HIGH AFFINITY, SPECIFIC BINDING OF [^{125}I] β NERVE GROWTH FACTOR TO GLASS BEADS

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SUMMARY. The binding of [^{125}I] β nerve growth factor to glass beads was studied. It was found that [^{125}I] β nerve growth factor exhibited high affinity, specific binding to glass beads. This binding cannot be explained as radioactivity being occluded in the spaces between the glass beads. The binding appears to be non-saturable under the conditions used. Binding is complete in less than ten minutes with a half-time of two minutes. The binding appears similar to that seen for receptor binding on responsive cells.

INTRODUCTION

The presence of specific receptors for [^{125}I]βNGF[1] has been detected in a number of whole cell and cell membrane systems from responsive nervous tissues (1-3). βNGF appears to be a trophic factor responsible for the maintenance of sensory and sympathetic neurons. This maintenance role has been studied both *in vivo* (4-7) and *in vitro* (4, 8, 9). Although an experimental link has not been firmly established, the specific binding of βNGF to the plasma membrane has been implicated as the triggering event in the trophic response.

A recent report showed that [^{125}I] low density lipoprotein exhibited specific binding to glass beads (10). This report was prompted by the observation that microscopic glass particles are present in a tissue homogenate when a glass homogenizer is used to dissociate cells (10). βNGF is known to "stick" to several

[1]The abbreviations used are: NGF, nerve growth factor; 7S NGF, the high molecular weight form of the nerve growth factor; βNGF, the β-subunit of 7S NGF; [^{125}I]βNGF, iodinated βNGF; BSA, bovine serum albumin; LDL, low density lipoprotein; PBG, phosphate buffered Gey's balanced salts; and PBG-BSA, phosphate buffered Gey's balanced salts containing 1 mg/ml BSA.

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materials[2] and since a glass homogenizer has been used in some NGF binding studies, we felt that it may be of interest to investigate the glass-NGF interaction.

This communication presents the results of our study on the binding of [^{125}I] βNGF to glass beads. As stated by Dana, et al. (10), the correlation of [^{125}I]-ligand binding data with physiological function must be undertaken with care, as underscored again by our findings.

MATERIALS AND METHODS

Isolation of βNGF: The βNGF was isolated by the method of Varon et al. (11) from 7S NGF. The 7S NGF was isolated by the method of Stach et al. (12). After isolation, the βNGF was stored in 0.2% acetic acid at a concentration of approximately 4 mg/ml.

Iodination of βNGF: βNGF was iodinated by the procedure of Herrup and Shooter (1) as modified by Rice and Stach (13) except 2 mCi of [^{125}I] NaI was used. The [^{125}I]βNGF was stored at 4°C and any protein remaining after 3 weeks was discarded and a new reaction performed. In the experiments performed, samples were counted for either 10 minutes or until 10,000 counts were reached on a Nuclear Chicago gamma counter.

Binding of [^{125}I]βNGF to Glass Beads: The binding assay was a modification of that used by Dana et al. (10). The buffer was phosphate buffered Gey's balanced salts (PBG), pH 7.4, ionic strength 0.1 (1) containing 2 mg/ml BSA. The incubations were performed using 100 µl of solution, in Beckman Microfuge tubes, at room temperature or 4°C and for the times indicated. Each value represents the average of triplicate assays with the subtraction of a blank tube, which was carried through the assay but contained no glass beads. The beads were Sigma type 1, 75-150 microns and were new, acid washed, or siliconized as indicated.

Bioassay: The NGF activity was determined using 8 to 10 days old embryonic chick dorsal root ganglia in Gey's balanced salts solution (14) containing 1 mg/ml BSA, on collagen-coated cover slips as previously described (15). The biological activity of the βNGF was approximately 1 biological unit per ng of protein.

Other Chemicals: Bovine serum albumin, cytochrome c and lactoperoxidase were purchased from Sigma Chemical Company, St. Louis, Mo. [^{125}I] sodium iodide was purchased from New England Nuclear, Boston, Mass. in a "combi-v-vial" in approximately 4 µl of solution, pH 8-10. Hydrogen peroxide, 30% solution, was purchased from Matheson, Coleman and Bell, Norwood, Ohio. Other chemicals were reagent grade.

RESULTS

When glass beads were incubated with [^{125}I]βNGF at room temperature for various lengths of time, maximum binding was achieved in less than 10 minutes with a half-time of 2 minutes (Figure 1). In the presence of a greater than 500 fold excess of unlabelled βNGF the binding of [^{125}I]βNGF was significantly

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[2] Without at least 1 mg/ml cytochrome c or BSA, 60 to 90% of the βNGF binds noncovalently (sticks) to glass, polyethylene, etc. whereas less than 1% "sticks" in the presence of these proteins.

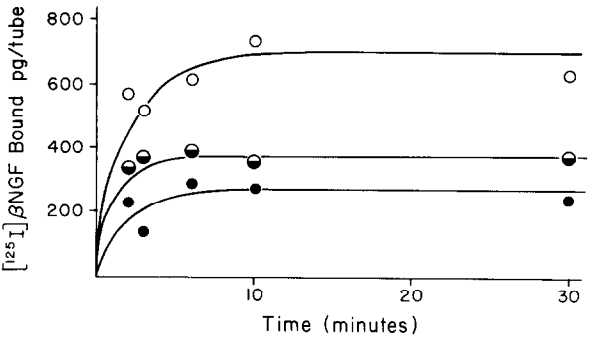


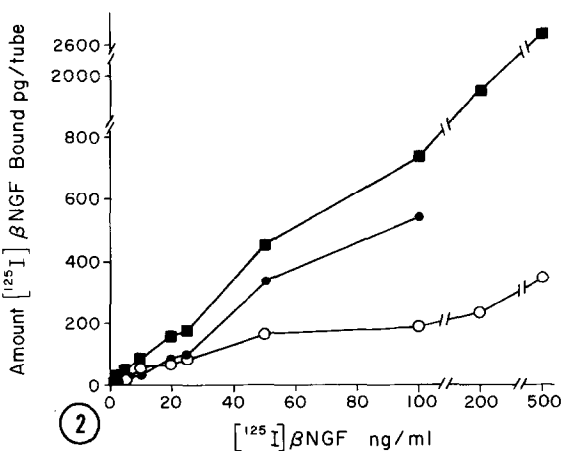
Figure 1: Time course of binding of [^{125}I]βNGF to glass beads. Each binding assay performed in triplicate contained 10 mg of glass beads and 100 ng/ml of [^{125}I]βNGF in the presence (⊖) (nonspecific binding) and absence (O) (total binding) of 100 µg/ml of unlabeled βNGF. After incubation at room temperature for indicated intervals, the amount of [^{125}I]βNGF bound to the beads was determined as described (10). Specific binding (●) is the difference between total and nonspecific binding.

reduced, suggesting the labelled and unlabelled ligands were competing for the same "binding" sites. A similar time course for binding was obtained at 4°C (data not shown). When 10 to 60 mg of glass beads were incubated with [^{125}I] βNGF, the amount of [^{125}I]βNGF bound was linearly proportional to the mass of glass beads present. In subsequent studies, 10 mg of glass beads were used.

When glass beads were incubated at various physiological concentrations of [^{125}I]βNGF, in the presence or absence of unlabelled βNGF, high affinity, specific, and apparently non-saturable binding was observed (Figure 2). This is similar to the binding reported in some receptor binding studies (3).

To exclude the possibility that the observed binding may be due to protein which is occluded in the spaces between the beads, [^{125}I] NaI was used in a glass bead binding study. Binding studies were performed as before, in tubes with or without glass beads. After the 30 minute incubation time, the solution was removed and the tubes were washed as previously described (10). There was no significant difference in binding between the tubes containing glass beads and those containing no glass beads.

A study to show that, like responsive nerve cells, the glass "receptors"



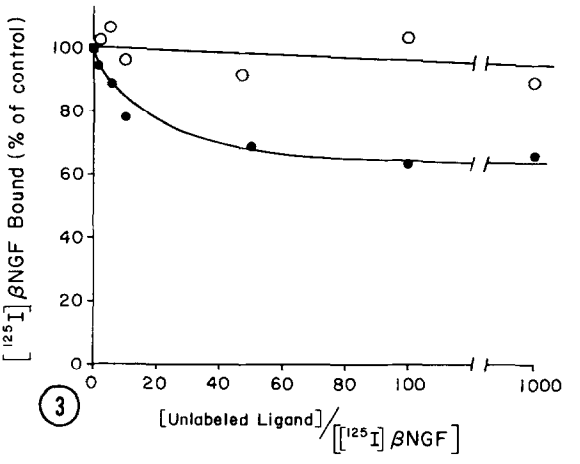


Figure 2: Binding of [^{125}I]βNGF to glass beads as a function of [^{125}I]βNGF concentration. Each binding assay, done in triplicate, contained 10 mg of beads and was incubated for 30 minutes at room temperature with various concentrations of [^{125}I]βNGF, either in the presence (●) or absence (■) of 250 µg/ml of unlabeled βNGF. The amount of [^{125}I]βNGF bound to the beads was determined as described (10). Nonspecific points not shown; 1683 pg/tube and 2282 pg/tube at [^{125}I]βNGF concentrations of 200 and 500 ng/ml, respectively.

Figure 3: Effect of unlabeled proteins on [^{125}I]βNGF binding to glass beads. Each binding assay contained 10 mg of glass beads, 50 ng/ml of [^{125}I]βNGF and the indicated proportion of unlabeled βNGF.(●) or unlabeled cytochrome c (○). Assays were performed in triplicate with 30 minutes of incubation at room temperature. The total amount of [^{125}I]βNGF bound to the beads was determined as described (10). Data reported as percent of control value, containing no unlabeled ligand. Control value was 480 pg/tube.

appear specific for βNGF was performed. Glass beads were incubated with [^{125}I] βNGF in the presence of increasing concentrations of unlabelled βNGF or cytochrome c (Figure 3). Cytochrome c was used because its physiochemical properties are similar to those of the NGF monomer. The unlabelled βNGF competed for the specific binding sites, but cytochrome c did so much less effectively (Figure 3).

The dissociation of [^{125}I]βNGF from the glass beads was also studied. After preincubation with [^{125}I]βNGF, 200 µl of PBG–BSA or 200 µl of PBG–BSA containing unlabelled βNGF was added. Table 1 shows that after 2 hours there was no significant dissociation of [^{125}I]βNGF from the glass beads under either condition as determined by Dunnett's t-test analysis (16).

TABLE I

Dissociation of Bound [^{125}I]βNGF from Glass Beads After Incubation in the Presence or Absence of Unlabeled βNGF

Incubation Period (minutes)	Additive	Binding pg/tube
0	none	616 ± 35
30	βNGF	510 ± 66
30	buffer	511 ± 92
60	βNGF	602 ± 120
60	buffer	575 ± 109
120	βNGF	599 ± 50
120	buffer	620 ± 53

Each binding assay contained 10 mg of beads and 50 ng/ml of [^{125}I]βNGF. After preincubation at room temperature for 30 minutes, the beads were washed as described (10) and the amount of [^{125}I]βNGF bound to the glass beads was determined for 3 tubes (zero time value). The remaining tubes received 100 µl of buffer containing either no βNGF or 25 µg/ml of βNGF. The tubes were incubated at room temperature and at the times indicated the total binding of [^{125}I]βNGF was determined on triplicate samples.

Table 2 shows the results of the binding of [^{125}I]βNGF to glass beads after the glass beads were either washed with acid or siliconized. The acid wash did not affect the total or specific binding to the glass beads, but siliconization reduced both types of binding by approximately 50%.

The effect of various concentrations of BSA in the incubation medium was also studied and no change in binding was observed in the concentration range of 2-8 mg/ml. However, at 10 mg/ml, a statistically significant decrease in non-specific binding was observed. The total binding was unchanged from control levels, causing an increase in specific binding to be observed.

TABLE 2

Effects of Various Treatments of Glass Beads on the Binding of [^{125}I]βNGF

Treatment	Binding of [^{125}I]βNGF pg/tube		
	(-) unlabeled βNGF	(+) unlabeled βNGF	Specific
None	134 ± 5	54 ± 3	80 ± 3
Acid Washed	163 ± 32	91 ± 19	72 ± 21
Siliconization	63 ± 24	19 ± 4	44 ± 14

Siliconization of the glass beads was accomplished as follows: 5 g of beads were suspended briefly in 25 ml of 1% dichlorodimethylsilane in benzene, then rinsed with benzene, air dried and washed extensively with water. Acid washing was accomplished by suspending the beads in 12 N HCl, neutralizing with NaOH and washing extensively with water before use. Each binding assay was performed in triplicate with 10 mg of glass beads and 25 ng/ml of [^{125}I]βNGF in the presence or absence of unlabeled βNGF as indicated. After incubation at room temperature for 30 minutes, the amount of binding was determined as described (10).

DISCUSSION

In the current studies, [^{125}I]βNGF was shown to bind to glass beads with high affinity, specific binding at concentrations which are physiologically significant even in the presence of BSA (2-10 mg/ml). This binding was rapid and similar to that seen for [^{125}I]βNGF binding to its receptor on responsive cells (1). This binding cannot be explained as protein which has been occluded in the spaces between the beads, since we have shown that our washing procedures remove all the unbound radioactivity.

The inconsistencies between various reports on the specific binding of [^{125}I]βNGF (1-3, 17, 18) may in part be due to binding to microscopic glass particles contaminating the tissue homogenates (10). Low, but significant levels of specific binding to non-neuronal tissues have been reported where glass homogenization was used in the tissue preparation; however, no specific binding was reported for rat red blood cells where homogenization is unnecessary to obtain

single cells (17). Consistent with this is the observation that non-neuronal tissue that has not been dissociated using a glass homogenizer shows no specific binding for [^{125}I]βNGF (1). Preliminary experiments in our laboratory have shown an apparent increase in binding (total, specific, and nonspecific) when glass homogenization was used in tissue preparation compared to parallel studies where cell dissociates were obtained by the method previously described (1).

The increase in [^{125}I]βNGF specific binding to glass beads at a BSA concentration of 10 mg/ml suggests that the use of very high concentrations of BSA causes anamolous binding behavior in our studies. It is possible that high concentrations of BSA also may have effects on receptor binding studies. Therefore, when large quantities of BSA are employed in a binding assay, the question of possible anamolous behavior should be investigated. It is interesting to note that Dana *et al.* (10) used a BSA concentration of 20 mg/ml in their studies and obtained quite high (75-90%) specific binding.

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REFERENCES

1. Herrup, K. and Shooter, E.M. (1973) Proc. Nat'l. Acad. Sci. USA 70, 3884-3888.
2. Banerjee, S.P., Snyder, S.H., Cautrecasas, P., and Greene, L.A. (1973) Proc. Nat'l. Acad. Sci. USA 70, 2519-2523.
3. Frazier, W.A., Boyd, L.F., and Bradshaw, R.A. (1974) J. Biol. Chem. 249, 5513-5519.
4. Levi-Montalcini, R. and Angeletti, P.W. (1968) Phys. Rev. 48, 534-569.
5. Bjerre, B., Björkland, A., and Stenevi, U. (1973) Brain Res. 60, 161-176.
6. Stenevi, U., Bjerre, B., Björkland, A., and Mobley, W. (1974) Brain Res. 69, 217-234.
7. Herrup, K., and Shooter, E.M. (1975) J. Cell Biol. 67, 118-125.
8. Phillipson, O.T. and Moore, K. (1975) J. of Neurochem. 25, 295-298.
9. Frazier, W.A., Boyd, L.F., and Bradshaw, R.A. (1973) Proc. Nat'l. Acad. Sci. USA 70, 2931-2935.
0. Dana, S.E., Brown, M.S., and Goldstein, J.L. (1977) Biochem. Biophys. Res. Comm. 74, 1369-1376.

11. Varon, W., Nomura, J., and Shooter, E.M. (1968) Biochemistry 7, 1296-1303.
12. Stach, R.W., Wagner, B.J. and Stach, B.M. (1977) Anal. Biochem. (in press).
13. Rice, B.L. and Stach, R.W. (1976) Biochem. Biophys. Res. Comm. 73, 479-484.
14. Gey, G.O. and Gey, M.K. (1936) Amer. J. Cancer 27, 45-76.
15. Stach, R.W. and Shooter, E.M. (1974) J. Biol. Chem. 249, 6668-6674.
16. Dunnett, C.W. (1955) J. Am. Stat. Assn. 50, 1096-1121.
17. Frazier, W.A., Boyd, L.F., Szutowicz, A., Pulliam, B.W., and Bradshaw, R.A. (1974) Biochem. Biophys. Res. Comm. 57, 1096-1103.
18. Frazier, W.A., Boyd, L.F., Pulliam, M.W., Szutowicz, A., and Bradshaw, R.A. (1974) J. Biol. Chem. 5918-5923.