Neurotrophin-3 Increases Intracellular Calcium in a Rat Insulin-secreting Cell Line through Its Action on a Functional TrkC Receptor*

(Received for publication, November 28, 1995, and in revised form, January 17, 1996)

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Pancreatic beta cells and neuronal cells show a large number of similarities. For example, functional receptors for nerve growth factor are present in beta cells. Here we investigate whether TrkC, a neuronal high affinity receptor for neurotrophin-3, is expressed in the insulin-secreting cell line INS-1. We demonstrate the expression in INS-1 cells of mRNAs coding for TrkC identical in size to those found in the brain. As in neuronal cells, different alternatively spliced forms of TrkC mRNA, differing by the insertion of an alternative exon in their kinase domain, were expressed in INS-1 cells. TrkC protein is also expressed in INS-1 cells and functional. Indeed, when INS-1 cells were treated with neurotrophin-3, TrkC became phosphorylated on tyrosine residues, and the expression of early response genes was induced. This activation of the receptor was paralleled by a rapid and transient increase in cytosolic free calcium due to an influx of extracellular calcium. Functional receptors for NT-3 are thus expressed in INS-1 cells. This cell line provides a new model for the study of NT-3 signal transduction and should be useful in the understanding of the role of neurotrophins in insulin-secreting cells.

The insulin-secreting beta cells of the pancreas are similar in many ways to neuronal cells (1–3). We had thus previously postulated that neurotrophic factors, which are implicated in the differentiation, growth, and survival of neuronal cells, could act on beta cells. We have shown that, indeed, both the high and low affinity receptors (TrkA and p75NFR) are expressed in a number of insulin-producing cell lines and in fetal and adult rat islets (4, 5). In different beta cell lines, these receptors are functional, as demonstrated by binding studies using iodinated NGF and by phosphorylation of TrkA on tyrosine residues and induction of early response genes by NGF treatment (6).

The family of neurotrophic factors contains NGF, the first neurotrophin to be identified (7), but also brain-derived neurotrophic factor (BDNF) (8) and neurotrophin-3 (NT-3) (9, 10). Signal transduction by these neurotrophins is initiated by high affinity binding to and activation of specific tyrosine kinase receptors. TrkA and TrkC bind NGF and NT-3, respectively, whereas TrkB binds BDNF (11). In addition, all neurotrophins bind to the low affinity receptor p75NFR (12). The precise role of p75NFR in NGF signal transduction is not fully understood.

Different roles have been recently attributed to NT-3 in the central and peripheral nervous system. It has been shown to support the survival of sensory neurons isolated from nodose ganglia, of oligodendrocytes, and of noradrenergic neurons in vivo (9, 10, 13, 14), and to promote the proliferation of neural crest cells (15). NT-3 has also been implicated in short term effects, such as potentiation of neuronal activity (16, 17). Moreover, NT-3 has been shown to induce intracellular free calcium ([Ca2+]i) elevation in hippocampal neurons within seconds following treatment (18).

Whereas the expression of NGF and BDNF has been detected only in a limited number of peripheral tissues, NT-3 mRNAs have been detected in all the tissues tested, including brain, heart, skin, gut, muscle, lung, spleen, and liver (9, 10). This ubiquitous pattern of expression suggests that NT-3 could act outside the nervous system. In fact, it has been shown recently that melanocytes constitutively express low levels of TrkC and that NT-3 prevents cell death of melanocytes (19).

The aim of the present study was to investigate whether the insulin-producing cells, INS-1, express functional TrkC receptors and whether NT-3 plays a role in these cells, similar to that which has previously been described for neurons. We therefore studied 1) the expression of TrkC mRNA in INS-1 cells by reverse transcriptase-polymerase chain reaction (RT-PCR) and Northern blot analysis and determined which isoforms of TrkC were expressed; 2) signal transduction steps, such as phosphorylation of TrkC after NT-3 treatment, and induction of the expression of early response genes, such as c-fos and NGFI-A; 3) cytosolic free calcium as it is known that, in hippocampal neurons, NT-3 increases cytosolic free calcium; and 4) finally, whether NT-3 affects insulin secretion in INS-1 cells.

Our data demonstrate that INS-1 cells represent a new experimental system for studying the different steps of signal transduction by NT-3 in endocrine cells.

EXPERIMENTAL PROCEDURES

Cell Culture—Two different cell lines were used in this study. PC12 cells derived from a rat pheochromocytoma (20) were grown in RPMI 1640 supplemented with 100 units/ml penicillin, 100 mg/ml streptomycin, 10% fetal calf serum, and 5% horse serum. INS-1 cells derived from a rat insulinoma (21) were grown in RPMI 1640 supplemented with 100 units/ml penicillin, 100 mg/ml streptomycin, 10% fetal calf serum, 1 mM sodium pyruvate, 10 mM HEPES, and 50 μM 2-mercaptoethanol. Cultures were maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO2.

RNA Isolation and Northern Blot Analysis—Total cellular RNAs

*This work was supported by grants from the Juvenile Diabetes Foundation and from Association pour la Recherche contre le Cancer. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; NT-3, neurotrophin-3; RT, reverse transcriptase-PCR, polymerase chain reaction; kb, kilobase pair(s).
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from rat brain or from cell lines were prepared by homogenization in guanidinium thiocyanate solution (22) or by LiCl-urea precipitation (23). Cytoplasmic RNAs were extracted as described previously (4). Ten μg of RNAs were analyzed by gel electrophoresis in a 1% agarose-formaldehyde gel and transferred to a Hybond-N nylon membrane (Amersham Corp.). Membranes were cross-linked by exposure to ultraviolet light. Hybridization mixture was prepared as described above. The probes were radioactively labeled by random priming. Membranes were then washed three times for 15 min at 65 °C in 0.5 × SSC containing 0.1% SDS. Each experiment was performed at least twice. The cDNAs used as probes in this study were prepared from the Trk-Cout antibody. The membranes were then washed in phosphate-buffered saline, containing 0.1% Tween 20, and then incubated with either the anti-phosphotyrosine monoclonal antibody 4G10 (Upstate Biotechnology Inc.) or with the Trk-Cint antibody. The membranes were then washed in phosphate-buffered saline, containing 0.1% Tween 20, and with a 1:2000 dilution of horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG antibodies. Immunoreactivities were determined using the ECL chemiluminescence reaction (Amersham).

Intracellular Calcium Measurements—The concentration of cytosolic free calcium was measured by dual emission microspectrofluorometry using Indo 1 as the intracellular fluorescent calcium probe (27). INS-1 cells were cultivated on coverslips for 3 days. The cells were then loaded for 30 min at room temperature with 5 μl Indo 1 penta-acetoxyxymester (Indo 1-AM) (Molecular Probes Inc.), in a solution containing 134 mM NaCl, 4.7 mM KCl, 1.2 mM KH2PO4, 1.2 mM MgSO4, 1.5 mM CaCl2, 5 mM NaHCO3, 3 mg glucose, and 25 mM HEPES, adjusted to pH 7.4, 300 mosm. At the end of incubation, the coverslips were mounted on the recording chamber of an inverted microscope (Nikon Diaphot, Japan) with epi-fluorescence (40 × glyceral immersion fluorescence objective) and equipped for microfluorometry (Phocal System, Life Science Resources Ltd., Cambridge, UK). The recording medium contained 134 mM NaCl, 4.7 mM KCl, 1.2 mM KH2PO4, 1.2 mM MgSO4, 5 mM CaCl2, 5 mM NaHCO3, 3 mg glucose, and 25 mM HEPES, adjusted to pH 7.4, 300 mosm. For the assay, solution light was provided at 560 nm by a 75 watt xenon arc lamp. Emitted fluorescence signals were passed through a pinhole diaphragm to eliminate stray and background light and, after being split by a dichroic mirror, were detected simultaneously by two photomultipliers at 405 and 490 nm in a dual channel photon counting mode. The recording field (INS-1 cells cluster of two to five cells) was delimited by an iris diaphragm positioned along the optical pathway inside the microscope. Quantitative calcium values were estimated from Indo 1 fluorescence by ratio method and were calculated as described previously (27). The base line was recorded for 3 min. The tested agents NT-3, NGF, KCl, and cobalt were prepared in the bath solution and were injected by Hamilton syringe into the chamber containing the test cells. K252a (Calbiochem-Novabiochem Corp., La Jolla, CA), a specific inhibitor of Trk tyrosine kinase receptors was used (28). In some cases, 100 μM K252a, dissolved in dimethyl sulfoxide, was injected into the chamber containing the cells 30 min prior to the addition of NT-3.

Insulin Secretion Studies—INS-1 cells were plated at 2 × 105 cells/ well into 24-well plates. Three days later, the medium was removed, and the cells were washed twice with a Krebs-Ringer buffer containing 134 mM NaCl, 4.7 mM KCl, 1 mM CaCl2, 1.2 mM MgSO4, 1.2 mM KH2PO4, 10 mM HEPES, and 0.4% bovine serum albumin. Precubation was performed for 30 min at 37 °C in Krebs-Ringer buffer supplemented with 2.5 mM glucose. Following precubation, the cells were washed with Krebs-Ringer buffer and incubated for 30 min at 37 °C in Krebs-Ringer buffer at various glucose concentrations (5–20 mM) in the presence or the absence of 50 ng/ml NT-3. The precubation and incubation were performed in an humidified atmosphere of 95% air and 5% CO2. Insulin secretion was determined by radioimmunoassay, using an anti-insulin antibody raised in guinea pig against porcine insulin (a gift from Dr. Van Schravendijk, Brussels, Belgium) and rat insulin as a standard. The total cellular insulin contents were measured after acid-ethanol extraction (concentrated HCl (1.5%), ethanol (75%)).

RESULTS

Expression of Trk-C mRNA in INS-1 Cells—To examine the expression of Trk-C mRNA in INS-1 cells, RT-PCR was performed using two specific pairs of primers, TrkC355-TrkC694 and TrkC1760-TrkC2563 corresponding to part of the extracellular and kinase domains of rat Trk-C, respectively. The PCR amplifications were performed on cDNA prepared from INS-1 cells, PC12 cells, and rat brain. PCR was also performed on samples prepared from INS-1, PC12 cells, and rat brain RNAs for which reverse transcriptase was omitted during the reverse transcription reaction, but which were otherwise processed identically to the first samples. The products of amplification were analyzed on a 1% agarose gel electrophoresis (Fig. 1a). When primers corresponding to the extracellular domain of Trk-C were used, a single band of the predicted size was amplified from both rat brain and INS-1 cDNAs. This band was absent when PC12 DNA was used and in all the samples for which reverse transcriptase was omitted. The specificity of the generated band was demonstrated by Southern blot analysis and by enzymatic digestion using specific restriction enzymes (data not shown). PCR was performed in parallel using cyto phosphin primers as a control (Fig. 1a). Thus, at least part of the extracellular domain of Trk-C is expressed in INS-1 cells.

Various truncated forms of Trk-C, lacking a kinase domain, and unable to transduce a signal into the cell, have previously been described (25, 29–31). Thus, to determine whether mRNA...
TrkC1761 and TrkC2563 primers were used; the major amplification product spanning the kinase domain. As shown in Fig. 1, PCR analysis was also performed using oligonucleotides coding for the TrkC kinase domain was expressed by INS-1 cells, when oligonucleotide primers spanning the TrkC kinase domain were used, the major amplification product obtained using rat brain cDNA had the predicted 685-base pair size. The same sized amplification product was obtained when INS-1 cDNA was used. No amplification was obtained from cDNA prepared from PC12 cells when reverse transcriptase was omitted. In addition, a second major product of higher molecular weight was coamplified from INS-1 cDNA. In the brain, different TrkC tyrosine kinase isoforms containing variable-sized amino acid insertions within the tyrosine kinase domain have been recently described (25, 31). TrkC forms of TrkC, were revealed by Northern blot analysis. In NT-3-responsive cells, the response is initiated by the phosphorylation of TrkC on tyrosine residues. To determine whether TrkC tyrosine residues were phosphorylated in INS-1 cells with or without insert (TrkC1) being the most potent (25, 30, 31). To determine whether the TrkC1 isoform was expressed in INS-1 cells, the PCR products corresponding to the kinase domain were subcloned and representative clones were submitted to sequence analysis. Two different sequences with or without 42 bases encoding a 14-amino acid insert were detected in INS-1 cells (Fig. 1b). Thus, the two products of different molecular weight, which were coamplified from INS-1 cells cDNA when oligonucleotide primers spanning the TrkC kinase domain were used, are identical to TrkCK1 and TrkCK14 previously detected in the brain.

TrkC expression was then examined by Northern blot analysis of total RNA extracted from INS-1 cells, PC12 cells, and adult rat brain. Two different probes, specific for the extracellular and kinase domain of TrkC, respectively, were used. Analysis with the TrkC extracellular probe (Fig. 2a) revealed a similar pattern of expression in rat brain and INS-1 cells with five major transcripts of different molecular sizes: 14, 7, 4.7, 4, and 1.1 kb. No signal was detected in PC12 cell RNA. Analysis with the TrkC kinase domain probe (Fig. 2b) showed the presence in both INS-1 cells and brain of four transcripts of different molecular sizes: 14, 10, 5, and 2.9 kb. A 2.9 kb band was also detected in RNA from PC12 cells. This band, which is absent when the extracellular probe is used, could represent cross-hybridization with TrkA. The 14-kb band is recognized by both probes in brain and INS-1 cells and represents thus a full-length TrkC receptor. In addition, a number of other transcripts in INS-1 cells, which could encode different truncated forms of TrkC, were revealed by Northern blot analysis.

Expression of TrkC Protein in INS-1 Cells—The expression of TrkC protein in INS-1 cells was then studied by immunoprecipitation using TrkCout antibody followed by Western blot analysis using the same antibody. As shown in Fig. 3, a protein of the expected molecular weight is detected in INS-1 cells and is absent from PC12 cells. Thus, TrkC protein is expressed in INS-1 cells.

NT-3 Phosphorylates TrkC in INS-1 Cells—In NT-3-responsive cells, the response is initiated by the phosphorylation of TrkC on tyrosine residues. To determine whether TrkC tyrosine residues were phosphorylated in INS-1 cells in response to NT-3, 2.5 x 10^4 INS-1 or PC12 cells were treated with 50 ng/ml NT-3 or 50 ng/ml NGF for 5 min and immunoprecipitated using TrkCin antibody that is TrkC-specific and does not recognize the other members of the Trk family (25). The immunoprecipitates were then analyzed by Western blot analysis using an anti-phosphotyrosine antibody. Fig. 4 shows that, within 5 min following NT-3 treatment, TrkC becomes phosphorylated on tyrosine residues in INS-1 cells. Induction of TrkC phosphorylation was performed in parallel in PC12 cells, known to express TrkA but not TrkC. We did not observe any phosphorylation of...
TrkC in PC12 cells in response to NT-3 or to NGF, demonstrating the specificity of the receptor/ligand interaction and the specificity of TrkC antibody.

Induction of Early Response Genes—To determine whether NT-3 transduces an intracellular signal in INS-1 cells, the effect of NT-3 on the expression of c-fos and NGFI-A, two early response genes, was studied in INS-1 cells and compared with the results obtained in PC12 cells. For that purpose, INS-1 and PC12 cells were serum-starved for 16 h and then treated for 30 or 60 min with either 50 ng/ml NT-3 alone or with NT-3 and 10% fetal calf serum. Fig. 5 shows that, in INS-1 cells, the addition of NT-3 induced a rapid increase in c-fos and NGFI-A mRNA steady state levels. On the other hand, whereas in PC12 cells both c-fos and NGFI-A mRNAs steady state levels are increased when the cells are treated with fetal calf serum, no such effect is detected upon NT-3 treatment. Thus, INS-1 cells possess the intracellular transduction mechanisms necessary for the response to NT-3.

Effect of NT-3 on Cytosolic Free Calcium \([Ca^{2+}]_i\) in INS-1 Cells—Since NT-3 has been reported to increase cytosolic free calcium in hippocampal neurons (18), and since variations in \([Ca^{2+}]_i\) are important for the function and survival of beta cells (32), we investigated whether NT-3 induces variations of \([Ca^{2+}]_i\) in INS-1 cells. The concentration of \([Ca^{2+}]_i\) was measured by dual emission microspectrofluorometry using Indo-1 as the intracellular fluorescent calcium probe. Fig. 6A shows that, in INS-1 cells, NT-3 induced an immediate and transient increase in cytosolic free calcium. To determine whether this elevation of \([Ca^{2+}]_i\) corresponded to a mobilization of the stored calcium in endoplasmic reticulum or to an influx of extracellular calcium, the same experiments were performed in the absence of extracellular calcium or in the presence of 1 mM cobalt, which blocks calcium channels. Fig. 6, B–C, shows that the effect of NT-3 on cytosolic free calcium was abolished in the absence of extracellular calcium or in the presence of cobalt, demonstrating that the effect of NT-3 on \([Ca^{2+}]_i\) was due to an influx of extracellular calcium. To determine whether the effect of NT-3 on \([Ca^{2+}]_i\), in INS-1 cells required the tyrosine phosphorylation of TrkC, INS-1 cells were pretreated with K252a, a specific kinase inhibitor of the Trk family members (28). Pretreatment of the Indo 1-loaded INS-1 cells with 100 nM K252a for 30 min resulted in a complete inhibition of the NT-3-induced rise in \([Ca^{2+}]_i\), but had no effect on the increases in \([Ca^{2+}]_i\) caused by KCl (Fig. 6D). Interestingly, whereas in addition to TrkC, INS-1 cells express functional TrkA receptors (4, 6), no such increase in \([Ca^{2+}]_i\) was detected upon NGF treatment (Fig. 6E), demonstrating different roles for NGF and NT-3 in INS-1 cells.

Insulin Measurements—In beta cells, the elevation of \([Ca^{2+}]_i\) mediates various responses, including insulin release (32). Since NT-3 induced an increase in \([Ca^{2+}]_i\), in INS-1 cells, we investigated whether it also had an effect on insulin release. No significant difference in insulin secretion was observed between control and NT-3-treated INS-1 cells with 0, 2.5, 5, 10, 15, and 20 mM glucose.
DISCUSSION

Here, we report that the insulin-secreting cell line INS-1 expresses functional receptors to NT-3. By Northern blot analysis and RT-PCR, mRNAs coding for TrkC, a high affinity NT-3 receptor, are detected in INS-1 cells, identical in size to those found in the brain. Upon addition of NT-3, TrkC is phosphorylated on tyrosine residues, and the expression of early response genes is induced. This activation of the receptor is paralleled by a rise in intracellular free calcium.

Most of the data available concerning the expression of TrkC derive from the nervous system, in which it is expressed at its highest levels (25, 31). In the brain, different forms of TrkC have been identified (25, 30, 31). Some contain an intracytoplasmic kinase domain, while others lack a kinase domain and are thus thought to be unable to transduce an intracellular signal. This type of truncated form lacking a kinase domain has previously been demonstrated in the case of other members of the Trk family, such as TrkB, a high affinity receptor for BDNF and NT4 (29, 33). For TrkB receptors, both full-length and truncated forms are detected in the brain (29, 33), whereas only truncated forms are detected in non-neuronal tissues, such as spleen, submaxillary gland, testes, kidney, or pituitary (33, 34).

In the case of TrkC, the situation is slightly more confusing. Indeed, when RNAs prepared from different tissues are analyzed, a weak signal is detected using a probe recognizing TrkC in the thymus, lung, kidney, stomach, and testes. Whereas some reports propose that, as in the case of TrkB, only truncated forms of TrkC are expressed in non-neuronal tissues (31), others detect mRNA coding for full-length TrkC forms in non-neuronal tissues (25). It was thus important to define whether full-length TrkC mRNA was expressed in INS-1 cells, which are thought to derive from the endoderm. Parallel analyses using probes that recognize the extracellular and the kinase domain of TrkC demonstrate the presence of a similar 14-kb transcript with each probe in total RNA prepared from brain and INS-1 cells, demonstrating the expression of full-length TrkC mRNA in the non-neuronal cell line INS-1. The other transcripts at 7.2, 4.7, and 4 kb, which were detected using the extracellular probe but not using the kinase domain probe, probably correspond to truncated forms of TrkC lacking a kinase domain as previously suggested by Valenzuela et al. (31).

In addition to forms lacking an intracytoplasmic tyrosine kinase domain, other TrkC isoforms have recently been described in the brain (25, 30, 31). These isoforms contain or not an insertion in their kinase domain of 14, 25, or 39 amino acids and have been shown to mediate different responses within the target cells. Whereas all of the TrkC tyrosine kinase isoforms have the ability to autophosphorylate after NT-3 treatment, the form with no insert is the only one able to mediate proliferation or neuronal differentiation in fibroblasts or PC12 cell lines that have been engineered to express the different forms of TrkC (25, 30, 31). We demonstrate in the present study that the pancreatic beta cell line INS-1 expresses, in addition to other forms, TrkCK1, which is, as described above and according to different groups, the most potent receptor for NT-3 (25, 30, 31). Thus INS-1 cells express, in addition to other TrkC isoforms, the full-length NT-3 receptor TrkCK1, known to be functional in neuronal cells.

**FIG. 6.** Effect of NT-3 on cytosolic free calcium \([Ca^{2+}]_i\) in INS-1 cells. The concentrations of \([Ca^{2+}]_i\) were measured by dual emission microspectrofluorometry using Indo 1 as the intracellular fluorescent calcium probe. A, the effect of NT-3 on cytosolic free calcium was researched in the presence of 1.5 mM CaCl2. B, the effect of NT-3 on cytosolic free calcium was measured in the absence of extracellular calcium. C, the cells were preincubated with 1 mM cobalt before NT-3 addition. D, the cells were preincubated with K252a before NT-3 stimulation. E, the effect of NGF on cytosolic free calcium was researched in the presence of 1.5 mM CaCl2.
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In INS-1 cells, NT-3 receptors are functional as demonstrated by tyrosine autophosphorylation of the receptor and induction of early response gene expression, such as c-fos and NGF1-A after NT-3 treatment. Thus, INS-1 cells represent a new experimental system for the study of Trk-mediated signal transduction. In fact, whereas PC12 cells have been used for years to study NGF signal transduction, to our knowledge, no such system exists for the study of NT-3 signal transduction. The only available cell lines are fibroblasts or PC12 cells engineered to express TrkC. Whereas these cell lines are very useful for the study of some steps of neurotrophin signal transduction, Trk receptors behave differentially, depending on the cellular context. For example, NT-3 is comparable to BDNF in its ability to induce TrkB autophosphorylation in fibroblasts transfected with TrkB, but not in PC12 cells transfected with TrkB (35). Moreover, according to the experimental system used, neurotrophins can act as growth factors and induce cell proliferation or act as differentiation factors and induce growth inhibition (36). Thus the insulin-producing cell line INS-1, which constitutively expresses TrkC, will represent a new experimental system for the study of the effects of NT-3.

In INS-1 cells, cytoplasmic free Ca2+ plays a fundamental role in signal transduction. For example, short term variations in [Ca2+]i, are implicated in insulin release by glucose (32). When glucose is taken up by beta cells, there is a rise in ATP, inducing the closure of ATP-dependent potassium channels. This leads to opening of voltage-dependent calcium channels, increase in [Ca2+]i, and insulin release. Since it has been shown that NT-3 induces an elevation in [Ca2+]i (18), we investigated whether such a variation could be produced in INS-1 cells. We demonstrated that NT-3 induces an elevation of cytosolic free calcium mediated by Trk. This elevation is due to an influx of extracellular calcium and requires the phosphorylation of TrkC on tyrosine residues. No such calcium influx is seen when INS-1 cells are treated with NGF. INS-1 cells represent thus an experimental system in which two neurotrophins acting via two different receptors produce different effects. It would now be interesting to compare the signal transduction pathway of NGF and NT-3 via TrkA and TrkC, respectively, in INS-1 cells and to define at which point they diverge.

Because elevation in [Ca2+]i has been implicated in insulin secretion, we tested whether NT-3, which induces a raise in [Ca2+]i, would induce insulin release. In the experimental conditions tested, we were unable to detect any effect of NT-3 on insulin release. There are different possible explanations for the discrepancy between the rise in [Ca2+]i, and its expected effect on insulin secretion. Application of various peptides as a2-adrenergic agonists have been shown to suppress insulin exocytosis, despite sufficient high levels of intracellular calcium (37). Thus, in certain conditions, increase in [Ca2+]i, and insulin secretion can be dissociated. The absence of insulin release upon NT-3 treatment could also be due to the experimental system itself; although it has been reported that INS-1 cells secrete insulin after glucose treatment (21), in our hands, after a given number of passages, we were not able to reproducibly observe a significant increase of insulin secretion using the range of glucose concentrations reported under “Experimental Procedures.” Therefore, caution is necessary in concluding that NT3 has no effect on insulin secretion. Obviously, more information is needed using mature islets of Langerhans.

Whereas TrkC is a receptor for NT-3, it seems that, in certain experimental systems, NT-3 can signal through TrkA. Indeed, in fibroblasts engineered to express TrkA, both NGF and NT-3 induce the rapid phosphorylation of TrkA and the transient expression of c-fos protein (36). In PC12 subclones that express TrkA but not TrkC and that have been engineered to express different levels of p75NGFR, NT-3 is fully capable of inducing neurite outgrowth in the clones deficient in p75NGFR but not in the parental clones expressing p75NGFR. Finally, in PC12 cells, when an anti-p75NGFR antibody is used to block NGF and NT-3 binding to the p75NGFR, NT-3 induces tyrosine autophosphorylation of TrkA, transcription of zif268, and cellular differentiation (38, 39). Thus, in different cell lines and in the absence of p75NGFR expression, NT-3 transduces a signal via TrkA. The possibility for NT-3 to signal via TrkA has also been studied in neurons, and it has been shown that, at certain stages of development, NT-3 can signal through TrkA (40). Because we have previously shown that INS-1 cells express functional TrkA receptors (4, 6), it was important to demonstrate that NT-3 signals through TrkC in INS-1 cells. We have now accumulated much evidence demonstrating that, in INS-1 cells, NT-3 signal transduction is mediated by TrkC. First, full-length TrkC mRNAs, identical to those present in the brain, are detected in INS-1 cells; second, INS-1 cells express high levels of p75NGFR; third, TrkC is specifically phosphorylated upon NT-3 treatment of INS-1 cells; and finally, NT-3 induces an elevation in [Ca2+]i, in INS-1 cells, while no effect of NGF on [Ca2+]i, is detected in the same cell type. Taken together, the data demonstrate that TrkC is implicated in NT-3 signal transduction in INS-1 cells.

Beta cell lines, such as INS-1, can thus respond to NGF via TrkA and to NT-3 via TrkC. These neurotrophin receptors belong now to the increasing list of molecules shared by both beta and neuronal cells. For example, enzymes such as tyrosine hydroxylase and glutamic acid decarboxylase (1, 2), molecules of the cytoskeleton such as neurofilaments (3), and type II sodium channels (41), first thought to be specific of neuronal and neural crest-derived cells, are expressed by both beta and neuronal cells. These similarities suggest that identical tissue-specific transcription factors could be expressed in beta and neuronal cells. It is in fact interesting to note that isl-1, a homeobox gene which was originally identified for its ability to transactivate the insulin gene, is also expressed in motoneurons in the embryonic spinal cord, where it could be involved in motoneuron fate (42). Pax-6, a homeodomain-containing protein, also belongs to the family of specific transcription factors shared by neuronal and islet cells; it is expressed in the neuroretina and in the neural tube, but also in insulin- and glucagon-producing cells (43). Another hypothesis exists to explain the large number of similarities between neurons and beta cells; it is based on the possible common absence in these two cell types of a specific repressor. Such a repressor, present in all non-neuronal cells, would inhibit the expression of neuron-specific proteins. This is substantiated by the finding of a 25-base pair element in certain murine genes such as SCG10, synapsin I, and type II sodium channels (44). This 25-base pair element is believed to play the role of silencer after binding a specific repressor expressed in non-neuronal cells. Recently, such a repressor has been cloned (44). The demonstration of the lack of expression of this repressor in beta cells would help to understand the expression of a large number of neuronal markers within beta cells.

Acknowledgments—We thank Genentech, Inc. for supplying us with neurotrophin-3 and David Kaplan for anti-TrkC antibodies. We also thank Dr. Van Schravendijk for iodinated insulin and anti-insulin antibody.

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J. Biol. Chem. 1996, 271:10154-10160.
doi: 10.1074/jbc.271.17.10154

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