Eur. J. Clin. Chem. Clin. Biochem.
Vol. 31, 1993, pp. 375–380

Determination of Nerve Growth Factor Concentrations in Human Samples by Two-Site Immunoenzymometric Assay and Bioassay

By *Martina Näher-Noé, H. Gnahn, A. Grundler, J. Klingelhöfer, A. Weindl* and *B. Conrad*

Neurologische Klinik der Technischen Universität München, München

(Received July 7, 1992/February 3, 1993)

Summary: Nerve growth factor is a neurotrophic protein which is known to act on sympathetic and sensory neurons and on the magnocellular cholinergic neurons of the basal forebrain. We quantified nerve growth factor in human tissues and body fluids by two methods, a rapid and sensitive two-site immunoenzymometric assay and a bioassay using dissociated chick dorsal root ganglion neurons. The two-site immunoenzymometric assay detects nerve growth factor in concentrations as low as 0.5–2.5 ng/l. Using a monoclonal antibody to mouse nerve growth factor, we found that the signal of the antibody for recombinant human nerve growth factor is about 60–90% of the signal for mouse nerve growth factor. As a control for the specificity of our data, a bioassay for nerve growth factor was performed and the results showed a good correlation. The highest nerve growth factor concentrations were found in sciatic nerve (2.5 ng/g wet weight), cardiac atrium muscle (1.5 ng/g wet weight) and in the central nervous system in the hippocampus (1.9 ng/g wet weight). Lower nerve growth factor concentrations were measured in human sera (0.2 ng/g wet weight). No nerve growth factor was detectable in cerebrospinal fluid. The distribution of human nerve growth factor-rich tissues is similar to that reported for rat tissues.

Introduction

Nerve growth factor is a neurotrophic protein which is synthesized by target tissues of nerve growth factor-sensitive neurons, selectively taken up by the nerve terminals and transported retrogradely to the cell bodies. Nerve growth factor has been shown to act on sympathetic and neural crest-derived sensory neurons in the peripheral nervous system as well as on the magnocellular cholinergic neurons of the basal forebrain (for review, see l.c. (1–3)). It has been suggested that nerve growth factor is involved in the neuropathology of *Alzheimer*'s disease (4). In the peripheral nervous system nerve growth factor may play a role in the development of autonomic and sensory neuropathies (5). To clarify whether nerve growth factor is of physiological or pathological significance in humans the sensitive and reliable quantification of endogenous nerve growth factor in human tissues and body fluids is a prerequisite.

The genomic sequence of human nerve growth factor has been determined (6) and small quantities of biologically active recombinant human nerve growth factor have already been produced (Genentech, USA). Since the sequence homology between murine and mature human nerve growth factor is 86% on the nucleotide level and 90% on the amino acid level (6), a relatively high immunological cross-reactivity is to be expected. Since the levels of nerve growth factor in tissues are extremely low in general, there is a need for a highly sensitive test system. The bioassay for nerve growth factor, which is based on the neurotrophic effect of nerve growth factor on embryonic sensory neurons (7), requires time-consuming cell culture procedures and is not suitable for measurements in a large number of samples. In competitive immunoradiometric assays, problems arise from the unspecific binding of nerve growth factor, for example by α_2-macroglobulin (8, 9). A two-site immunoradiome-