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Binding Characteristics of Brain-Derived Neurotrophic Factor to Its Receptors on Neurons from the Chick Embryo

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Brain-derived neurotrophic factor (BDNF), a protein known to support the survival of embryonic sensory neurons and retinal ganglion cells, was derivatized with ^{125}I-Bolton-Hunter reagent and obtained in a biologically active, radioactive form (^{125}I-BDNF). Using dorsal root ganglion neurons from chick embryos at 9 d of development, the basic physicochemical parameters of the binding of ^{125}I-BDNF with its receptors were established. Two different classes of receptors were found, with dissociation constants of 1.7×10^{-11} M (high-affinity receptors) and 1.3×10^{-9} M (low-affinity receptors). Unlabeled BDNF competed with ^{125}I-BDNF for binding to the high-affinity receptors with an inhibition constant essentially identical to the dissociation constant of the labeled protein: 1.2×10^{-11} M. The association and dissociation rates from both types of receptors were also determined, and the dissociation constants calculated from these kinetic experiments were found to correspond to the results obtained from steady-state binding. The number of high-affinity receptors (a few hundred per cell soma) was 15 times lower than that of low-affinity receptors. No high-affinity receptors were found on sympathetic neurons, known not to respond to BDNF, although specific binding of ^{125}I-BDNF to these cells was detected at a high concentration of the radioligand. These results are discussed and compared with those obtained with nerve growth factor on the same neuronal populations.

Brain-derived neurotrophic factor (BDNF) is a small basic protein known to support the survival of some embryonic vertebrate neurons *in vitro* and *in vivo* (Barde et al., 1982, 1987; Hofer and Barde, 1988). In some respects, it resembles the well-known protein nerve growth factor (NGF): both its size (M_r 12,300) and charge (pI, ~10.0) are similar to the monomer of NGF. However, many, but not all, neuronal populations supported by these 2 proteins are different. For example, NGF typically supports the survival of sympathetic neurons, whereas BDNF does not. Conversely, BDNF supports the survival of placode-derived sensory neurons or rat retinal ganglion cells, whereas NGF does not (see Barde et al., 1987, for a review). Some sensory neurons in the dorsal root ganglia of the chick embryo are affected by both proteins. In this case, it has been hypothesized that NGF is transported to the cell bodies from the peripheral nerve endings and BDNF from the central ones and that this dual supply is necessary for the survival of some primary sensory neurons (Lindsay et al., 1985; Davies et al., 1986). Support for this idea has come from the following experiments: using embryonic chick spinal sensory neurons of 6 d, a large proportion of the neurons can be kept alive *in vitro* by using saturating amounts of either factor alone (Lindsay et al., 1985). *In vivo* also, both NGF and BDNF are able, when given separately, to increase neuronal numbers (by prevention of naturally occurring neuronal death) in quail dorsal root ganglia (Hofer and Barde, 1988). In addition, cutting the peripheral or the central axons of the newborn rat spinal ganglia results in the death of neurons (Yip and Johnson, 1984). In the chick, when the central target structure (the neural tube) of these ganglia is removed, or the connection between the DRG neurons and the spinal cord is interrupted by a mechanical barrier, the corresponding neurons degenerate (Teillet and Le Douarin, 1983; Kalcheim and Le Douarin, 1986). The presence of BDNF adsorbed onto such a barrier results in the transitory survival of dorsal root ganglion cells distal to the barrier, provided this barrier is first coated with laminin (Kalcheim et al., 1987).

Since the binding of BDNF to its receptor is the first step in the mediation of its physiological effects, it is important to determine the basic physicochemical characteristics of this interaction. We report here on the presence of 2 types of receptors: high-affinity ones, present on spinal sensory but not sympathetic neurons, and low-affinity ones on both. These results are discussed and compared with those obtained with NGF, also known to have 2 types of receptors (Sutter et al., 1979; Landreth and Shooter, 1980; Bernd and Greene, 1984; Sonnenfeld and Ishii, 1985).

Materials and Methods

Preparation of dorsal root ganglia (DRG) neurons. Fertilized chicken eggs were incubated in a humidified atmosphere at 38°C. Nine-d chick embryos were used throughout this work. DRG were dissected out and neurons prepared as described by Sutter et al. (1979) and Vale and Shooter (1985) with some modifications. Briefly, about 300–600 ganglia were collected into ice-cold, Ca^{2+}, Mg^{2+}-free Gey's buffer (Gey and Gey, 1936). Ganglia were washed by a short centrifugation and resuspended into 5 ml of the same buffer (37°C). DNase II (Sigma) and trypsin (Worthington) were then added at final concentrations of 0.012 and 0.12 mg/ml, respectively, and digestion was for 10 min at 37°C. Trypsin action was terminated by the simultaneous addition of soybean trypsin inhibitor (0.15 mg/ml; Sigma) and 0.55 ml horse serum (Gibco). Ganglia were then mechanically dissociated by gentle aspiration through a wide-bore 5 ml pipet (5 strokes). Undissociated ganglia were allowed to settle for 3–5 min, the supernatant decanted and saved, and the undissociated ganglia resuspended in 2.7 ml Gey's buffer containing 0.3 ml horse

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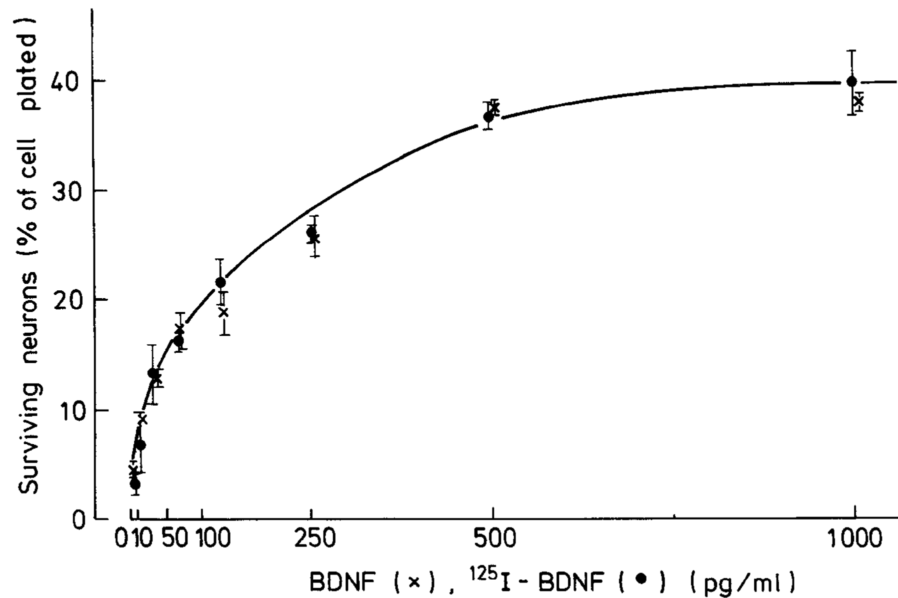

Figure 1. Comparison of the biological activities of ^{125}I-BDNF (●; mean ± SD of triplicate determinations) and unlabeled BDNF (×; mean ± SD of duplicate determinations). DRG neurons from 9-d chick embryos were prepared and plated on laminin as described (Lindsay et al., 1985) and counted after 24 hr. Both labeled and unlabeled BDNF display the same biological activity: half-maximal survival is obtained with about 80 pg/ml (6.6×10^{-12} M) and maximal with 500 pg/ml (4.1×10^{-11} M).

serum. Mechanical dissociation was carried out as before (8–9 strokes). The latter dissociation procedure was repeated twice more (10 and 10 strokes). Combined supernatants were filtered through a nylon net (40 μm pore size) in order to remove the remaining clumps. The cell suspension was then preplated onto four to six 10 cm Nunc (uncoated) tissue culture dishes for 2 hr at 37°C in a water-saturated atmosphere containing 10% CO_2. This preplating step was used to enrich the cell population for neurons and also to allow for recovery of cell-surface receptors possibly affected by the trypsin digestion. Dissociation of the ganglia without prior digestion with trypsin led to much lower yield of viable cells. The nonattached cells were collected and centrifuged at 100 × *g* for 10 min. Pelletted cells were resuspended in the binding assay buffer (see below) at a concentration of $1–1.5 \times 10^6$ cells/ml. The average of cell yield was considered satisfactory: 8000 cells per ganglion were obtained on average. It has been reported that there are 12,000 neurons in the 25th DRG (one of the largest DRG) of 9-d chick embryo (Levi-Montalcini and Levi, 1943). Cell viability was 80–90% as judged by trypan blue exclusion, and >90% of the cells were neurons by morphological criteria.

Preparation of sympathetic neurons. Both lumbar and thoracic sympathetic chains were dissected and dissociated using the procedure described for the DRG neurons.

Preparation of BDNF. BDNF was purified from adult pig brains as described by Barde et al. (1982) with the modifications described in Hofer and Barde (1988).

Preparation of ^{125}I-BDNF. One microgram of BDNF was dissolved in 30 μl 50 mM sodium borate buffer, pH 8.75. The solution was transferred to a vial containing 2 mCi radioiodinated Bolton-Hunter reagent (2000 Ci/mmol; New England Nuclear) and dried before use. The reaction solution was first incubated at 1.5 hr at 0°C and then 1.5 hr at room temperature. The reaction was terminated by the addition of 10 μl of a glycine solution (10 mM final concentration). Fifteen minutes later, 50 mM sodium acetate buffer, pH 4.0, containing 150 mM NaCl and 0.5% (wt/vol) BSA, was added to the reaction solution to yield a final volume of 200 μl. The final mixture was filtered through a Biogel P6 (Biorad) column (20 × 0.3 cm) previously equilibrated with the acetate buffer. Elution was performed with the same buffer at a flow rate of 0.2 ml/min. Fractions of 150–170 μl were collected. The derivatized protein appeared as a clearly defined peak of radioactivity corresponding to the exclusion volume of the column. Ninety percent of this material was TCA precipitable and the yield (when compared with the TCA precipitable fraction before the column) ranged between 25 and 35%. Around 8% of the eluted radioactivity was found in the exclusion volume. In 8 different preparations, the specific activity was between 300–600 cpm/pg BDNF. The radioiodinated factor was stored dissolved in the elution buffer at 4°C. No loss of biological activity of ^{125}I-BDNF was detected within 4 weeks after iodination. However, in the experiments reported here, ^{125}I-BDNF was used only up to 10 d after preparation.

Binding assays. Either sensory or sympathetic neurons were resuspended in Krebs-Ringer-HEPES buffered solution, pH 7.37 (Herrup and Thoenen, 1979), containing 5 mg/ml BSA and 0.1 mg/ml horse heart cytochrome C (Serva), which slightly decreased the nonspecific binding of ^{125}I-BDNF. In addition, cytochrome C is a particularly good control for the specificity of the binding of BDNF to its receptors: both on SDS-gel electrophoresis and gel filtration in the presence of 0.1% trifluoroacetic acid, its apparent molecular weight is identical to that of BDNF. The isoelectric points of both proteins are very basic, and finally, on reverse-phase HPLC, both proteins elute at very similar acetonitrile concentrations. In all experiments reported here, incubations of the cell suspension with ^{125}I-BDNF were carried out at 5 ± 1°C, with gentle shaking in order to prevent sedimentation of the cells. Binding mixtures, usually 0.4–1.0 ml, were contained in 1.5-cm-wide, round-bottom, polypropylene tubes. Cell-bound radioactivity was collected by rapidly sedimenting the cells (50–150 μl binding mixture) through a 2-step sucrose gradient: 100 μl 0.15 M sucrose in Krebs-Ringer-HEPES without added proteins (upper layer) and 100 μl 0.3 M sucrose in the same buffer (lower layer) (Herrup and Shooter, 1973; Vale and Shooter, 1985). After centrifugation in a Beckman microfuge (10,000 rpm for 60 sec), the tubes were immediately frozen on an acetone–dry ice mixture. The bottom (6 mm), of each tube was cut off and counted (cell-bound radioactivity) separately from the rest of the tube (unbound radioactivity). Samples were counted in a Berthold gamma-counter with a counting efficiency of 60%. At least 3000 counts were measured. The amount of ^{125}I-BDNF bound is expressed here as fmoles bound/10^6 cells. Specific binding was determined by subtracting the nonspecific binding from the total binding. Nonspecific binding was measured by incubating the cells with a 100-fold excess of unlabeled BDNF for 30 min before adding ^{125}I-BDNF. It was found that nonspecific binding increased linearly with the concentrations of ^{125}I-BDNF and was subtracted from the total counts. All experimental points (both samples and controls) were done in 3 or 4 determinations.

Steady-state binding. Cell suspensions were incubated with different concentrations of ^{125}I-BDNF (from 4×10^{-12} to 10^{-9} M) for 50 min. The incubation was terminated by centrifugation as described above.

Kinetics of association. ^{125}I-BDNF, at concentrations ranging from 4×10^{-12} to 3×10^{-10} M, was added to the cell suspension previously incubated for 30 min either with or without a 100-fold excess of cold BDNF. Aliquots of 3 or 4 × 100 μl were taken from the reaction mixture at different times after the addition of the radioactive factor. The aliquots were immediately overlaid on the sucrose gradients and centrifuged.

Kinetics of dissociation. ^{125}I-BDNF, at concentrations ranging from 5×10^{-11} to 8×10^{-10} M, was added to the cell suspension. The binding mixtures were incubated for 50 min in order to achieve equilibrium conditions. Then, a 100-fold excess of cold BDNF was added and aliquots (4 × 100 μl) were taken and immediately centrifuged.

Treatment of steady-state and kinetic data. Data were treated using the standard formula for the binding of a ligand to its receptor:

$$(L) + (R) \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}},$$

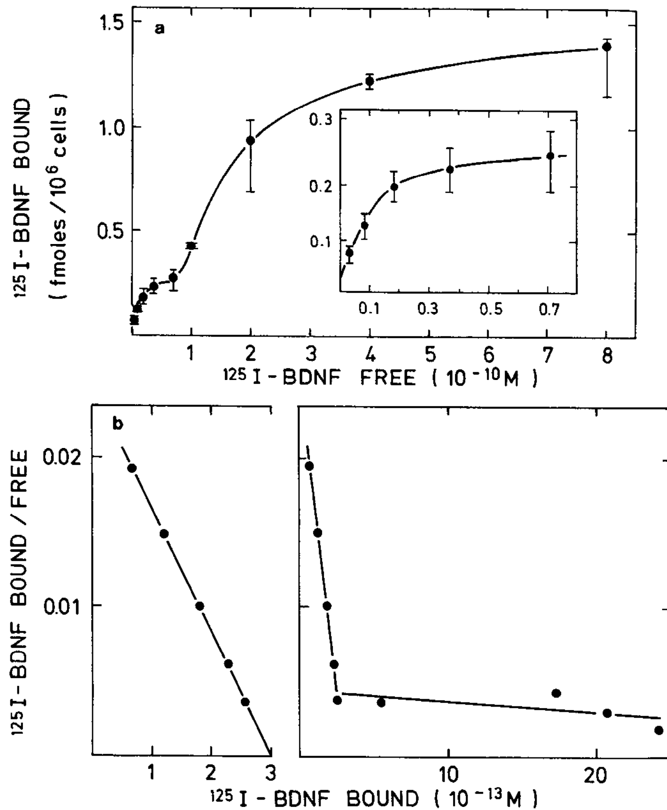

Figure 2. Binding of ^{125}I-BDNF to sensory neurons. Cells (10^6/ml) were incubated at 5°C for 50 min with various concentrations of ^{125}I-BDNF. For each experimental point, 3 aliquots of 100 μl each were processed. Specific binding was determined by subtracting nonspecific binding (measured in the presence of a 100-fold excess of unlabeled BDNF) from total binding. *a,* Saturation curve. For each point, the mean and the range value are indicated. *b,* Scatchard plot of the data. The average values (3 experiments) for the dissociation constants were: K_d(I), 1.7×10^{-11} M; K_d(II), 1.3×10^{-9} M. The mean value of high-affinity binding sites was 234 per cell and that of low-affinity, 3175 per cell.

where L is BDNF (native or iodinated), R its receptor on neurons, k_{+1} the on-rate, and k_{-1} the off-rate. (L) was always in excess over (R) and considered not to change throughout the duration of the experiment. Therefore, the binding process was considered a pseudo-first-order reaction. The experimental determination of (RL) at different (L) allowed the construction of Scatchard plots (Scatchard, 1949). From them, dissociation constants, as well as number and classes of receptors, were deduced. Association rates were calculated as follows: (LR) was determined at several reaction times before the equilibrium was reached using also several concentrations of L. From the exponential curves obtained, the observed rates (k_{ob}) were obtained by determining the time at which (RL) reached half of the equilibrium value according to the equation $kob = \ln 2/t$. In some cases, k_{ob} was deduced from the slope of $\ln[(RL)_{eq}/(RL)_{eq} - (RL)]$ versus time. Applying $k_{ob} = k_{+1}(L) + k_{-1}$, the association constant, k_{+1} was determined by plotting k_{ob} versus (L). In one case (low-affinity receptors), k_{+1} was determined according to the equation $\omega = k_{+1}t = [\ln(\beta(2x + \gamma)/\gamma(2x + \beta))]/\sqrt{Q}$, where $Q = D^2 - 4ab$, $\beta = D + \sqrt{Q}$, $\gamma = D - \sqrt{Q}$, $D = (-a-b-c)$; a = the initial concentration of ^{125}I-BDNF (7×10^{-10}M); b = the initial concentration of low-affinity receptors (5.3×10^{-12} M, from Fig. 2*b*); $c = (a - x_e)(b - x_e)/x_e$, where x_e = ^{125}I-BDNF bound at equilibrium, t = time (min), x = ^{125}I-BDNF bound at time t (Shiu and Friesen, 1974). This equation allows k_{+1} to be determined from a time course of binding at a single ligand concentration. Given the relatively high number of low-affinity receptors (Fig. 2) and the slow kinetics of association (Fig. 5*a*), it was felt that the data points could be determined with a precision justifying the use of this equation (see also Godfrey and Shooter, 1986). The dissociation rates (k_{-1}) were deduced by direct experimental measurements as described above. (RL) decay was measured at different times after the addition of an excess of cold BDNF. k_{-1} was obtained from $\ln[(RL)/(RL)_{eq}] = -k_{-1}t$. Where necessary, straight lines were fitted to the experimental points by the least-squares method using a linear-regression program.

Results

Preparation of ^{125}I-BDNF

Preliminary experiments using solid surface iodination with both chloramin T and lactoperoxidase revealed that, even with the mild oxidative condition used for the direct derivatization of tyrosine residues, BDNF lost its biological activity. However, the coupling of ^{125}I-Bolton-Hunter reagent to the amino groups of BDNF resulted in a fully active preparation (Fig. 1) with a specific activity that was high enough to be used for binding studies (300–600 cpm/pg).

Steady-state binding of ^{125}I-BDNF to DRG neurons

We used whole-cell preparations at 5°C in order to avoid complex kinetics resulting from internalization following binding (Carpenter and Cohen, 1976; Goldstein et al., 1979; Sutter et al., 1979; Olender and Stach, 1980; Olender et al., 1981). Neurons were incubated with ^{125}I-BDNF for 50 min, allowing equi-

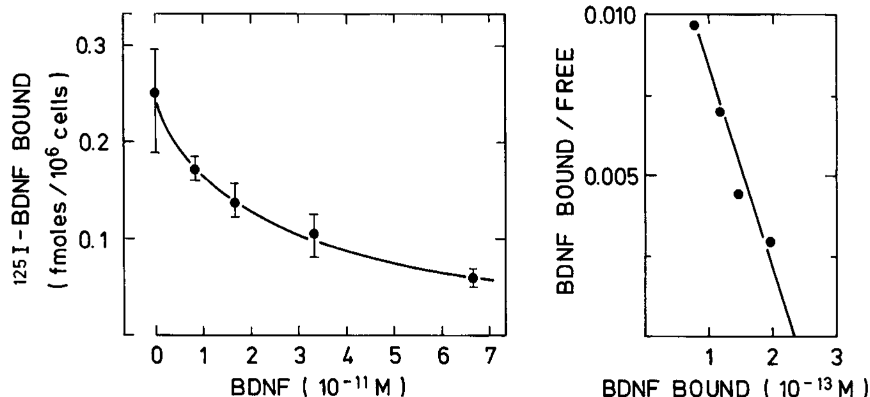

Figure 3. *a,* Inhibition of binding of ^{125}I-BDNF to DRG neurons by various concentrations of unlabeled BDNF (mean and range values). *b,* Scatchard plot of the data. Cells (1.5×10^6/ml) were first incubated with unlabeled BDNF at different concentrations for 50 min at 5°C. Then, ^{125}I-BDNF at a concentration of 7×10^{-11} M (sufficient to saturate the high-affinity binding sites only) was added to the binding mixture, and the incubation was continued for a further 30 min. Free and bound ^{125}I-BDNF were separated by centrifugation. For each experimental point, 4 aliquots of 100 μl each were processed. No specific binding was measured in the presence of 8×10^{-9} M unlabeled BDNF. The average values (4 experiments) for K_d(I) was 1.2×10^{-11} M and the average number of binding sites was 192.

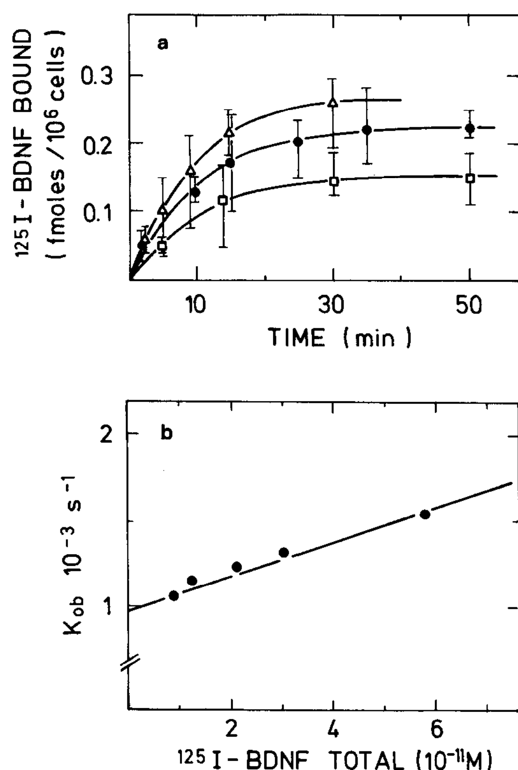

Figure 4. Association rates of ^{125}I-BDNF to the high-affinity receptors on DRG neurons. *a*, Time-course curves of the specific binding of ^{125}I-BDNF to neurons. Cells (10^6/ml) were incubated at 1.1×10^{-11} (□), 2.1×10^{-11} (●), and 5.8×10^{-11} M (△). At the times indicated, 4 aliquots of 100 µl each were processed for the separation of free and bound ^{125}I-BDNF. *b*, Observed association rates (k_{ob}) of ^{125}I-BDNF as a function of the concentration of ^{125}I-BDNF. A second-order association rate constant, k_{+1}, of 1.0×10^7 M^{-1} sec^{-1} was obtained from the slope.

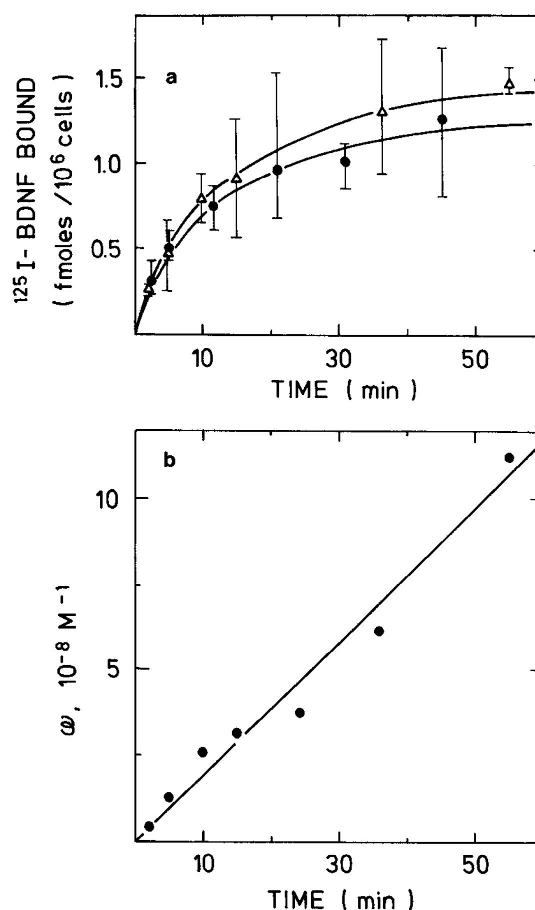

Figure 5. Association rates of ^{125}I-BDNF to the low-affinity receptors on DRG neurons. *a*, Time-course curves of the specific binding of ^{125}I-BDNF at concentrations of 4.8×10^{-10} (●) and 7.1×10^{-10} M (△). (See legend of Fig. 4 for further details.) *b*, Data from the upper curve replotted to obtain k_{+1}(II) according to Shiu and Friesen (1974) (see also Material and Methods). The value obtained from the slope of the line was 3.1×10^5 M^{-1} sec^{-1}.

librium to be reached (Fig. 3). Figure 2*a* shows the results obtained with ^{125}I-BDNF at concentrations between 4×10^{-12} and 8×10^{-10} M; Figure 2*b* shows the Scatchard plot (Scatchard, 1949) of the binding data of Figure 2*a*. The analysis revealed 2 saturable binding components with equilibrium dissociation constants (K_d) differing by 2 orders of magnitude. The average values (3 experiments) were 1.7×10^{-11} M, K_d(I), and 1.3×10^{-9} M, K_d(II). Scatchard plot analysis further revealed that there were 234 high-affinity binding sites per cell soma, while there were considerably more low-affinity binding sites, about 3175. In order to know if unlabeled BDNF and ^{125}I-BDNF behave similarly, neurons were incubated with various concentrations of cold BDNF for 50 min and then with a concentration of ^{125}I-BDNF high enough to saturate the high-affinity binding sites only (when not filled by the unlabeled factor). Figure 3*a* shows the results of such an experiment, with the Scatchard analysis of the data (Fig. 3*b*). The mean value of 4 experiments of this kind revealed a dissociation constant of 1.2×10^{-11} M for unlabeled BDNF, which is essentially identical with that determined for ^{125}I-BDNF, 1.7×10^{-11} M (see above). The mean number of high-affinity receptors was also very similar, 192.

Kinetics of association of ^{125}I-BDNF to DRG neurons

The association of ^{125}I-BDNF to neurons was quantified as a function of time using concentrations of ^{125}I-BDNF ranging between 0.9 and 5.8×10^{-11} M (Fig. 4*a*). Within this range, again mostly the high-affinity receptors were analyzed. The experimental observed rates (k_{ob}) deduced from the type of curves shown in Figure 4*a* for 3 ^{125}I-BDNF concentrations were plotted as a function of the concentration of ^{125}I-BDNF used (Fig. 4*b*, 5 different concentra-tions). As can be seen, the observed association rates were dependent on the concentrations of the ligand. An association rate constant, k_{+1}(I), of 1.0×10^7 M^{-1}sec^{-1} was obtained from the slope of the line of Figure 4*b*. This value is close to the rate of a diffusion controlled step according to the size of the ligands and the temperature used for the binding studies.

Using a higher concentration of ^{125}I-BDNF (7×10^{-10} M), the association rate constant to the low-affinity binding sites (present in considerably larger numbers, see Fig. 2*b*) was determined. The top time-course curve of Figure 5*a* was replotted (Fig. 5*b*) to determine the second-order association constant, k_{+1}(II) (Shiu and Friesen, 1974). A low value of 3.1×10^5 M^{-1}sec^{-1} was obtained [compared with k_{+1}(I)]. The slow association rate of ^{125}I-BDNF to the low-affinity binding sites of sensory neurons seems to be, therefore, a factor responsible for the considerably lower affinity of the low-affinity binding sites. However, it should be borne in mind that, in determining this association constant, there must be a component due to the (much more rapid) bind-

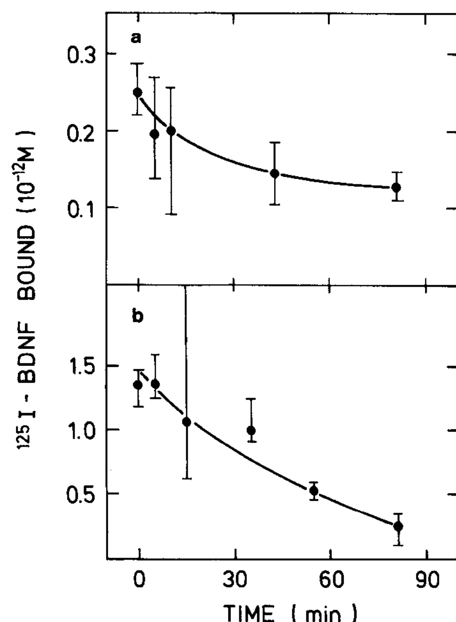

Figure 6. Dissociation rates of bound ^{125}I-BDNF from DRG neurons. Cells (1.5 × 10^6/ml) were incubated with (*a*) 6.6 × 10^{-11} and (*b*) 7.7 × 10^{-10} M ^{125}I-BDNF for 50 min at 5°C. Dissociation was started in both cases by the addition of a 100-fold excess of unlabeled BDNF. Four aliquots of 100 μl each were taken at different times and processed for the separation of bound from free ^{125}I-BDNF. For each point, the mean and range values are indicated. Dissociation rate constants were deduced from the exponential decay of bound ^{125}I-BDNF. The average values (2 experiments each) were k_{-1}(I) 1.1 × 10^{-4} and k_{-1}(II) 3.0 × 10^{-4} sec^{-1}.

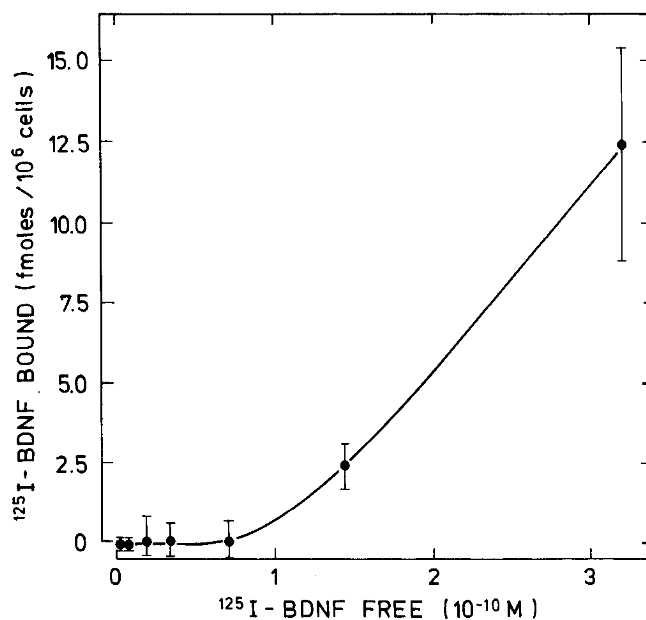

Figure 7. Specific binding of ^{125}I-BDNF to sympathetic neurons from chick embryos. Cells (10^6/ml) were incubated at 5°C for 50 min with various concentrations of ^{125}I-BDNF. For each experimental point, 4 aliquots were processed.

ing of ^{125}I-BDNF to the high-affinity binding sites. Therefore, the value assigned to k_{+1}(II) may be somewhat overstimated.

Kinetics of dissociation of ^{125}I-BDNF from DRG neurons

Dissociation of labeled BDNF from its receptors was determined after the addition of a large excess of unlabeled BDNF to a suspension of neurons with bound ^{125}I-BDNF after equilibrium conditions had been reached. Figure 6 shows 2 experiments done using 2 different concentrations of ^{125}I-BDNF. In the first (Fig. 6*a*), with 6.6 × 10^{-11} M, it was expected that the radioligand was mostly bound to high-affinity binding sites. The observed dissociation occurred with a first-order rate, k_{-1}(I), of 1.1 × 10^{-4} sec (mean of 2 experiments). The division of this value by that of k_{+1}(I) gave a theoretical K_d(I) of 1.1 × 10^{-11} M, which is very close to the experimental K_d(I) obtained from equilibrium binding studies, 1.7 × 10^{-11} M. When cells were incubated with ^{125}I-BDNF at a higher concentration, 7.7 × 10^{-10} (Fig. 6*b*), it was predicted that 80–85% ^{125}I-BDNF bound at the equilibrium would occupy low-affinity binding sites (see Fig. 2). The rate of dissociation was clearly faster than that observed when the radioligand dissociated from high-affinity binding sites: a k_{-1}(II) of 3.0 × $10^{-4}$$sec^{-1}$ was obtained. The division of this value by the experimentally determined k_{+1}(II) (3.1 × 10^5 M^{-1} sec^{-1}) gave a theoretical K_d(II) of 9.7 × 10^{-10} M, which is very close to the experimental value of 1.3 × 10^{-9} M deduced from equilibrium binding experiments. But again, it should be pointed out that the dissociation curve from the low-affinity receptors should be somewhat influenced by the slower dissociation of ^{125}I-BDNF from the high-affinity receptors (15–20% of the total). However, the experimental data do not allow discrimination of a biphasic exponential.

Binding at the equilibrium of ^{125}I-BDNF to sympathetic cells

Sympathetic cells are not responsive to BDNF in terms of survival or neurite outgrowth (Barde et al., 1982; Lindsay et al., 1985). Therefore, it was of interest to see if these neurons had receptors for ^{125}I-BDNF. Figure 7 shows that no binding was seen in the range of concentrations where ^{125}I-BDNF binds to the high-affinity receptors on sensory neurons. At higher concentrations, specific binding was observed in a range that could correspond to the low-affinity receptors found on sensory neurons. Saturation was not achieved using this range of concentrations, the highest being 3 × 10^{-10} M.

Discussion

The main result of this study is that BDNF binds to one of its typical targets cells, DRG neurons, by interacting with 2 classes of receptors: high-affinity (K_d = 1.7 × 10^{-11} M) and low-affinity (K_d = 1.3 × 10^{-9} M). No high-affinity receptors were found on sympathetic neurons.

A prerequisite to the present study was the labeling of BDNF with a sufficiently high specific activity. This could be accomplished using the derivatization of BDNF with the Bolton-Hunter reagent. This procedure allowed a full preservation of BDNF's biological activity (Fig. 1). In addition, labeled and unlabeled BDNF behaved in a similar way in binding experiments (Fig. 3). It is important to note that the dissociation constants for the 2 receptors have been established using both steady-state and kinetic experiments and that the values obtained in the 2 sets of experiments are essentially identical. Whereas the significance of low-affinity receptors cannot be assessed at present, that of high-affinity ones seems easy to explain. First, this affinity is well within the range of concentrations at which BDNF exerts its activity (half-maximal neuronal survival is seen with about 7 × 10^{-12} M BDNF). Second, sympathetic neurons, which are not affected by BDNF in terms of survival or fiber outgrowth, do not display such high-affinity receptors. Thus, it is tempting

to speculate that the survival and fiber outgrowth-promoting activity of BDNF requires the presence of these high-affinity receptors.

Some DRG neurons represent not only a common target for BDNF and NGF (see introductory remarks), but they also have been shown to possess 2 classes of NGF receptors. Furthermore, the dissociation constants for NGF reported by Sutter et al. (1979) are very close to those reported here for BDNF: 2.3×10^{-11} and 1.7×10^{-9} M. In fact, in terms of binding parameters, there seems to be only one major difference between the BDNF and the NGF receptors: the association and dissociation rates to and from the NGF low-affinity receptors are so rapid (even at 4°C) that they cannot be measured accurately (Vale and Shooter, 1985). This is not the case for the low-affinity BDNF receptors, the association and dissociation rates of which are much slower. Also, the number of high-affinity receptors for both ligands on the cell soma does not appear to be very different: We found about 230 receptors. At a temperature comparable to ours (2°C), Sutter et al. (1979) found about 1000 receptors per cell soma. However, it is known that at the stage of development used for these NGF binding studies (8 d), essentially all neurons have NGF receptors as quantified by autoradiographic studies (Rohrer and Barde, 1982). This is not the case with BDNF: results from our laboratory (Rodriguez-Tébar et al., unpublished observations) show that only about 40% of the neurons can be labeled with ^{125}I-BDNF in culture. Assuming that all BDNF receptors-bearing neurons have a similar number of receptors, there are about 500 receptors per cell soma.

In addition to the many and striking similarities between the NGF and the BDNF receptors, there is one major difference: Whereas high- and low-affinity NGF receptors have also been found on chick sympathetic neurons (Godfrey and Shooter, 1986), no high-affinity receptors for BDNF have been found on sympathetic neurons. Thus, both with NGF and BDNF, there seems to exist a simple correlation between the presence of high-affinity receptors and the typical biological response elicited by these neurotrophic proteins, i.e., neuronal survival and fiber outgrowth.

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