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Nerve growth factor (NGF) is present in human placenta and semen, but undetectable in normal and Paget's disease blood: measurements with an anti-mouse-NGF enzyme immunoassay using a recombinant human NGF reference

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An enzyme immunoassay (EIA) originally developed for mouse ß-nerve growth factor (NGF) and commercially available was validated for human NGF. Cell culture medium containing bioactive recombinant human NGF was used as a reference, and mouse 2.5S ß-NGF as a standard. One of three human placentas contained measurable NGF (70 pg/g of tissue of mouse ß-NGF equivalents), a second detectable, and a third undetectable NGF. In three human semen samples NGF content ranged from 0.13 - 1.4 ng/ml. NGF could not be detected in normal human serum and in plasma from patients with Paget's disease, although mouse 2.5S ß-NGF added to human blood could be completely recovered from the serum.

Nerve growth factor (NGF) is a 118 amino acid polypeptide that is essential for the differentiation and maintenance of sensory, sympathetic, and central cholinergic neurons (1,2). A wealth of data and information exists on NGF in animals, but little is known about NGF in the human. The prinicipal reasons are the low abundance of NGF in most tissues (0.5-3 ng/g wet weight (3)), and lack of a valid and sensitive detection method.

The human haploid genome contains a single NGF gene on the short arm of chromosome 1 (4). A fragment of human chromosomal DNA containing a partial human NGF gene has been cloned and structurally

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characterized (5). Using the cloned human DNA as a probe, the mRNA encoding human NGF (NGF mRNA) was detected by Northern blot hybridization in human placenta and brain (6). Immunoreactive material with the physical characteristics of mouse ß-NGF and possessing its neurotrophic activities has been described in human placenta (7). Saide et al. (8) detected immunoreactive NGF (60 - 80 ng/ml) in normal human serum, and 3-6 fold higher levels in serum from patients with Paget's disease.

The relative lack of knowledge about NGF in the human, and recent evidence from animal studies that suggests a possible therapeutic role for NGF in the treatment of Alzheimer's disease prompted us to prepare hNGF by recombinant DNA techniques (9). Using the recombinant human NGF (rhNGF) as a reference, we show in this report that a commercially available EIA for mouse NGF can be used to measure NGF in human tissues and fluids.

Materials and Methods. A monoclonal antibody against mouse 2.5S ß-NGF, a conjugate of ß-galactosidase with the same antibody, the enzyme substrate o-nitrophenol-ß-D-galactopyranoside, and the proteinase inhibitor aprotinin were obtained from Boehringer-Mannheim (Indianapolis, IN). 2.5S mouse NGF was purchased from Collaborative Research (Waltham, MA). Phenylmethylsulfonyl fluoride was obtained from Sigma (St. Louis. MO). Recombinant hNGF was obtained by transfecting a recombinant DNA containing exon 4 of the human NGF gene into COS cells and collecting the cell culture medium (5). Aliquots of the medium that had been used to characterize the rhNGF by EIA, bioassay and column chromatography were used as reference.

Placentas were obtained at delivery, frozen, and stored at -70°C. Normal human blood was drawn, aprotinin added to 4 u/ml, and the blood allowed to clot at room temperature. Serum was collected 2-8 h later. Human semen was collected, allowed to liquify, and stored at -20°C until assayed. Plasma samples from two patients with untreated Paget's disease and two age-matched controls were obtained from the Arthritis Service at Massachusetts General Hospital. The plasma had been stored at -20°C for two years.

The EIA was performed essentially as recommended by the distributor, including preparation of samples. Placenta and semen were homogenized with a Polytron homogenizer. Antibody was used at 0.5 µg/ml, and galactosidase conjugate at 10 munits/well. Substrate concentration was 1 mg/ml. The EIA was carried out in Nunc Immunoplates (Nunc, Denmark). Each sample was assayed in triplicate.

Results and Discussion. The monoclonal antibody 27/21 used for the NGF assays was raised against mouse ß-NGF for which it has high affinity (10). The EIA therefore allows detection of mouse ß-NGF at 5 pg/ml in the assay, and at 0.05-0.1 ng/g wet weight of tissue. The

epitope recognized by Mab 27/21 must be exposed in the ß-NGF dimer because Mab 27/21 is used in the "sandwich" both to immobilize the antigen and, linked to ß-galactosidase, for enzymatic detection of the antigen. Little is known about the structure of the ß-NGF dimer. However, because the amino acid sequences of mouse and human ß-NGF are exceedingly similar (5), it is likely that human NGF dimerizes in a similar fashion, exposing a closely related or perhaps identical epitope for interaction with Mab 27/21. Since we could detect hNGF with the EIA, hNGF must dimerize to some extent. However, the stability of the dimer remains to be established and compared with that of the mouse NGF dimer. In addition, it will be important to identify the epitopes recognized by Mab 27/21 in the two NGFs and their dimers so their structures can be compared. These factors will influence the affinity of Mab 27/21 for hNGF and therefore the sensitivity of the EIA for measurements of hNGF.

Although we have not yet established the sensitivity of the EIA for hNGF, it must be rather high, and clearly within the same order of magnitude as for mouse NGF, i.e close to 5 pg/ml. This can be concluded from measurements of rhNGF in cell culture medium (9). Precise comparison of the sensitivities of the Mab 27/21-based EIA for mouse and human NGF will require utilization of a pure hNGF standard such as hNGF produced by recombinant DNA methods.

Because at present there is an insufficient supply of rhNGF to allow the preparation of pure hNGF, we have used the medium of COS cells transfected with recombinant DNA as a reference. The medium contains bioactive rhNGF that is a dimer since it is detectable by the EIA and migrates as a molecule of 26-29 kd on a gel permeation chromatography column (9).

Expressed in mouse equivalents, the level of NGF was 70 (+/- 17) pg/g in one human placenta. In another sample NGF was detected but was too low to be measured accurately. A third placenta contained no detectable NGF. In three human semen samples, NGF was 0.8 (+/- 0.06) ng/ml, 1.4 (+/- 0.11) ng/ml and 0.13 (+/- 0.015) ng/ml, respectively. No NGF could be detected in serum, although mouse NGF added after collection of blood was completely recovered. Placental NGF is probably synthesized locally because NGF mRNA is present in placenta (6), and NGF is absent from normal blood. The source of NGF in semen is unknown. Hyperinnervation of hypertrophic prostate (11) suggests that it may be secreted from this gland. The level of NGF in semen is relatively low, but is in the range that could activate NGF receptors. Recent evidence indicates that NGF receptors are present in cervix (12). Guinea pig

prostate secretes large amounts of NGF (13), as does bull seminal vesicle (14).

NGF was not expected in blood inasmuch as normally NGF is efficiently taken up by NGF receptor-bearing cells, and evidence in rodents suggests that the uptake system is operating below saturation (3). However, human blood may contain NGF in diseases, particularly in cancer. NGF was discovered in a mouse sarcoma (15). Plasma from two patients with Paget's disease was assayed because a previous report suggested elevated levels of NGF in Paget's disease (8). In this study, no NGF could be detected in plasma of two patients with active, untreated Paget's disease. The samples had been stored. Although the stability of hNGF in stored plasma is not yet known, it is unlikely that the plasma samples contained the elevated levels of NGF (180 - 480 ng/ml) previously reported (8).

In conclusion, the presently available EIA developed for mouse NGF assays is valid for measurements of hNGF and is of similar albeit as yet imprecisely known sensitivity. Application of the EIA to several human tissues revealed significant levels of NGF in human placenta and semen, and no detectable NGF in normal human serum or plasma from two patients with Paget's disease. Thus, a previously unrecognized tool is available now for studies of NGF gene expression in human brain and other tissues.

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