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NERVE GROWTH FACTOR DERIVED FROM SNAKE VENOM AND MOUSE SUBMAXILLARY GLANDS ARE SIMILAR AND SHARE SOME IMMUNOLOGICAL PROPERTIES

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ABSTRACT

Nerve growth factor (NGF) is an important molecule in neurobiology, which studies the structure, function, and activity of the nervous system in animal species. Subcutaneous injections of NGF in rodents cause an increase in the sympathetic ganglia of the para- and prevertebral chains until it reaches a volume 10-12 times that of the untreated ganglia. NGF communicates with the immune system by increasing the maturation, survival, and number of immune cells, particularly of mastocytes. In lymphocytes cultured *in vitro*, NGF exerts a proliferation action by binding to its TrkA receptor, induces the expression of T cell growth factor (IL-2), and modifies the shape of platelets. The antiserum from the mouse submaxillary gland counteracts NGF and exerts an inhibition identical to that caused by snake venom-derived NGF. The amino acid compositions of NGF derived from snake venom and mouse submaxillary glands are remarkably similar and sulfhydryl groups are absent in both. Here, we report that these two NGFs deriving from two different sources are the same molecule.

KEYWORDS: *nerve growth factor, NGF, venom, submaxillary gland, immunity*

INTRODUCTION

Nerve growth factor (NGF) is a neurotrophic peptide capable of influencing the development and growth of the nervous system (1). NGF can induce neurite growth in explants from sympathetic and sensory ganglia (2). This molecule is important for the role it plays in connecting the nervous system with the immune and endocrine systems. At the immune level, NGF provokes the maturation and survival of mast cells (MCs), the chemoattraction of basophilic granulocytes, and the proliferation of lymphocytes, induces the expression of the IL-2 receptor, causes the differentiation, survival, and chemotaxis of immune cells, and changes the shape of the platelets (3). There are various immune cells that interact with NGF and among these are lymphocytes and MCs. In both immune and endocrine cells, NGF uses the tropomyosin kinase (TrkA) receptor to transduce signals. Previous research has indicated the striking similarities between NGF derived from snake venom and NGF synthesized from mouse submaxillary glands (4,5).

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DISCUSSION

The NGF which is derived from snake venom and the NGF synthesized from mouse submaxillary glands share several biological properties. Both forms of NGF act specifically on sympathetic cells and embryonic sensory cells, producing an identical *in vivo* response that involves increases in both the number and size of nerve cells (6). Their specific activity *in vitro* is of the same magnitude, 10-8 to 10-9 g protein per biological unit.

Also from a chemical viewpoint, the NGF molecules have many characteristics in common. They are proteins with molecular weights all ranging from 25 Kd to 35 Kd. The amino acid compositions are remarkably similar and sulfhydryl groups are absent. Their biological activity is not lost when treated with organophosphorus compounds, although there is a progressive loss of biological and immunological activity with progressive oxidation of tryptophan residues.

Immunological data also favor a partial identity of some NGF preparations. Cross-reaction of mouse salivary gland NGF antiserum with crude Agkistrodon piscivorus venom NGF has already been shown by other authors, based on inactivation of the biological activity *in vitro* (7). Later, a degree of cross-reactivity for Crotalus adamanteus venom NGF was confirmed in micro-complement fixation experiments (8).

These immunological studies were extended by using NGF from mouse submaxillary glands and the venom of poisonous snakes. Specific monovalent antiserums were used in these preparations. NGF from mouse salivary glands and snake venom were prepared according to procedures designed in the laboratory. Mouse preparations met several homogeneity criteria. NGF from venom was a purified preparation obtained by isoelectric focusing of crude venom (9). Antiserums to mouse salivary gland NGF and to snake venom NGF were prepared in rabbits following an immunization.

Immunological activity was investigated by micro-complement fixation immunodiffusion, and by *in vitro* inhibition of NGF activity. The results were obtained from *in vitro* inhibition experiments. The antiserum to mouse salivary gland NGF inhibited the biological activity of the venom tested. The degree of cross-reactivity was between 10% and 20%. The antiserum to snake venom NGF, although showing a higher titer with its homologous antigen, inhibited the NGF activity from the other sources to a lesser extent. The percentage of cross-reactivity with other venom NGF was between 5 to 10%.

With mouse salivary gland NGF, the cross-inhibition was less than 1%. When tested on agar plate, the antiserum to venom NGF gave distinct bands with the NGF of the venoms studied. The reactions between homologous and heterologous antigens were identical. No precipitation reaction was obtained when the venom antiserum was confronted with NGF from the mouse salivary gland (did not precipitate the NGF that was used). However, with venom from different snakes, NGF at a higher concentration than the mouse salivary gland NGF, a precipitation band was obtained, which fused with that of the homologous antigen.

The immunological relationship between the snake venom NGF and mouse submaxillary gland NGF was further explored using micro-complement fixation. A sharp peak was obtained against its diluted antiserum, but no complement fixation was obtained even when the antiserum concentration was increased using mouse salivary gland NGF as an antigen. When the antigen to the mouse salivary gland NGF was used, 80% complement fixation was obtained against its homologous antigen. Under these conditions, venom NGF showed traces of complement-fixation activity. When the antiserum concentration was raised ten-fold, a clear-cut peak of complement fixation approaching that found with the homologous antigen was obtained.

The results reported above further support the concept that the NGFs from all animal sources belong to a family of closely related proteins whose mechanism of action on the responsive nerve cells must be essentially the same. The antibodies elicited by the salivary gland NGF are clearly able to cross-react with identical antigenic sites on the venom NGF molecule (10). The two molecules must, in fact, be very similar if one takes into consideration the identical reaction obtained by immunodiffusion and the similarity index obtained by fixation of the micro complement with NGF from venom and the antiserum for NGF from mouse salivary glands.

CONCLUSIONS

NGF is a neurotrophic peptide discovered approximately 60 years ago by Rita Levi-Montalcini et al. as a protein that induces the growth of nerves (11). NGF works by binding to its TrkA receptor and is considered a target for the treatment of neurodegenerative diseases. NGF can be synthesized from mouse submaxillary glands and from snake venom. In this article, it is shown that the two NGF molecules synthesized from two different sources, the snake venom and mouse submaxillary glands, have the same biochemical size and are therefore, the same protein. In addition, the NGFs derived from the two sources act similarly on embryonic cells, producing both an increase in the number and size of nerve cells. Therefore, it can be concluded that the two molecules are the same molecule that exerts the same biological effect on nerve cells.

Conflict of interest
The authors declare that they have no conflict of interest.

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