Pancreatic acinar AR42J cells express functional nerve growth factor receptors

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Abstract

The factors regulating the differentiation of the endocrine cells of the pancreas are still unknown. In previous studies, we have demonstrated that, like neurones, various β -cell lines express functional neurotrophin receptors. Moreover, Trk-A, the nerve growth factor (NGF) high-affinity receptor, is expressed *in vivo* in mature rat islets and early during development in the pancreatic ductal network that represents the source of putative stem cells. Rat pancreatic AR42J cells possess both exocrine and neuroendocrine properties. Recent studies have shown that these cells can differentiate either into acinar cells or into insulin-

expressing cells. In this study, we demonstrate that AR42J cells, in common with the embryonic ductal cells, do express Trk-A. Moreover, on treatment with NGF, Trk-A is phosphorylated and early responsive genes such as NGFI-A, c-fos and c-jun are induced. These results clearly show that the Trk-A receptor expressed in AR42J is functional. AR42J cells provide a model system with which to study the role of NGF in the development of the pancreatic cells.

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Introduction

The endocrine cells of the pancreas exhibit many features in common with those of neurones (Scharfmann 1997). For example, one of the major targets in the immune reaction leading to type I diabetes is the enzyme, glutamic acid decarboxylase, which is present in β cells and neurones and is involved in the synthesis of γ -aminobutyric acid, the main inhibitory neurotransmitter in the brain (Baekkeskov *et al.* 1990). Given the similarities between β cells and neurones, we postulated that these cells and their precursors could be sensitive to the same growth and differentiation factors. Among the factors implicated in neurone differentiation and survival, the best known are the members of the neurotrophin family, which comprises: nerve growth factor (NGF) (Levi-Montalcini 1987), brain-derived neurotrophic factor (BDNF) (Leibrock *et al.* 1989), neurotrophin-3 (NT-3) (Hohn *et al.* 1990) and neurotrophin-4/5 (NT-4/5) (Halbrook *et al.* 1991). Two kinds of transmembrane glycoproteins have been identified as receptors for neurotrophins: p75^{NGFR} (Chao *et al.* 1986) and members of the Trk family of tyrosine kinases (Barbacid 1994). p75^{NGFR} has been described as a low-affinity receptor for all neurotrophins, whereas Trk tyrosine kinases are high-affinity receptors that display neurotrophin specificity: Trk-A acts as a receptor for NGF and NT-3, Trk-B is a receptor for BDNF, and Trk-C binds exclusively NT-3.

Recently, our laboratory has demonstrated that both the low- and high-affinity NGF receptors (p75^{NGFR} and Trk-A respectively) are expressed in different insulinoma-derived cell lines (Scharfmann *et al.* 1993). Furthermore, we have shown that the NGF receptors expressed by these insulin-secreting cells are functional (Tazi *et al.* 1995). We have also shown that Trk-A is present in rat pancreas during development and adult life (Kanaka-Gantenbein *et al.* 1995). During fetal life, a low level of Trk-A expression can be detected in insulin- or glucagon-positive cells by immunohistochemistry, whereas high levels are detected in the ductal cells. In the pancreatic ducts, Trk-A expression progressively decreases as pancreas maturation progresses; in the adult pancreas, Trk-A expression is restricted to the islet cells. Thus the expression and localization of Trk-A in the rat pancreas is developmentally regulated.

Pancreatic AR42J cells are derived from a chemically induced rat pancreatic acinar carcinoma. These cells secrete amylase and other digestive enzymes, but they also possess some of the characteristics of neuroendocrine cells (Christophe 1994). For example, AR42J cells have electrically excitable membranes, and express neuroendocrine markers such as synaptophysin, the protein, SV2, and glutamic acid decarboxylase (Christophe 1994). Logsdon *et al.* (1985) reported that, after exposure to dexamethasone, these cells lost their neuroendocrine properties, whereas the amylase contents and the number

of secretory granules increased. Thus dexamethasone induces AR42J to differentiate into a more acinar phenotype (Logsdon *et al.* 1985). Recently, it has been demonstrated that, on treatment with activin A and betacellulin, AR42J cells differentiate into insulin-secreting cells (Mashima *et al.* 1996a). AR42J cells are also able to differentiate into β cells when exposed to hepatocyte growth factor (HGF) (Mashima *et al.* 1996b). Moreover, the effect of HGF is markedly increased if the cells are previously treated with activin A (Mashima *et al.* 1996b). Thus AR42J cells provide an excellent model with which to study *in vitro* the factors implicated in the differentiation of pancreatic endocrine cells. In this study, we have examined the expression of neurotrophin receptors in AR42J cells and analysed the effect of neurotrophic factors on the neuroendocrine properties of these cells.

Material and Methods

Materials

Recombinant mouse NGF was purchased from Boehringer Mannheim, Meylan, France. NT-3 and NT-4/5 were generously provided by Genentech Inc., San Francisco, CA, USA. Recombinant human activin was obtained from the National Hormone and Pituitary Program of the NIDDK, Bethesda, Maryland, USA.

Cell culture

Three different cell lines were used in this study. PC12 cells derive from a rat pheochromocytoma (Greene & Tischler 1976), and were grown in RPMI 1640 supplemented with 100 U/ml penicillin, 100 μ g/ml streptomycin, 10% fetal calf serum (FCS) and 5% horse serum. INS-1 cells derive from a rat insulinoma (Asfari *et al.* 1992) and were maintained in RPMI 1640 supplemented with 100 U/ml penicillin, 100 μ g/ml streptomycin, 10% FCS, 1 mM sodium pyruvate, 10 mM HEPES and 50 mM 2-mercaptoethanol. AR42J cells derive from a rat pancreatic adenocarcinoma (Christophe 1994). The BxPC-3 cells (Tan *et al.* 1986) were obtained from the American Type Culture Collection (CRL1687), Rockville, MD, USA. The SOJ-6 cell line (Fujii *et al.* 1990) is a subclone of the line SOJ, and was kindly provided by Dr Fujii, Institute of Medical Science, University of Tokyo, Japan. AR42J, BxPC-3 and SOJ-6 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 100 U/ml penicillin, 100 μ g/ml streptomycin and 10% FCS. The cell cultures were maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO₂.

RNA isolation and Northern blot

Cytoplasmic RNA was prepared as previously described (Scharfmann *et al.* 1993). Briefly, the cells were washed

twice in PBS solution, precipitated, and resuspended in a lysis buffer containing 100 mM Tris-HCl pH 8.6, 140 mM sodium chloride, 1.5 mM magnesium chloride and 0.5% Nonidet P-40. After 5 min at 4 °C, the nuclei were pelleted by centrifugation and the supernatant was diluted with one volume of 200 mM Tris-HCl pH 7.5, 300 mM sodium chloride, 25 mM EDTA and 2% SDS and incubated with proteinase K (250 μ g/ml) at 37 °C for 30 min. RNA was then phenol-extracted, precipitated and resuspended in water. Ten micrograms RNAs were analysed by electrophoresis in a 1% agarose formaldehyde gel and transferred to Hybond-N nylon membrane. Membranes were cross-linked by exposure to u.v. light. Hybridization was carried out according to the method of Church & Gilbert (1984), using probes labeled by random priming. Filters were then washed three times for 15 min in 0.5 $\times$ SSC containing 0.1% SDS. Autoradiography was performed at -70 °C with Kodak XAR5 film. Probes were removed from the membrane by washing at 95 °C for 15 min in 0.5% SDS, 1% glycerol and 1 mM EDTA.

Probes

The probes used in this study were: a 2.8-kb BamHI-EcoRI fragment from the p5b plasmid corresponding to rat p75^{NGFR} (Buck *et al.* 1987), a 350-base pair (bp) insert and a 464-bp XbaI-SacI fragment from the plasmid pDM97 corresponding to the kinase and the extracellular domain of rat Trk-A, respectively, as described previously (Scharfmann *et al.* 1993), a 2.2-kb insert corresponding to rat c-fos (Curran & Morgan 1985), a 1.9-kb c-jun cDNA (Lamph *et al.* 1988), a 3-kb NGFI-A cDNA (Milbrandt 1987), and a 1.1-kb BamHI fragment derived from a rat vgf genomic clone (Levi *et al.* 1985).

Immunocytochemistry

For immunofluorescence cell staining, the cells were cultured for 24 h on poly(L-lysine)-coated glass coverslips. The cells were rinsed in PBS, and fixed for 4 min in 4% paraformaldehyde. When Trk-A detection was performed, the cells were permeabilized for 5 min in 0.5% Triton X-100, 10 mM Tris pH 7.4, 300 mM sucrose, 3 mM MgCl₂ and 50 mM NaCl. The cells were then preincubated for 20 min with 0.1% BSA and 5% horse serum in PBS, and finally incubated overnight at 4 °C with 1:100 dilution of Trk antibodies (Trk(763), Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA; used here as the anti-Trk-A antibody) diluted in 0.1% BSA and 5% horse serum in PBS. After washing, the cells were incubated with the appropriate fluorescein-conjugated (second) antibodies (Dako, Copenhagen, Denmark), diluted 1:200, for 1 h at room temperature. As controls, the first antibody was either omitted, or preincubated using an excess of the peptide used to raise it.

Immunoprecipitation and Western blot analysis

PC12, AR42J, BxPC-3 and SOJ-6 cells were grown in 100-mm dishes. The cells were then washed twice with cold PBS and lysed in 500 μ l detergent lysis buffer containing 50 mM Tris pH 8.0, 1% Nonidet-P40, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 0.15 U/ml aprotinin, and 1 mM sodium orthovanadate. Insoluble material was removed by centrifugation at 10 000 g at 4 °C for 10 min. The lysates were immunoprecipitated with the anti-Trk antibody or a rabbit anti-p75^{NGFR} for 16 h at 4 °C (this antibody, known to recognize specifically the p75^{NGFR} receptor (Benedetti *et al.* 1993) was a generous gift from Dr Chao, Cornell University, New York, USA). Protein A–Sepharose beads were used to collect the antigen–antibody complexes. The complexes were then washed twice with 50 mM Tris pH 8.0, 150 mM NaCl, 0.1% NP-40 and once with 50 mM Tris pH 8.0, 0.1% NP-40. Immune complexes were solubilized in 30 μ l sample buffer containing 62 mM Tris–HCl pH 6.8, 10% glycerol, 2% SDS, 0.25% bromophenol blue and 1% β -mercaptoethanol, and boiled for 5 min.

For Western blot analysis, proteins were run on a 7.5% SDS gel. Proteins were next transferred to nitrocellulose membranes (Amersham, Les Ulis, France), subsequently preincubated for 1 h in blocking solution (3% BSA, 0.1% Tween-20 in PBS) and incubated with the anti-Trk, anti-p75^{NGFR} or anti-phosphotyrosine (Upstate Biotechnology Inc., New York, USA) antibodies. The membranes were then washed in PBS, containing 0.1% Tween-20, and reincubated with a 1:2000 dilution of horseradish-conjugated anti-rabbit IgG antibodies. Immunoreactivities were revealed using the ECL chemi-luminescence reaction (Amersham).

Induction of early response genes by neurotrophins

AR42J and PC12 cells were seeded into 100-mm tissue culture plates in their respective growth medium. When the cells reached 50–70% confluence, the medium was exchanged for 10 ml fresh medium without FCS. The experiments were initiated 16 h after serum deprivation by the addition of the neurotrophins or 1 ml FCS (positive control). All the neurotrophins (NGF, NT-3 and NT4/5) were used at final concentration of 50 ng/ml. After 30 and 60 min of incubation, cytoplasmic RNAs were extracted and Northern blot analysis performed.

Reverse transcriptase PCR analysis

Total RNAs were first treated with RNase-free DNase. The synthesis of the first-strand cDNA was performed with 8 μ g RNA using random hexamers as primers as previously described (Scharfinann *et al.* 1993). The reaction was performed in the presence or the absence of

Figure 1 Northern blot analysis of Trk-A and p75^{NGFR} mRNA transcripts in AR42J cells. (A) 10 μ g total RNAs from AR42J, PC12, INS-1, BxPC-3 and SOJ-6 cells were hybridized using a probe recognizing the extracellular domain of Trk-A. (B) After dehybridization, the same membrane was hybridized with a probe corresponding to p75^{NGFR}. Ethidium bromide staining allowing comparison of the total amount of RNA per sample, is shown at the bottom.

reverse transcriptase and allowed to proceed for 1 h at 37 °C. The following oligonucleotide primers were used for the PCR: cyclophilin primers (sense) 5'-ATGGTCAACCCACCGTGTT-3' and (anti-sense) 3'-CGTGTG

Figure 2 Analysis of Trk-A and p75^{NGFR} protein expression in AR42J cells. (Aa) Immunofluorescence staining of AR42J cells using anti-Trk antibodies. (Ab) No staining is observed when the cells are incubated solely with the fluorescein-conjugated second antibody. (B, C) Western blot analysis of Trk-A and p75^{NGFR} expression (molecular mass markers are shown at the left). AR42J and PC12 cells were lysed as described in Materials and Methods, and immunoprecipitated using anti-Trk antibodies. Immunoprecipitates were separated by 7.5% SDS-PAGE, transferred to a nitrocellulose membrane and immunodetected with the anti-Trk antibody (B). The same procedure was used to analyze the expression of p75^{NGFR}. In that case, immunoprecipitation and immunodetection were performed with a polyclonal anti-p75^{NGFR} antibody (C).

AAGTCACCACCCT-5'; Trk-A primers (sense) 5'-GGCGGATCCATGGCTGCCTTTATGGACAACC-3' and (anti-sense) 3'-GGCCGAATTCGACCCCAAAAGGTGTTTCGTCC-5'; Trk-B primers (sense) 5'-ACCTGGCATCCCAACACTTC-3' and (anti-sense) 3'-AGGAAGTCTTGAACCGCTTC-5'; and Trk-C primers (sense) 5'-CAGCCCAGAGCCTTTGCTAAG-3' and (anti-sense) 3'-TTACCGAGACCGAGAGGAAACGG-5'. Thirty-five cycles of amplification were performed on a water-cooled cyler. Amplification parameters included a 1-min denaturing step at 92 °C, a 1-min annealing step at 54 °C and a 2-min extension step at 72 °C. The products

of PCR amplification were separated by electrophoresis on 1% agarose and revealed by ethidium bromide staining.

Results

Expression of TrkA in AR42J cells

To determine whether AR42J cells express the high-affinity NGF receptor, Trk-A, we first used Northern blot to detect the presence of mRNA coding for this receptor. Cytoplasmic RNAs from AR42J cells were hybridized

using a probe specific to the extracellular domain of Trk-A. In this experiment, cytoplasmic RNA from PC12 and INS-1 cells were used as positive controls, as both cell lines are known to express Trk-A mRNA (Meakin *et al.* 1992, Scharfmann *et al.* 1993). The RNAs prepared from BXPC-3 and SOJ-6 cells, two exocrine cell lines, were used as negative controls. Both cell lines are derived from human acinar carcinomas and were expected not to express Trk-A mRNA, because our immunological studies on pancreatic sections indicated that acinar cells do not express Trk-A (Kanaka-Gantenbein *et al.* 1995). As shown in Fig. 1A, a specific Trk-A signal of the expected size was detected in RNA derived from AR42J, PC12 and INS-1 cells. In contrast, and as expected, the Trk-A probe did not hybridize to RNAs prepared from BxPC-3 and SOJ-6 cells. This Northern blot experiment also indicated that Trk-A mRNA steady-state levels appear to be lower in AR42J cells than in PC12 and INS-1 cells.

To demonstrate that Trk-A protein is expressed in AR42J cells, immunocytochemistry and immunoprecipitation followed by Western blot analysis were performed. By immunocytochemistry using the anti-Trk antibody, positive cell staining was observed in the AR42J cells (Fig. 2Aa). The fluorescence was not detectable when the antibody was preincubated with an excess of the peptide used to raise the antibody (data not shown) or when the cells were incubated only with second antibody (Fig. 2Ab). Trk-A expression was then studied by immunoprecipitation with the anti-Trk antibody followed by Western blot. The AR42J cells were lysed and the protein extracts were incubated with anti-Trk antibody in order to immunoprecipitate Trk-A. The immunoprecipitated material was then submitted to Western blot analysis using the same anti-Trk antibody. As shown in Fig. 2B, this antibody recognized the same doublet in PC12 and AR42J cells. The specificity of this signal was confirmed by the absence of signal in immunoprecipitates from BxPC-3 cells, which do not express Trk-A mRNAs (data not shown).

Expression of the low-affinity receptor for NGF, p75^{NGFR}, in AR42J cells

When cytoplasmic RNAs from AR42J cells were submitted to Northern blot analysis using a probe specific for p75^{NGFR} mRNA, no hybridization signal could be detected (Fig. 1B). This suggests that p75^{NGFR} is probably not expressed by those cells.

To confirm further the absence of p75^{NGFR} expression in AR42J cells, Western blot analysis was performed. The low-affinity receptor for NGF could not be detected on protein extracts of AR42J cells previously immunoprecipitated with an antibody specific to p75^{NGFR} (Fig. 2C).

Figure 3 NGF-induced phosphorylation of Trk-A in AR42J and PC12 cells. Semiconfluent AR42J and PC12 cells were treated with different concentrations of NGF (0, 5, 10 and 50 ng/ml) for 5 min, lysed and immunoprecipitated using the anti-Trk antibodies. The immunoprecipitates were separated by a 7.5% SDS-PAGE, blotted onto a nitrocellulose membrane, incubated with an anti-phosphotyrosine antibody and revealed using the ECL system as described in Materials and Methods. P-Trk-A, phosphorylated Trk-A.

Functionality of the Trk-A tyrosine kinase receptor expressed in AR42J cells

In NGF-responsive cells, binding of NGF to Trk-A induces a rapid tyrosine phosphorylation of the receptor (Kaplan *et al.* 1991). To determine whether tyrosine residues of Trk-A were phosphorylated in AR42J cells in response to NGF, semiconfluent AR42J cells were treated with different concentrations of NGF for 5 min and Trk-A was immunoprecipitated using the anti-Trk antibody. Subsequently, the immunoprecipitates were analysed by Western blot using an anti-phosphotyrosine antibody. NGF at a concentration of 5 ng/ml induced phosphorylation of Trk-A in both AR42J and PC12 cells (Fig. 3). To determine whether the Trk-A receptor expressed by AR42J cells is able to transduce a signal to the cell nucleus after its interaction with NGF, we checked the capacity of NGF to induce the expression of NGFI-A, an early response gene, in AR42J cells. For this purpose, PC12 and AR42J cells were serum-deprived for 16 h and then treated for 30 or 60 min with 50 ng/ml NGF, the NGF carrier buffer, or 10% FCS. Northern blot analysis (Fig. 4) shows that NGF induces a rapid increase in NGFI-A mRNA steady-state levels in both INS-1 and AR42J cells. However, it is interesting to note that, whereas the induction of NGFI-A in PC12 reached its maximum after 30 min of NGF treatment, this effect was delayed in AR42J cells, in which the maximal induction was obtained after 60 min. Nevertheless, this experiment clearly demonstrates that the Trk-A protein expressed by AR42J acts as a functional receptor able to transduce the signal of NGF.

Induction of other NGF-responsive genes

Northern blot analysis was next performed to study the capacity of NGF to induce in AR42J the expression of c-fos, c-jun and vgf, three genes known to be regulated by

Figure 4 Effect of NGF on NGFI-A expression in PC12 and AR42J cells. (Top) The cells were cultured in medium depleted of serum for 16 h and then treated for 30 or 60 min with NGF (50 ng/ml), its carrier, or with 10% FCS. Cytoplasmic RNAs were isolated and hybridized using an NGFI-A probe as described in Material and Methods. (Bottom) Ethidium bromide staining, allowing comparison of the total amount of RNA per sample.

this neurotrophin in PC12 cells. NGF had a clear effect on c-jun mRNA steady-state levels in AR42J cells (Fig. 5A). A more moderate effect was observed on the c-fos mRNA levels (Fig. 5B), and no effect could be observed on vgf mRNA steady-state levels (Fig. 5C).

Expression of other members of the Trk family in AR42J

To examine the expression of other members of the Trk tyrosine kinase family in AR42J cells, we performed RT-PCR analysis using primers specific for Trk-A, Trk-B, and Trk-C. The PCR experiments (Fig. 6) were conducted on cDNA samples prepared from AR42J and INS-1 cells. The cDNAs from INS-1 cells were used as positive controls, because these cells are known to express the three Trk family members (Tazi *et al.* 1996). After amplification, the PCR products were analysed by electrophoresis on a 1% agarose gel. As expected, the three neurotrophin receptors could be amplified from cDNA prepared from INS-1 cells; however, only Trk-A could be amplified from cDNAs prepared from AR42J cells.

Transduction of the signals of NT-3 or NT-4/5

It has been reported that Trk-A, although acting as a preferential receptor for NGF, can also, in certain con-

ditions, act as a receptor for other neurotrophins (Benedetti *et al.* 1993). In order to determine if the Trk-A receptor expressed by AR42J cells can transduce the signal of these neurotrophins, we checked the capacity of NT-3 and NT-4/5 to induce the expression of NGFI-A. AR42J cells were serum-deprived overnight and then treated with 50 ng/ml NGF, 100 ng/ml NT-3, 100 ng/ml NT-4/5 or 10% FCS. The induction of NGFI-A mRNA steady-state levels was analysed by Northern blot (Fig. 7). This experiment showed that NGFI-A mRNA steady-state level was induced when AR42J cells were treated with NGF or 10% FCS, but not when the cells were treated with NT3 or NT-4/5. Thus Trk-A in AR42J cells cannot transduce the signals of NT3 or NT-4/5.

Effect of NGF on the neuroendocrine properties of AR42J cells

Having established that AR42J cells are able to respond to NGF, we next examined whether this neurotrophin can induce AR42J cells to differentiate into endocrine cells. For this purpose, the cells were cultured in the presence of NGF (50 ng/ml) alone or in combination with activin A (2 nM). After a 7-day culture period, the expression of mRNA coding for insulin or glucagon was examined by RT-PCR. Neither NGF treatment, alone or in

Figure 5 Effect of NGF on the induction of c-jun, c-fos and vgf gene expression. AR42J cells were cultured in a medium depleted of serum for 16 h and then treated for 30 or 60 min with NGF (100 ng/ml). Cytoplasmic RNAs were isolated and hybridized using a c-jun (A), c-fos (B) and vgf (C) probes. Ethidium bromide staining, allowing comparison of the total amount of RNA per sample, are also shown for (A) and (B).

combination with activin was able to induce the expression of mRNA for the pancreatic endocrine hormones (insulin or glucagon) in AR42J cells (data not shown).

Discussion

In this work we have analysed the expression of functional neurotrophin receptors in the rat cell line

AR42J. First, we studied the expression of Trk-A mRNA in AR42J cells. Northern blot analysis indicated that mRNA encoding Trk-A is expressed by these cells, although at smaller levels than in PC12 and INS-1 cells, two cell lines already known to express Trk-A. Western blot analysis confirmed that Trk-A protein is expressed by AR42J. Both results clearly demonstrate that AR42J cells line express Trk-A, the high-affinity receptor for NGF.

Figure 6 RT-PCR analysis of Trk mRNA transcripts in INS-1 and AR42J cells. Total RNAs from INS-1 and AR42J cells were reverse-transcribed in the presence (+) or in the absence (–) of reverse transcriptase and amplified by PCR using oligonucleotide primers specific for Trk-A, Trk-B or Trk-C. The PCR products were resolved in a 1% agarose gel and revealed by ethidium bromide staining.

It is known that NGF can also bind to a low-affinity receptor known as p75^{NGFR}. Therefore, we addressed the question of the expression of p75^{NGFR} in AR42J cells. Both Northern and Western blot analyses indicated that p75^{NGFR} is not expressed in these cells. Although there has long been controversy regarding the respective roles of both receptors, it seems that Trk-A is sufficient to ensure transduction of the NGF signal (Cordon-Cardo *et al.* 1991). It is currently believed that p75^{NGFR} may be necessary to enhance the affinity of the cells to NGF (Hempstead *et al.* 1991). In this sense, AR42J appears to be an interesting model with which to analyse the role of p75^{NGFR}. The transfection of AR42J cells with p75^{NGFR} cDNA would allow comparison of the response to NGF of the same cell type, in the presence or absence of this low-affinity NGF receptor.

As Trk-A is sufficient to transduce the NGF signal, we investigated whether the Trk-A receptor expressed in AR42J is functional. In both PC12 and AR42J cells, NGF induced tyrosine phosphorylation of Trk-A in a similar dose-dependent manner. Moreover, the induction experiments conducted on PC12 and AR42J cells also demonstrated that, when the cells were exposed to NGF, the expression of the early response genes, NGFI-A, c-jun, and c-fos, was induced. Thus AR42J cells respond to NGF stimulation in the same way as do PC12 cells. However, some differences in the response to NGF were

found between the two cell lines. First, the induction of NGFI-A in AR42J appears to be delayed. Secondly, NGF could not induce the expression of vgf mRNA in AR42J cells. This gene is restricted to cells of neuronal origin and is rapidly induced by NGF in PC12 (Levi *et al.* 1985). Thus it is possible that this pathway of induction is not functional in AR42J.

NGF belongs to a family of neurotrophic factors known as neurotrophins, which bind different members of the Trk family. RT-PCR analysis conducted with cDNA from AR42J cells and INS-1 cells (which express the three neurotrophin receptors: Trk-A, Trk-B and Trk-C (Tazi *et al.* 1996)) indicated that only Trk-A is expressed in AR42J. Because it has been reported that Trk-A could also act in some systems as a functional receptor for other neurotrophins (Benedetti *et al.* 1993), we also checked whether NT-3 and NT-4/5 could induce the expression of NGFI-A mRNA in AR42J cells. Our induction studies indicated that, in AR42J cells, NT-3 and NT-4/5 cannot induce the expression of early response genes, strongly suggesting that in AR42J cells, Trk-A does specifically act as a receptor for NGF.

Histomorphometric studies have revealed that the endocrine cells of the pancreas, in addition to the acinar cells, originate from the epithelial cells of the pancreatic ducts (Pictet & Rutter 1972). On the basis of their observations in transgenic mice expressing interferon-γ

Figure 7 Effect of neurotrophic factors on the NGF-A mRNA steady-state levels of AR42J cells. (Top) AR42J cells were cultured for 16 h in a medium devoid of serum and then treated for 30 min with NGF (50 ng/ml), NT-3 (100 ng/ml), NT-4/5 (100 ng/ml) or 10% FCS. Cytoplasmic RNAs were isolated and hybridized using a probe specific for NGF-A. (Bottom) Ethidium bromide staining, allowing comparison of the total amount of RNA per sample.

under the control of the insulin promoter, Gu *et al.* (1994) have proposed the following hypothetical model. The ductal cells would first differentiate into cells expressing the properties of both ductal and acinar cells. These cells would next differentiate into transitional cells expressing acinar and endocrine properties. Finally, these transitional cells would differentiate into either endocrine or exocrine cells. AR42J cells resemble the transitional precursors described by Gu *et al.* (1994). Indeed, AR42J cells possess both endocrine and exocrine properties (Christophe 1994). We demonstrate here that AR42J cells express a functional receptor for NGF, as do ductal cells during early pancreatic development (Kanaka-Gantenbein *et al.* 1995), which are believed to be able to differentiate into endocrine or exocrine cells.

Mashima *et al.* (1996a,b) have recently shown that AR42J cells can differentiate into β cells when treated with activin in combination either with betacellulin or with HGF. It was thus interesting to define whether NGF could mimic the effect of betacellulin or of HGF. In our experimental conditions, we could not observe any differentiation of AR42J cells when treated with NGF, alone or in combination with activin. This does suggest that NGF may not be necessary for the endocrine differentiation of these cells. However, it is known that, in other systems,

NGF and the other neurotrophic factors are implicated not only in neuronal differentiation, but also in the survival of neurones and neuronal precursors (Hamburger *et al.* 1981, Johnson *et al.* 1980), protecting these cells from apoptotic death (ElShamy & Ernfors 1996, Ferrari 1996). Such a protective role of NGF in AR42J should now be tested.

In summary, we have demonstrated that the acinar AR42J cells express a functional Trk-A receptor that efficiently transduces the signal of NGF. AR42J cells could thus represent an interesting model through which to understand the role of NGF during pancreatic development.

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