Formation of Heterodimers from Three Neurotrophins, Nerve Growth Factor, Neurotrophin-3, and Brain-derived Neurotrophic Factor*  

Tatsutomu Arakawa, Mitsuhiro Haniu, Linda O. Narhi, James A. Miller, Jane Talvenheim, John S. Philo, Hilary T. Chute, Christine Matheson, Josette Carnahan, Jean-Claude Louis, Qiao Yan, Andrew A. Welcher, and Robert Rosenfeld  

From Amgen Inc., Thousand Oaks, California 91320-1789  

(Received for publication, June 7, 1994)  

Three neurotrophic factors, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and nerve growth factor (NGF) form noncovalent homodimers in solution. Since they are highly homologous proteins, it seemed probable that two monomers of these proteins might associate together to form a heterodimer. This was tested by denaturing the two different proteins together in 6 M guanidine HCl and refolding them in phosphate-buffered saline. When the refolded mixture of BDNF and NT-3 was subjected to Mono S cation exchange chromatography, a new peak was observed eluting between NT-3 and BDNF, which accounted for about 30% of the protein used. This new protein species migrated as a single band upon native gel electrophoresis with mobility between that of the NT-3 homodimer and the BDNF homodimer, indicating that a complex had been formed. Sedimentation equilibrium data show that the dissociation constant of this heterodimer is $< 10^{-10}$ M. The heterodimer was stable upon incubation at 37°C in phosphate-buffered saline over 11 days. Having determined that the heterodimer is highly stable, it was subjected to various biological assays. Autophosphorylation assay using TrkB receptor showed that the heterodimer is indistinguishable from the BDNF or NT-3 homodimer in the ability to induce phosphorylation of the receptor. It was also indistinguishable from the homodimers in the neurotrophic activity using chick dorsal root ganglion explant. In the sympathetic neuron survival assay, the heterodimer behaved more similarly to NT-3, whereas in the dopamine uptake assay, it was intermediate between the two homodimers. In addition, the heterodimer was shown to be retrogradely transported in the dorsal root ganglion neurons. A heterodimer between NGF and BDNF is formed but much less effectively than the NT-3-BDNF heterodimer, and it is not stable even at 4°C. These results indicate that BDNF and NT-3 have an intersubunit contact surface for dimerization resembling each other’s but different from the contact surface of NGF.

Neurotrophin-3 (NT-3),¹ brain-derived neurotrophic factor (BDNF), and nerve growth factor (NGF) are members of a family of neurotrophic factors with extensive homology in their primary structures and strong conservation of the residues around their 6 cysteines, which are thought to form intrachain disulfide bonds (Barde, 1986; Leibrock et al., 1989; Maisonnierre et al., 1990; Hohn et al., 1990; Rosenthal et al., 1990; Ernfors et al., 1990; Jones and Reichardt, 1990; Acklin et al., 1990). The members of this family also form strong noncovalent dimers in solution (Angletti et al., 1971; Bothwell and Shooter, 1977; Radziejewski et al., 1992; Narhi et al., 1993), such that they are dimers at physiologically active concentrations.

The x-ray structure of murine β-NGF revealed a secondary structure rich in β-sheet but with no α-helix (McDonald et al., 1991). It has a flat, very hydrophobic face involved in dimer formation, which contains residues from noncontiguous areas of the amino acid sequence. Since BDNF, NT-3, and NGF have similar, although not identical, CD and infrared spectra (Radziejewski et al., 1992; Narhi et al., 1993) and an extensive sequence homology, it is expected that they have a similar intersubunit contact surface, and hence a combination of two proteins may form a heterodimer. In fact, recently, the formation of the heterodimer was observed by Radziejewski and Robinson (1993). Therefore, we carried out a study to test this possibility and found that a combination of BDNF and NT-3 form a stable heterodimer, whereas the formation of NGF-BDNF is less favorable and that of NGF-NT-3 heterodimer is marginal. Here we report the preparation and extensive characterization of the BDNF-NT-3 heterodimer. In this paper, we report studies of the interactions of this heterodimer and its parent homodimers with receptor extracellular domains.

EXPERIMENTAL PROCEDURES

Materials—Human recombinant NT-3 and BDNF were expressed in Chinese hamster ovary or Escherichia coli cells and purified by a combination of ion exchange, and reverse phase chromatographies.

Native Gel Electrophoresis—The native gel electrophoresis in a reversed polarity (migration toward anode) was performed using a Qiangen temperature gradient gel electrophoresis system and a 5% continuous polyacrylamide gel in 20 mM sodium acetate, 2 M urea, pH 4.5. The gel was cast onto a gel-supporting film supplied by Qiangen. Inclusion of urea does not affect the dimerization and conformation of the proteins, but it reduced smearing of protein bands. Samples were prepared by mixing the protein solution with concentrated sample buffer (without urea) to obtain final concentrations of 10 mM sodium acetate, pH 4.5, and 5% glycerol. Methyl green was included as a tracking dye. The gel was placed on a temperature-controlled plate and connected with electrode buffers using wicks. About 5–10 µl was loaded onto each well and subjected to electrophoresis at 20°C until the dye front migrated at least 2 cm into the gel. Then the gel was tightly covered with a plastic sheet and a plastic wrap to minimize evaporation. After the dye migrated to the bottom of the gel, the protein was fixed and stained with Coomassie Blue. The native gel electrophoresis was also performed using 2% agarose gel in 0.135 M glycine, 0.05 M sodium citrate, pH 5.6.
Samples were prepared similarly except for the buffer. The electrophoretic apparatus was immersed in an ice bath.

_Sedimentation Equilibrium—_Freshly prepared NT-3-BDNF heterodimer in PBS was further diluted into PBS, and six 120-μl samples were loaded into two charcoal-filtered Epon sedimentation equilibrium cells at loading concentrations of 20,000, 10,000, 5,000, and 2,500 μg/ml (five 1-ml samples were duplicated). The cells were centrifuged at 20,000 rpm for 24 h at 25 °C in a Beckman XL-A centrifuge with an AN-60Ti analytical rotor to establish sedimentation equilibrium. Scans of absorbance at 215 nm versus radius were taken at 20, 22, and 24 h, and the fact that they were indistinguishable proves that equilibrium was reached. The rotor speed was then increased to 32,000 rpm, and data were recorded at the new equilibrium after another 24 h. Finally, the rotor speed was increased to 48,000 rpm for 16 h in order to reduce the concentration through most of the cell to zero. A data set at 48,000 rpm was then recorded for use in establishing the true zero absorbance level for each cell.

Data analysis was carried out by simultaneous fitting of all data sets by the nonlinear least squares technique, using the program KDALTON developed in house. This analysis is functionally equivalent to that used in our earlier studies of the neurotrophin homodimers (Narhi et al., 1993). The data sets used in the analysis were for two samples each at loading concentrations of 10 and 5 μg/ml, at both 20,000 and 32,000 rpm and a 2.5 μg/ml sample at 32,000 rpm. For each sample, the data set at 48,000 rpm was fitted to a constant through the region where the meniscus had been completely depleted in order to establish the true zero absorbance level. This constant was then subtracted from the corresponding 20,000 and 32,000 rpm data to correct them for base-line offsets. The partial specific volume for the heterodimer was calculated from the amino acid composition as 0.7265 g/ml at 25 °C, and the density of PBS was measured to be 1.00394 g/ml.

_Circular Dichroism_—CD was performed on a Jasco J-810C spectropolarimeter controlled by a Samtron computer. Cuvettes with a path length of 1 cm (near UV) and 0.02 cm (far UV) were used. The results are expressed as molar ellipticity, assuming a mean residue weight of 113 for BDNF, 114.5 for NT-3, and 113.8 for the heterodimer. Protein concentrations were determined from the absorbance at 280 nm and 1-cm path length in PBS, using previously determined values of 1.6 for BDNF and 2.17 for NT-3 for 0.1% protein solutions and assuming a value of 1.88 for the heterodimer.

_Reverse Phase HPLC_—rp-HPLC was performed using a Waters 600E solvent delivery module, a 715 autosampler, and a Hewlett Packard 1040 M diode array detector with an HP Chemstation for data acquisition. The following buffers were used to develop gradients for elution: buffer A (0.1% (v/v) trifluoroacetic acid in water) and buffer B (90% acetonitrile, 0.1% trifluoroacetic acid in water). Samples were analyzed on a VyDAC C4 reverse phase column, 214TF54 (0.46 x 25 cm).

_Biological Activity_—For the chick DRG explant assay, ganglia were dissected from day 8 chick embryos and plated in a collagen matrix in a final volume of 2 ml. The samples were assayed by adding them directly to the culture medium in the test dish. For control samples, either pure BDNF or NT-3 (at 5 and 10 ng/ml) or PBS containing 1 mg/ml bovine serum albumin alone was added to the test dish. After 24 h at 37 °C, the ganglia were scored from 0 to 5 based on the basis of neurite length and density. The score for each sample is the average of five ganglia.

For the autophosphorylation assay, NIH3T3 cells expressing the human TrkB receptor were serum-starved then treated for 15 min at 37 °C with varying amounts of neurotrophin. The cells were lysed in radiolabeled precipitation buffer (0.15 M NaCl, 1% Nonidet P-40, 50 μM Tris- HCl, pH 8.0, 2 mM EDTA, 0.2 mM Na3VO4, 10 μg/ml aprotinin), and lysates were centrifuged at 10,000 g for 20 min. The supernatant was incubated with 1 μg of anti-TrkB antibodies, and complexes were detected using horseradish peroxidase-conjugated secondary reagents and chemiluminescence (ECL, Amersham).

The neurotrophins were also examined for their ability to sustain the survival of sympathetic neurons. Embryonic day 8 sympathetic nerve chains were dissected and placed in Ham’s F-14 nutrient mixture supplemented with 10% heat-inactivated horse serum and 1% l-glutamine-penicillin-streptomycin. Sympathetic chains were washed twice with PBS and trypsinized in 1 x trypsin-EDTA solution. After dissociation and washing, cells were plated on a 35-mm Petri dish for 3 h. After preplating, cells were counted, and 150 cells were plated in polycarbonate-laminin-coated chamber slide wells. Cells were plated in the presence of 10 ng/ml of BDNF, NT-3, or the heterodimer. Cells were also plated in the absence of any factors. Cell counts were made 3 days postplating and 7 days postplating. Survival activity was determined by the ratio of these two counts.

To examine the dopamine uptake of neurons grown in the presence of the neurotrophins, cultures of dopaminergic neurons were obtained from embryonic day 15 rats. The substantia nigra was microdissected and enzymatically dissociated, using a mixture of papain (20 units/ml) and DNase (100 units/ml). The dissociated tissue was brought into a single cell suspension by trituration through fire-polished Pasteur pipets, followed by sieving through 25-μm nylon mesh. After centrifugation (300 x g, 5 min), the cell pellet was resuspended in complete culture medium, containing of B27-supplemented neurobasal medium (Life Technologies, Inc.) containing 5% heat-inactivated horse serum, and then seeded at a density of 40,000 cells/6-mm well (96-well Falcon microplates) in 90 μl. The microplates were sequentially precoated with polyornithine (0.1 mg/ml) in 0.1 N boric acid for 1 h at room temperature) and mouse laminin (1 μg/ml) in PBS for 2 h at 37 °C. The neurotrophins were then added (10 μl/well) in PBS containing 0.1% bovine serum albumin. The following concentrations of BDNF, NT-3, and the heterodimer were used: 5, 50, 500, 1,250, 3,125, and 0.1 ng/ml.

For 5 days in culture, the substantia nigra cultures were tested for their ability to uptake dopamine. The cultures were rinsed in PBS and then incubated for 1 h at 37 °C in the presence of [3H]dopamine (50 nM; 100,000 cpm/well) in Krebs-Ringer solution supplemented with 1 mM ascorbic acid. After three washes with uptake buffer, the cells were lysed in 100 μl of 0.5% ethanol, and the radioactivity was determined by liquid scintillation in Ecolite.

The uptake of dopamine reflects the number of high affinity uptake sites per culture. Enhanced uptake can reflect an increase in the number of dopaminergic neurons per culture and/or the increased expression of dopamine transporters per neuron, and the dopamine uptake measurement is an indicator of both survival and maturation of dopaminergic neurons in substantia nigra cultures.

For the retrograde transport assay, the protein was iodinated by the lactoperoxidase method of Marchalonis (1969), and approximately 2–3 × 106 cpm in a 2-μl volume were injected into the right footpad of 2-day-old rat pups (n = 3). After approximately 23 h, the pups were perfused transcardially with fixative, and lumbar spinal columns containing the L4-L5 DRG (which send axon terminals to the hind limb) were processed for cryostat sectioning and cut in serial. Slides were dipped in photographic emulsion and allowed to expose for 2 weeks before developing and counterstaining.

**RESULTS AND DISCUSSION**

Whereas NT-3, NGF, and BDNF are very basic proteins with high isoelectric points, their charge states are slightly different at pH 8.5. During ion exchange chromatography using a Pharmacia Mono S column in 40 mM Tris-HCl, pH 8.5, NT-3 and NGF elute at a lower NaCl concentration than BDNF, whereas the difference in NaCl concentration for elution is very small between NGF and NT-3. This property was utilized to isolate heterodimers from their parent homodimers.

NT-3, NGF, and BDNF form extremely stable homodimers and do not exchange their subunits to form heterodimers at an appreciable rate as long as they are in nondenaturing buffers. Rosenfeld and Benedek (1993) and Narhi et al. (1993) have shown that the BDNF homodimer dissociates slowly even under strongly denaturing conditions. However, after dissociation BDNF reversibly assembles into a homodimer simply by diluting the dissociated protein into PBS (Phil et al., 1993). Therefore, the proteins were first denatured and dissociated and then mixed and refolded together to form the heterodimers as well as their respective homodimers.

**Formation of NT-3-BDNF Heterodimer**—A mixture of BDNF and NT-3 was denatured by 6 M guanidine-HCl (neutral pH or pH 3.0) at 37 °C for at least 4 h. The denatured molecules were then refolded by diluting 10-fold into PBS and dialyzed versus

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2 A. A. Welcher, manuscript in preparation.
The recovery of the protein depends little on the ratios is seen in all of the chromatograms and corresponds to a heterodimer of NT-3 and BDNF, as described below.

The percentages of these three peaks are summarized in Table I, are nearly the same. The value of 1.4 implies that $K_{AB}$ is not much different from $K_{AA}$ or $K_{BB}$. However, it is also possible that these fractions do not represent equilibrium values but rather simply reflect the probability of kinetic trapping of a folded monomer by another folded monomer during the rapid refolding process.

To further confirm the formation of heterodimers, the protein eluting in this peak was analyzed by native gel electrophoresis as shown in Fig. 2 (lane 4 of polyacrylamide gel or lane 6 of agarose gel). Only a single band is seen at a position between that of the NT-3 and BDNF bands (Fig. 2, lanes 2 and 1 of polyacrylamide gel or lanes 5 and 7 of agarose gel, respectively), indicating that this protein migrates in the native gel as a complex and that its charged state is the average of contributions of BDNF and NT-3. When BDNF and NT-3 were mixed at a 1:1 ratio in PBS and analyzed by native gel electrophoresis prior to denaturation and refolding, two bands corresponding to the individual homodimers were seen (Fig. 2, lane 3). The BDNF and NT-3 homodimers reformed during the refolding process migrated as their starting molecules. Amino acid sequence analysis further demonstrated that this protein peak contained BDNF and NT-3 in approximately equal ratios. When this species was treated with 6 M guanidine HCl and subjected to rp-HPLC analysis, both NT-3 and BDNF peaks were observed as shown in Fig. 3; NT-3 (first peak) and BDNF (second peak) are in almost equal amounts. When NT-3 or BDNF was analyzed in the same way, only the first or second peak was observed. (The guanidine HCl treatment was necessary to completely dissociate and unfold both proteins; without this treatment both BDNF and NT-3 give rise to two peaks in rp-HPLC caused by slow unfolding under these experimental conditions (Rosenfeld and Benedek, 1993).)

The stability of the NT-3-BDNF heterodimer in PBS at 37 °C was examined using mono S ion exchange column chromatography and the native gels. No detectable reconversion of the heterodimer to the corresponding homodimers was observed after up to 11 days of incubation. Radziejowski and Robinson (1993) also failed to see reconversion for this heterodimer in 24 h, unlike the other neurotrophin heterodimers they examined. However, they attributed this difference to lack of chromatographic resolution rather than to a true enhanced stability for the NT-3-BDNF heterodimer. We believe the present results

![Figure 1](image-url)
clearly show that this NT-3-BDNF heterodimer is unusually stable.

Biophysical Characterization of the NT-3-BDNF Heterodimer—Having established that NT-3 and BDNF form a stable heterodimer, we characterized the complex isolated as described above using CD and sedimentation equilibrium.

CD analysis of the heterodimer revealed a near UV CD spectrum equivalent to the average of the BDNF and NT-3 homodimers (Fig. 4). This suggests that the structures of the individual BDNF and NT-3 monomers are unaffected by the protein they are associated with in the dimers. The far UV CD spectrum of the heterodimer, however, is not the average, but more closely resembles BDNF than NT-3. The small amount of \( \alpha \)-helix seen in the NT-3 homodimer is missing from the heterodimer. This suggests that there is a slight perturbation of local secondary structure resulting either from heterodimer formation or from unfolding and renaturation.

The molecular weight of the complex and its possible dissociation into monomers were examined by sedimentation equilibrium. Data from nine combinations of concentration and rotor speed (2,043 total data points) were simultaneously analyzed using two different models. These data cover a range of concentration of heterodimer from approximately 0.7-40 \( \mu \)g/ml (3 \( \times \) 10^{-6} to \( 1.5 \times 10^{-6} \) M). The first model assumes that there is only a single species present, i.e. that there is no significant dissociation of the heterodimer over the concentration range studied. This model provides an excellent fit of the data and returns a best fit molecular mass for that single species of 27.11 kDa with a 95\% confidence interval of 26.91-27.31 kDa. Thus the best fit molecular weight is only 1.1\% lower than the sequence molecular weight of 27,401 for the heterodimer. Whereas the formal 95\% confidence interval from the fit does not quite include the sequence molecular weight, this confidence interval does not account for any uncertainty in the calculated partial specific volume of the protein used in the molecular weight calculation (see “Experimental Procedures”). Since using calculated partial specific volumes generally gives an uncertainty of 1-2\% in molecular weight, we conclude that the data are consistent with no dissociation of the heterodimer over this concentration range.

In the second analysis model, the heterodimer molecular weight is held fixed at the sequence molecular weight, but it is assumed to be in equilibrium with BDNF and NT-3 monomers, with a dissociation constant to be determined by fitting. This model provides a good fit to the data, with a variance 1.3\% higher than the single species fit. The best fit dissociation constant is \( 1.2 \times 10^{-10} \) M, with a 95\% confidence interval of \( 5.9 \times 10^{-11} \) to \( 2.9 \times 10^{-10} \) M. These dissociation constants imply that there is almost no dissociation over the range of concentrations measured (and are therefore consistent with the first analysis). In essence, this model tries to account for the measured molecular weight being \( \sim 1\% \) below the sequence molecular weight by allowing a small amount of dissociation. Again, this analysis does not account for possible errors in the assumed partial specific volume. Therefore, this analysis is best viewed as a way of estimating an upper limit for the dissociation constant of \( \sim 3 \times 10^{-10} \) M.

We conclude that the sedimentation equilibrium data are consistent with no detectable dissociation of the NT-3-BDNF heterodimer at concentrations down to <1 \( \mu \)g/ml (as low as can reliably be measured by this technique). It is important to note that because BDNF and NT-3 are so similar in molecular weight, these experiments cannot, in fact, distinguish between heterodimers and a 50:50 mixture of NT-3 and BDNF homodimers. However, since we have shown above that this heterodimer is stable for >11 days at 37 °C, it is reasonable to assume that little rearrangement into homodimers took place during these experiments.

Biological Activity of the NT-3-BDNF Heterodimer—Next, it was of interest to test whether the heterodimer retained biological activity and to compare this activity to that of the native BDNF and NT-3 homodimers. The neurotrophins generate responses in neurons that express specific receptors on the cell surface. Two types of receptors for the neurotrophins have been identified, the p75 receptor and the Trk family of tyrosine kinase receptors (Barbacid, 1993). Although the role of p75 in signal transduction is not understood, the Trk receptors are able to generate a cellular response in the absence of the p75
Characterization of NT-3-BDNF Heterodimer

One of the earliest responses of the Trk receptors upon ligand binding is the autophosphorylation of tyrosine residues in the intracellular domain of the receptor. This autophosphorylation of the receptor is responsible for generating intracellular responses (Ullrich et al., 1990). BDNF interacts with the TrkB receptor, whereas NT-3 can interact with either the TrkB or TrkC receptors (Ip et al., 1993). Since the TrkB receptor is capable of being activated by either BDNF or NT-3, this receptor was examined for its ability to be autophosphorylated in response to heterodimer binding. NIH3T3 cells, expressing the full-length human TrkB receptor, were briefly treated with increasing concentrations of the neurotrophins or heterodimer, and the autophosphorylation of the receptor was examined. The heterodimer was able to induce autophosphorylation at a concentration of 1 ng/ml (Fig. 5), which was comparable to the native BDNF and NT-3. Although this method is not quantitative, it is clear that the heterodimer has similar activity to the homodimers both in the dose-response curve and in the maximal response. In parallel experiments, NGF was unable to induce autophosphorylation of the TrkB receptor, as would be expected based on the binding selectivity of the Trk receptors (data not shown).

Since it appeared that the NT-3-BDNF heterodimer was able to induce TrkB autophosphorylation similar to that of BDNF and NT-3, it seemed possible that it would be active on neurons. To test this, the heterodimer was assayed for neurotrophic activity using the chick DRG explant assay. Prior to bioassay, the samples were diluted to 200 ng/ml in PBS containing 1 mg/ml bovine serum albumin. All samples were assayed at final concentrations of 5 ng/ml and 10 ng/ml; results are shown in Table II. Within the limits of the bioassay error, the activity of the heterodimer was not significantly different from the activity of native BDNF or NT-3.

Sympathetic neurons have been recently reported to survive in the presence of NT-3 in culture (Dechant et al., 1993), whereas no effect of BDNF has been reported. This provides a system where NT-3 could act as the positive control and BDNF could act as the negative control for the heterodimer. After 7 days in culture with or without 10 ng/ml neurotrophins, survival rates were as follows: BDNF, 14%; the heterodimer, 36%;...
TABLE II

The activity of the NT-3-BDNF heterodimer in the chick DRG explant assay

| Sample        | DRG response (mean ± S.D.) |
|---------------|-----------------------------|
|               | 5 ng/ml                     | 10 ng/ml                    |
| NT-3 control  | 4.4 ± 0.6                   | 4.4 ± 0.6                   |
| BDNF control  | 3.8 ± 0.3                   | 4.1 ± 0.2                   |
| Heterodimer 1 | 3.2 ± 0.6                   | 3.8 ± 0.7                   |
| Heterodimer 2 | 2.4 ± 0.4                   | 3.6 ± 0.4                   |

NT-3, 38%; no factors, <1%. The results demonstrate that the survival activity of the heterodimer is comparable to that of NT-3 in chick embryonic day 8 sympathetic culture. The NT-3 survival activity also agrees with the published data (Dechant et al., 1993).

The treatment of substantia nigra cultures with BDNF resulted in a dose-dependent increase of dopamine uptake after 5 days in culture, as shown in Fig. 6. The maximal activity was approximately 2-fold the level of control cultures and was reached with doses of 30–8,000 ng/ml. The half-maximal concentration for BDNF was approximately 0.5 ng/ml. NT-3 was also able to increase dopamine uptake, although it was considerably less potent than BDNF; the maximal activity was 1.5-fold the control level and required concentrations higher than 1,000 ng/ml. The half-maximal concentration for NT-3 was 20–30 ng/ml.

Treatment of the cultures with the NT-3-BDNF heterodimer resulted in a 1.6-fold increase in dopamine uptake. This maximal activity was obtained with heterodimer concentrations of 8–8,000 ng/ml. The half-maximal for the heterodimer was 0.1–0.2 ng/ml. These results indicate that the three neurotrophins are active in stimulating the uptake of dopamine in cultures of embryonic rat substantia nigra, with BDNF > heterodimer > NT-3. The comparison of the half-maximal concentrations suggests that the heterodimer may be closer to BDNF, probably by activating TrkB.

Members of the neurotrophin family are retrogradely transported in vivo by DRG neurons after injection into the footpad of neonatal animals (Yan et al., 1991). A demonstration of retrograde transport of ligands, therefore, suggests a potential for biological actions. We examined whether the heterodimer underwent retrograde transport by injecting iodinated heterodimer into the footpad of neonatal rats and examining histological preparations of the DRG using emulsion autoradiography.

Fig. 7 shows a dark field photograph of the right L5 DRG of a heterodimer-injected pup, indicating that DRG neurons retrogradely transported the iodinated heterodimer as demonstrated by the dense accumulation of silver grains over a subpopulation of the DRG neurons (magnification × 1,000).

Fig. 6. Effect of BDNF, NT-3, and NT-3-BDNF heterodimer on dopamine uptake in cultures of rat substantia nigra. [3H]dopamine uptake was determined in cultures treated for 5 days with various concentrations of the neurotrophins. Each value is the average of two determinations from two individual wells. ○, BDNF; △, heterodimer; NT-3.

Fig. 7. Dark field autoradiographs of the right L5 DRG injected with iodinated NT-3-BDNF heterodimer (A) or cytochrome c (B). Retrograde transport of the heterodimer is made evident by the dense accumulation of silver grains over a subpopulation of the DRG neurons (magnification × 1,000).
proteins used formed the heterodimer, suggesting that the formation of this heterodimer is less thermodynamically favorable than that of the BDNF-NT-3 heterodimer. The stability was also lower; about 20% of the heterodimer converted to the corresponding homodimers within 2 weeks at 4 °C. Freezing at -75 °C in PBS containing 4% glycerol showed no apparent conversion of this heterodimer.

CONCLUSION

It is evident that BDNF and NT-3 form a highly stable heterodimer, whereas the formation of heterodimers involving NGF is extremely unfavorable. This indicates that NT-3 and BDNF have intersubunit contact surfaces resembling each other's but different from that of NGF. In vitro receptor binding using the soluble TrkB and TrkC receptors showed that the heterodimer binds these receptors (in this issue) (Philo et al., 1994). It will be of interest to test these heterodimers on other types of neurons to explore the full range of activities they possess. In addition, the use of these heterodimers should shed light on the interaction of the neurotrophins with the Trk receptors as well as help to identify which mechanisms are responsible for the in vivo discrimination of different neurotrophins by their receptors.

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