Short Communications

Lipids in Ganglia Treated with Nerve Growth Factor

By G. D'AGNOLO, F. H. FOPPEN and ANTONIA LIUZZI

Laboratori di Chimica Biologica and Centro di Neurobiologia, Istituto Superiore di Sanità, Rome, Italy

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Sensory-nerve and sympathetic-nerve cells are stimulated by a protein (NGF*) isolated from mouse salivary glands (Cohen, 1960) and snake venoms (Banks *et al.* 1968). NGF elicits *in vitro* a vigorous nerve-fibre outgrowth in chick-embryo sensory ganglia, whereas according to Levi-Montalcini (1966) injection in newborn mice causes a striking hypertrophic and hyperplastic response of the sympathetic ganglia.

Experiments on the incorporation of radioactive precursors have demonstrated remarkable action of this protein on ganglionic biosynthetic processes (Angeletti, Gandini-Attardi, Toschi, Salvi & Levi-Montalcini, 1965; Toschi, Dore, Angeletti, Levi-Montalcini & de Haën, 1966; Liuzzi, Angeletti & Levi-Montalcini, 1965).

In the present work attention was paid to the effect of NGF on total lipid content of chick-embryo sensory ganglia, with special regard to fatty acids.

Methods. Sensory ganglia, as soon as explanted from 8-day-old chick embryos, were washed with cold 0.9% NaCl and then homogenized for zero-time analyses. Incubation experiments were carried out as described by Liuzzi & Foppen (1968), with NGF preparations purified by either the method of Bocchini & Angeletti (1968) or that of Varon, Nomura & Shooter (1967). Although important molecular divergences were found, no significant quantitative biochemical differences were registered.

After the 4 hr. period the ganglia were collected from the incubation medium and they were washed with cold 0.9% NaCl and then homogenized.

Total lipids were extracted by the method of Folch, Lees & Sloane-Stanley (1957). For quantitative analyses the chloroform-lipid phase was concentrated *in vacuo* at 40° under N₂. Thereafter the concentrate was subjected to the vanillin-H₂SO₄ colour reaction (Pilz, 1967) for total lipid determination, and to the hydroxamic acid reaction (Goddu, Leblanc & Wright, 1955) for measurement of esterified fatty acids. For the determination of free fatty acids ganglia were freeze-dried and then

homogenized in a known volume of ether. The ethereal suspension was divided into two portions, one of which was treated with diazomethane (de Boer & Backer, 1954). Both samples were analysed for fatty acid esters by the hydroxamic acid method. Values for fatty acid esters from the non-methylated solution were in good agreement with those found for the direct measurements described above.

Total glycerol, after saponification of the lipid extract for 3 hr. in 2M-KOH-methanol (1:1, v/v), was measured with the Boehringer enzyme test originally described by Garland & Randle (1962).

Results and discussion. A significant increase of total lipids in chick-embryo sensory ganglia was observed after treatment with the NGF (Table 1). This net increase in lipid formation was accompanied by a striking rise in esterified fatty acid content, whereas free fatty acids almost completely disappeared, diminishing from 6.0 to 1.3 µg./100 ganglia with NGF. Also total glycerol, measured after saponification, increased remarkably on treatment with this protein. Comparison of the results obtained between zero time and after incubation for 4 hr. shows clearly that the quantitative changes are due to the presence of NGF. The 70% increase of lipids comes near to the stimulating effect on the incorporation of labelled acetate into sensory ganglia described by Liuzzi *et al.* (1965). It is noteworthy that, although the total lipid increased in the treated ganglia, no comparable changes in ganglial dry weights were observed.

The physiological importance, in the nervous system, of complexes formed by an association of proteins with lipids is well established. Gandini-Attardi, Calissano & Angeletti (1967) have obtained evidence of a small differential stimulation of cell proteins, suggesting a possible effect on the proteins constituents of the nerve fibres. Our own results do not rule out such an interpretation, but indicate that the NGF possesses a higher specific activity. The present results, in fact, strongly indicate an action of the protein on the enzymes concerned with the acyltransferase processes. This is supported by the fact that there is a marked decrease in the concentration of free fatty acids when there appears

* Abbreviation: NGF, nerve growth factor.

Table 1. *Amount of lipids in sensory ganglia treated with NGF*

Total lipids were measured with the vanillin-H₂SO₄ colour reaction of Pilz (1967); esterified fatty acids and free fatty acids, after methylation, were determined with the hydroxamic acid reaction of Goddu *et al.* (1955); total glycerol, after saponification, was measured enzymically. The values are given in $\mu\text{g./100}$ ganglia, and are the means $\pm$ s.e.m. of at least five experiments.

	At 0 hr.	Control at 4 hr.	NGF-treated at 4 hr.	Difference between NFG-treated and control (%)
Dry weight	495 $\pm$ 31	454 $\pm$ 25	435 $\pm$ 20	-4
Total lipids	110 $\pm$ 12	103 $\pm$ 14	171 $\pm$ 14	+66
Esterified fatty acids	9.5 $\pm$ 1.7	8.6 $\pm$ 1.5	21.7 $\pm$ 3.4	+152
Free fatty acids	6.0 $\pm$ 0.5	6.0 $\pm$ 0.7	1.3 $\pm$ 0.3	-78
Total glycerol	57.6 $\pm$ 4.0	49.0 $\pm$ 3.4	76.6 $\pm$ 6.4	+56

to be an increased biosynthetic potential. It is not possible to attribute this decrease to an inhibition of fatty acid synthetase, limiting the amount of acyl thiol esters available for the synthesis of lipids, because both total lipids and total fatty acids are increased.

On the other hand the lower but still remarkable stimulation of total glycerol content could be explained either as a secondary effect or a more general mechanism triggered by the protein. Lipids, in fact, play a critical role in cell-surface structure and function, so that the NGF, which affected profoundly lipid synthesis, might be responsible for the nerve-fibre outgrowth through action on the cell membranes. This kind of action had been envisaged in our study on the sterol-like compound (Liuzzi & Foppen, 1968).

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