MICROBIOLOGY AND IMMUNOLOGY

Identification of Specific Binding Sites for Nerve Growth Factor on Human Blood Platelets and Membranes from Bovine Brain

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The presence of specific binding sites for nerve growth factor on membranes from bovine brain and human blood platelets is shown. The association and dissociation kinetics and the dissociation constants of receptor binding of nerve growth factor are analyzed.

Key Words: *platelet; nerve growth factor; nerve growth factor receptor*

Nerve growth factor (NGF), a protein necessary for the development and normal functioning of sensory and sympathetic neurons, exerts its physiological effect through interaction with specific membrane receptors [14]. NGF receptors have been identified and characterized in various tissues, including sympathetic and sensory ganglia [13,14], PC12 cells [9], melanoma cells [6], and neurofibroma and Schwann cells [4]. Previous results [1] on NGF-induced platelet aggregation and on the release of ^{3}H-serotonin and Ca^{2+} efflux [11] indirectly attest to the presence of specific NGF receptors on the platelet membrane. However, specific binding sites for NGF on these cells have not yet been identified.

In previous studies NGF specific binding sites (NGF receptors) in rat brain and human keratinocytes were identified by measuring the binding of ^{125}I-NGF from mouse submaxillary gland with a membrane fraction from rat brain or with native human keratinocytes [3,5,12]. In the present study for identification of NGF receptors we used the specific binding of ^{125}I-NGF with native human platelets and a membrane fraction from bovine brain. However, the NGF from bovine sperm used in our study is very similar to human NGF with respect to both the amino acid sequence and biological activity, whereas mouse NGF exhibits only about 90% homology and differs considerably in its immunological characteristics [10].

The aim of the present study was to identify NGF receptors on human platelets and a membrane fraction from bovine brain using a radioligand binding assay.

MATERIALS AND METHODS

NGF from bovine seminal plasma (2.5 S subunit) was isolated as described elsewhere [7]. Sodium dodecyl sulfate electrophoresis confirmed a 90% purity of the preparation [8]. The activity of NGF was tested in a PC12 culture.

We also used blood from healthy donors and a membrane fraction from bovine brain cortex. Platelets were isolated from the blood by gel filtration [2]. For isolation of the membrane fraction from bovine brain cortex the tissue was homogenized in cooled isolation

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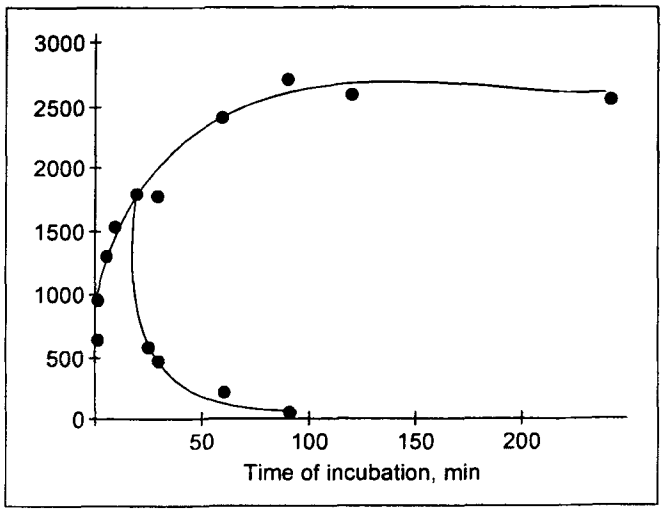

Fig. 2. Time course of specific binding of ^{125}I-NGF with human platelets (10 nM ^{125}I-NGF) and dissociation of the ligand upon dilution with a 50-fold volume of buffer.

medium (phosphate buffer saline, PBS, pH 7.4) containing 1 mM PMSF at a 1:10 weight:volume ratio and centrifuged at 750 g for 10 min. The supernatant was collected and recentrifuged at 40,000 g for 45 min. The pellet was resuspended in PBS/PMSF-buffer containing 1 mg/ml bovine serum albumin. NGF was iodinated using a modified lactoperoxidase method [4].

Interaction of ^{125}I-NGF with the platelets or with the membrane fraction was studied using a radioligand binding assay. Binding of ^{125}I-NGF was carried out at 0°C during 2-4 hours in PBS (pH 7.4) containing 1 mM PMSF, and 1 mg/ml bovine serum albumin, ^{125}I-NGF in concentrations of 0.07 to 20 nM, and the platelet suspension (18-24×10^6 cells/ml) or an aliquot of the membrane fraction (1.0-1.6 mg protein/ml). The total volume of the incubation mixture was 1 ml. The bound ligand was separated from the free by rapid filtration through GF/C filters (Whatman). The filters were washed 3 times with 3 ml cold PBS, pH 7.4, and transferred to counting vials. Specific binding was calculated as the difference between binding measured in the presence and absence of a 100-fold excess of unlabeled NGF.

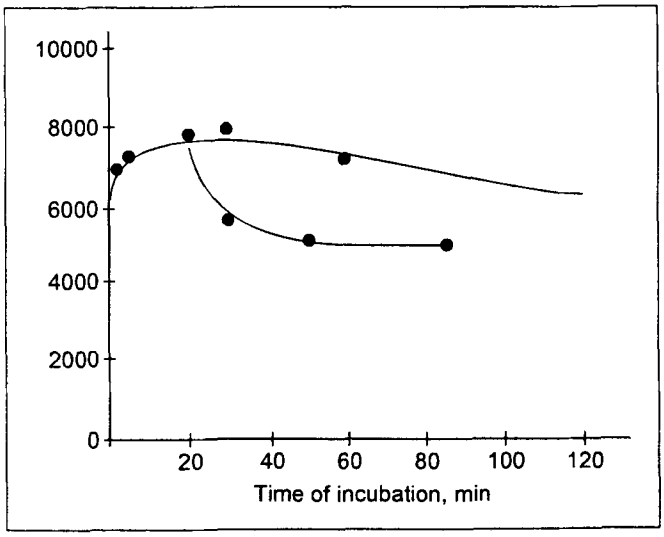

Fig. 1. Kinetics of specific binding of ^{125}I-NGF with membrane fraction from bovine cerebral cortex and kinetics of dissociation of ligand-receptor complex (4 nM ^{125}I-NGF) after dilution with a 50-fold volume of buffer. Here and in Fig. 2: the ordinate shows the specific binding of ^{125}I-NGF, thousand counts per minute. Temperature of incubation 0°C.

RESULTS

Kinetic studies showed that the parameters of ^{125}I-NGF binding with the membrane fraction from bovine brain (Fig. 1) differed from those with human platelets (Fig. 2). Binding of ^{125}I-NGF in a concentration of 4 nM with the membrane fraction attained the maximum after 1-2 hours and then remained at this level during at least another 2 hours. Half-saturation was attained within 5 min. A similar nature of binding was observed at higher concentrations of labeled NGF, but the process was more rapid. For instance, binding of ^{125}I-NGF in a concentration of 20 nM attained the maximum after a 20-30-min incubation, half-saturation being attained within 2 min. By contrast, binding of 10 nM ^{125}I-NGF with platelets attained the maximum after 15-20 min and the half-saturation time was shorter than 1 min, but then binding decreased and after 1 hour was 80-90% of the maximal level. An analogous curve was obtained for binding of 20 nM ^{125}I-NGF with the platelets: binding was maximal after 5-10 min, remained at this level for 40 min, and then decreased and after 2 hours constituted 80% of the maximal level. As is seen from the kinetic curves, ^{125}I-NGF binds with platelets more than 5 times more rapidly than with membranes from bovine brain.

Reversibility of binding was assessed by diluting the incubation mixture with a 50-fold volume of PBS (pH 7.4). The binding of NGF with the membrane fraction was completely reversible both after a 20-min (Fig. 1) and after a 2-hour incubation with the label. On the other hand, in platelets reversible binding accounts for only about 40% of the maximal binding.

In light of the above it may be assumed that the interaction of ^{125}I-NGF with bovine brain membranes is represented solely by reversible binding of NGF with specific sites, resulting in the formation of a ligand-receptor complex, whereas interaction with platelets may be assumed to include also nonreceptor processes such as transport of labeled ligand into the cell.

A study of the specific binding of ^{125}I-NGF with the membrane fraction from bovine brain as a function of the ligand concentration showed an unsaturable nature of the binding in the concentration range used (Fig. 3, *a*). This makes it impossible to

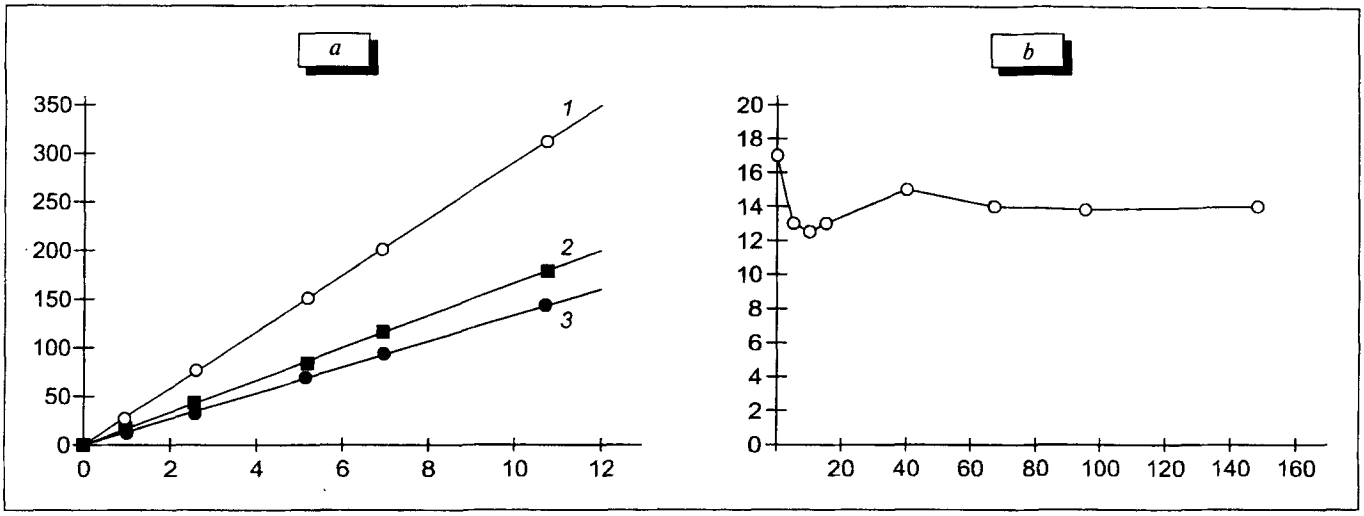

Fig. 3. Specific binding of ^{125}I-NGF with membrane fraction from bovine cerebral cortex as a function of the concentration of ligand. *a*) abscissa: concentration of free ^{125}I-NGF, nM; ordinate: binding, fmol/mg protein. *1*) total binding, *2*) nonspecific binding; *3*) specific binding. *b*) the same data in Scatchard coordinates; abscissa, specific binding of ^{125}I-NGF, fmol/mg protein; ordinate: ratio of specific binding (fmol/mg protein) to concentration of free ^{125}I-NGF (nM).

use Scatchard's analysis for determination of the equilibrium dissociation constant (K_d) and the maximal number of specific binding sites (B_{max}) (Fig. 3, *b*). Therefore, the equilibrium dissociation constant $K_d$eq was calculated using K_{ass} and K_{diss} values obtained from the kinetics of association and dissociation ($K_d$eq= K_{diss}/K_{ass}). Analysis of the kinetic curves showed that for the membranes from bovine brain K_{ass}=0.012 nM^{-1}min^{-1} and K_{diss}=0.046 min^{-1}, so $K_d$eq=4.1 nM. The half-life of the complex ($t_{1/2}$)=15 min. For human platelets these parameters are 0.065 nM^{-1}min^{-1}, 0.0025 min^{-1}, 38×10^{-3} nM, and 4.7 h, respectively. These values are comparable to published data. For instance, K_d has been reported to be equal to 45 pM and 21 nM, and 10 pM and 8 nM for rat brain and keratinocytes, respectively [3,5].

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