Catecholamine Levels of Mouse Sympathetic Ganglia Following Hypertrophy Produced by Salivary Nerve-Growth Factor. (26720)

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A protein fraction isolated from mouse submaxillary gland has been shown by Levi-Montalcini and Cohen to have a powerful stimulating effect on the growth of peripheral ganglion cells, both in tissue culture as well as *in situ*(1-3). Daily injection of this "nerve-growth factor" (NGF) in newborn mammals produced as much as 6-fold enlargement of the sympathetic ganglia, while other types of neural tissue (as well as all non-neural organs) showed no morphological changes. Protein, RNA and DNA content of the ganglia increased 6-fold, 3-fold and 2-fold, respectively. In spite of this dramatic structural alteration in the autonomic nervous system, no functional changes have been detected. The purpose of this paper is to confirm the NGF effects reported by Levi-Montalcini and Cohen, and to show that the hypertrophy of the ganglia involves proportionate increase in their catecholamine content.

Materials and methods. The NGF component of mouse submaxillary glands was purified using a procedure similar to that described by Cohen(3), omitting, however, the DEAE- and one of the CM-cellulose column extractions.* The resulting fractions were assayed in tissue cultures of chick embryo spinal ganglia(4) and showed potencies of 7-160 units/μg dry weight (1 unit of NGF being defined as that quantity per ml of culture medium which produces a "3+" neuritic outgrowth after 1 day of incubation). Newborn mice were given daily subcutaneous injections of 500-1000 NGF units/g body weight. The fractions were generally lyophilized for long-term storage, and dissolved in physiological salt solution for injections at 0.05 ml/g body weight. Litters were restricted to 8 mice—4 treated and 4 controls (the latter receiving equal volumes of salt solution alone). The mice were sacrificed one day after 13-24 daily injections. The stellate and superior cervical ganglia were dissected out of each mouse, weighed in groups of 8-12 (consisting of equal numbers of the 2 types of ganglia). The resulting 4 to 8 mg of tissue was submerged in 1.0 ml of 0.01 N HCl in conical ground-glass homogenizer tubes. After homogenizing and washing the tube with 1.0 ml of 0.01 N HCl, the combined acid phase plus 2 g NaCl was extracted with 25 ml n-butanol by shaking 5 minutes. The norepinephrine (NE) was extracted into 1 ml of 0.01 N HCl from 20 ml of the n-butanol phase plus 20 ml n-heptane, using the same extraction conditions. For analysis of NE and epinephrine (EP), 16 ganglia were extracted by the same procedure into a final volume of 1.6 ml acid. Standard solutions of the amine in 0.01 N HCl and an acid blank were run with each set of samples. Aliquots of 0.6 ml of the acid phase were used to determine NE or EP by the method of Shore and Olin(5), using an Aminco spectrophotofluorometer. Except for changes in volume and in homogenization details the same procedure was used for the other tissues.

Results. After about 2 weeks of NGF injections, the sympathetic ganglia of the mice were found to be enlarged as much as 5-fold (Fig. 1 cf. 1, Fig. 6-7). Catecholamine levels were determined on a series of litters involving smaller, but still clearcut, hypertrophy (Table I). The wet weights of the NGF-treated ganglia ranged from 1.6 to 3.2 times control levels, but these values included variable amounts of connective tissue. The ratios are likely to be low in view of the greater difficulty in "cleaning" the smaller, untreated ganglia. The increase in catecholamine content per ganglion ranged from 1.7- to 4.1-fold, this ratio being greater in

* Purification was carried out by Otto Walasek (Biochemistry Dept.) and by Ronald Gendrich and Dr. Wilfred White (Biochemical Development Dept.).

each of the 7 litters than the ratio of tissue mass increase. The NE *concentration* of NGF-hypertrophied ganglia has therefore been maintained (and possibly increased) during these dynamic conditions of growth. In litter 5, epinephrine analyses were made in addition to those for norepinephrine. In contrast to the 3-fold increase in NE per ganglion to 19 mμg, the EP content remained at the barely detectable level of about 1 mμg. On the basis of the specificity of this analytical procedure with reference to these and closely related amines(6), it seems clear that the observed NGF-evoked increase in ganglion catecholamine consists predominantly of NE. It should be pointed out that the data from the NGF-treated mice have been presented by litters in order to emphasize the correspondence between catecholamine increase and hypertrophy of these ganglia under a variety of experimental treatments. On the other hand, the weights and catecholamine content of the control ganglia from all 7 litters have been pooled since no significant differences as a function of body weight or age were detected. It is of interest, for example, that increases in ganglion mass and NE content were still substantial even when toxic side effects of relatively im-

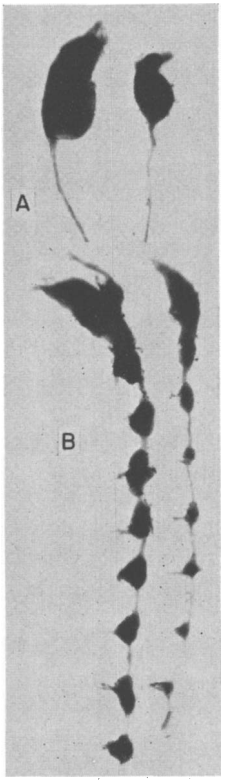

FIG. 1. Enlarged sympathetic ganglia (A: superior cervical; B: stellate and thoracic) of a 15-day NGF-treated mouse (left) in comparison with a 15-day control ganglion chain (right).

TABLE I. Catecholamine Levels of Sympathetic Ganglia from Control and NGF-Treated Mice.

	Litter						
	Treated						Control
	1	2	3, 4	5	6	7	
NGF potency (units/μg protein)	7	7	16	16	33	160	—
No. of daily injections (500-1000 units/g body wt)	16	14‡	13	24	13	22	—
Avg body wt (g) at detn. (1 day after last inj.)	3.7 (8.4)†	6.5 (7.0)	7.7 (8.8)	14 (16)	10 (10)	14 (14)	—
No. of ganglia used	8 (12)†	8 (10)	24 (23)	15 (16)	12	16 (16)	77
Avg wet wt of ganglion (mg)	.56	.70	.85	1.1	.63	.67	.34 ± .6§
Wt of NGF ganglion/control wt	1.6	2.1	2.5	3.2	1.8	2.0	—
Avg NE content of ganglion (mμg*)	7.9	12	13	19	10	14	4.6 ± .8§
NE content of NGF gangl./control content	1.7	2.6	2.8	4.1	2.2	3.0	—
Avg NE conc. of gangl. (mμg/mg tissue)	14	17	15	17	16	21	14 ±3 §

* mμg = millimicrograms = 10^{-9} g.
† Figures in parentheses refer to controls for specific litters.
‡ Somatotrophic growth hormone (Armour) was inj. along with NGF in these mice (*ca.* 15 μg/g body wt).
§ Mean ± stand. dev.

pure NGF fractions drastically curtailed body growth, *e.g.*, litter 1(cf. 1-3).

NE concentrations were determined in several other organs in litters 2, 5 and 7. A ratio of these concentrations in treated with respect to control mice was obtained using pooled organs from 2 to 4 treated animals and an equal number of controls. The mean and standard deviation of 3 to 4 ratios per organ were as follows: submaxillary gland, 2.3 ± 0.6; heart, 1.9 ± 0.4; brain, 1.0 ± 0.1; adrenal gland, 0.9 ± 0.1; spleen, 0.9 ± 0.2; and liver, 0.8 ± 0.2.

Discussion. The data suggest that NGF-induced hypertrophy of sympathetic ganglia involves increase not only in total protein and nucleic acids, but also in molecules which play a special role in the normal functioning of these neurons. It will be of interest to determine whether this hypertrophy leads to significant physiological effects, both in the normal as well as the neurologically damaged organism.

Our observation of 2- to 5-fold enlargement of sympathetic ganglia following daily injections of newborn mice with 500-1000 NGF units/g body weight is in good agreement with the work of Levi-Montalcini and Cohen. They reported use of 300-670 units/g to produce 3- to 6-fold ganglion hypertrophy. Considering the variations in our salivary purification procedures and tissue culture NGF-potency interpretations, the above differences in required dosage are quite small.

The lack of agreement between degree of ganglion hypertrophy produced in some of our litters by apparently similar dosage (*e.g.*, litters 5 *vs* 7 in Table I), is not surprising in view of the limitations in tissue culture assay of the various NGF fractions(4) as well as biological variations in NGF sensitivity that may well occur. Serious quantitation of the dose-response relations must await availability of larger amounts of purified NGF.

Our value of 14 ± 3 μg NE/g of mouse sympathetic ganglion tissue agrees well with the concentrations reported by Lissák(7) for adult cat and rabbit superior cervical ganglia, *i.e.*, 16 and 14 μg/g respectively.

The approximately 2-fold increase in NE concentration of heart and submaxillary gland in our NGF-treated mice may reflect the "hyperneurotization of the viscera" determined by histological methods(1). Although the lack of NE increase in brain and adrenal gland of the treated mice is quite reasonable in view of Levi-Montalcini's morphological observations(2), analysis of the spleen and liver results will require further investigation.

The recent report by Selye *et al.*(8) that isopropyl noradrenaline (isoproterenol) can evoke selective, 5-fold enlargement of (rat) salivary glands may be pertinent to analysis of the mechanism by which a (mouse) salivary gland component acts as a selective stimulant of growth and catecholamine content of sympathetic ganglia.

Summary. (1) Previous reports(1-3) of powerful growth-stimulating effects of a mouse submaxillary gland protein fraction (NGF) on certain peripheral ganglia have been confirmed. (2) Norepinephrine content of enlarged sympathetic ganglia in NGF-treated mice increased proportionate to tissue mass (3- to 4-fold), while epinephrine content remained barely detectable. (3) Norepinephrine concentrations of the heart and submaxillary gland increased about 2-fold in NGF-treated mice, while no significant increases were detected in brain, adrenal gland, spleen and liver.

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