently differentiates into a complex membranous labyrinth that contains the sensory epithelium of the inner ear and highly specific networks of neuronal connections between the epithelia and the central nervous system (Dechesne,1992). Several evidences suggests that cochlear and vestibular neurons of vertebrates such as chick, mouse and rat, require neurotrophic factors which are released from their natural targets in order to survive and differentiate during development (Van De Water et al; 1992; Ylikoski et al, 1993). From "in vitro" experiments and analysis of null mutant mice, the NGF family of neurotrophins emerges as molecules which are required for the inner ear innervation (Bianchi et al, 1996). However to date no information is available for human development.The NGF family of neurotrophins, which includes NGF, brain-derived neurotrophic factor (BDNF) and neurotrophins 3 and 4 (NT3, NT4), are a structurally homologous group of polypeptides that share about 60% of their aminoacid identity and have similar biological effects (Bothwell et al, 1996).

Two structural unrelated classes of receptors have been characterized for neurotrophins, the p75 that serves as the common low affinity receptor for all of them and the products of *trk* protooncogene family that serve as the high affinity receptors (Barbacid,1994). The present study was conducted to determine the expression of TrkA,TrkB,TrkC and p75 proteins in the human inner ear throughout development, and to assess the role of neurotrophins in the development of auditory and vestibular specific innervation in man.

The material studied comprised 32 human embryos and fetuses obtained from legally approved therapeutic abortions and spontaneous abortions. Informed consent was obtained according to the guidelines of Helsinki Declaration II. The aborted embryos and fetuses were subjected to autopsy, where radiograph, organ histology and chromosome examination were include. Only normal embryos were selected. The crown-rump length (CRL) of the specimens studied ranged from 10 to 220 mm, corresponding to 5 to 24 weeks of gestation (ovulation age). Tissue samples for this study were both the entire temporal primordia and isolated CVGs obtained by the microdissection of the inner ear primordia, which were analysed by using both Western blots and immunocytochemistry on frozen sections by standard techniques. Immunodetection was carried out using affinity purified rabbit polyclonal (IgG) antibodies (anti-TrkA, TraB and TrkC, Santa Cruz Biotechnology. USA) and anti p75 (Sigma). Blots were developed using the enhanced chemiluminescence (ECL) Western blotting detection system (Amersham) that utilized a species specific HRP tagged secondary antibody. Frozen sections (25µm) were processed with a wide spectrum universal phosphate-based immunohistochemical detection system (Biomeda Corp.USA).

Quantitative Western blot studies revealed that TrkB and C immunoreactivity appeared by embryonic week (ew) 5th in CVG neurons, increased at high levels between embryonic week 7th and 15th , and later on, in 18th week specimens and older began to decrease to low levels. This TrkB and TrkC expression reflects the presence of high affinity receptors for BDNF and NT3 during these gestational periods.

	W5	W7	W9	W10	W12	W15	W18	W22	W24
TRK-A	++	++	+	-	nd	-	nd	-	nd
TRK-B	+	++	+++	+++	+++	++	+	+	+
TRK-C	++	+++	+++	+++	+++	+++	++	+	+
p75	+++	++	++	++	++	nd	++	nd	++

Table 1 Neurotrophin receptor expression in developing cochleovestibular ganglion in human embryos and fetuses at the indicated gestational weeks (W). The table summarizing the the presence of Trk and p75 proteins in a series of Western blots of CVG extracts. Data are presented as average values of 3 human embryos or fetuses that were examined for the p75 and the Trk antibodies. The staining intensity was expressed with a rating of: (-) No expression, (+) low expression, (++) moderate expression, (+++) abundant expression, nd = not done

TrkA immunoreactivity was detected at just moderate levels during 5th and 7th weeks reflecting the presence of NGF high affinity receptors only at these earlier developmental ages. The p75 immunoreactivity was detected at abundant levels in the earliest stage of 5th (ew) and at moderate levels in all studied inner ears from 7th to 24 weeks.

These Western blot observations on Trk and p75 expression were corroborated by immunocytochemistry on frozen sections and specific TrkA,TrkB ,TrkC and p75 immunoreactivity was found in CVG sections in all examined human embryos in a similar temporal pattern of expression. Trk IR was found segregated to CVG neurons whereas supporting cells of these ganglion resulted unlabelled. On the contrary, p75 IR was localized on both neurons and supporting cells. Positive Trk IR was found in the neuronal somata of the labelled neurons, whereas peripheral processes remained unreactive. In all analysed times this cellular distribution pattern remained basically unchanged.

The present study demonstrates Trks and P75 immunoreactivity localized on the CVG during specific developmental periods, reflecting the presence of high and low affinity receptors for neurotrophins in this ganglion.

The Trk and p75 developmental timetable of expression in human CVG is not similar but equivalent to the schedule of expression in the CVG of other vertebrates, such as rat (Ylikoski et al, 1993) The stages at which Trks and P75 immunoreactivity is present appears to be tightly associated with a series of specific developmental events, suggesting that the corresponding neurotrophins play a major physiological role in these developmental events. There was a correlation between TrkB/TrkC and p75 expression in humans and the developmentally regulated neuronal cell death in the CVG and therefore BDNF/NT3 may serve in this system to support survival of CVG neurons (Bianchi et al, 1996). Based on these results, we hypothesize that the Trk´s ligands NT3 and BDNF may be an active signal for neuronal differentiation and target-dependent neuronal cell death in human inner ear (Van de Water et al, 1992; Pirvola et al, 1992) The equivalence between Trks developmental timetable in humans and in other vertebrates also suggests that BDNF and NT3 may have a similar role on the inner ear neurons throughout species: establishing and stabilizing the afferent innervation of inner ear hair cells during development. Nevertheless, additional roles for the other NGF gene-related neurotrophins and other defined factors (e.g. cytokines) and as yet undefined growth stimulating factors cannot be ruled out.

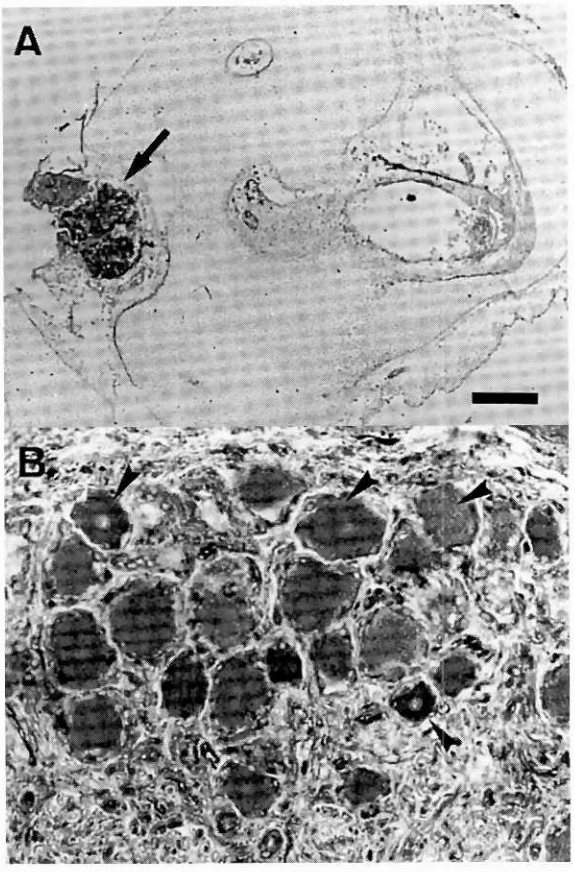

Figure 1 TrkB immunoreactivity (IR) in human inner ear. Light micrographs of 25-µm section through the temporal bone primordia of an 15th week human embryo. Vestibular ganglion is shown at low (A) and high magnifications (B). Tissue sections were processed with αTRK-B antibody (10µg/ml) or with preimmune serum as controls. Note the positive TrkB IR on the vestibular ganglion (arrow in A) and on the vestibular neurons (arrowheads in B), while supporting cells of this ganglion resulted unlabelled Scale bars = 300 µm (A) and 20 µm (B).

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