EFFECT OF A SPECIFIC NEURONAL GROWTH FACTOR ON RNA METABOLISM BY SENSORY GANGLIA FROM CHICK EMBRYO

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When sensory ganglia from 8-day old chick embryos are incubated in vitro, the growth of the nerve cells depends upon the presence in the medium of a specific protein factor (NGF) (Levi-Montalcini and Cohen, 1960; Levi-Montalcini and Angeletti, 1963). Under these conditions effects of NGF on the main processes of protein metabolism have been observed (Angeletti et al, in preparation): during the time preceeding the morphological phenomena (the first 6 hrs) ganglia incubated in the presence of NGF definitely showed higher rates of: a) amino acid incorporation into the cell proteins, b) loss of previously incorporated amino acids.

All these observations have raised our interest in a study of the connections between NGF and RNA metabolism. This is the subject of the present work.

EXPERIMENTAL

Preparations and assays of NGF, as well as the general experimental conditions, have been described elsewhere (Levi-Montalcini and Cohen, 1960). In the present experiments, the ganglia from one embryo were collected and pooled in order to compare 15 ganglia from one side ("experimental") with their contralateral counterparts ("control"). As a rule, "pairs" from 4 to 5 embryos were run in each experiment. Media and times of incubation are indicated in the legends. Uridine-H^3 with a specific activity of 2.1 c/mM and l-leucine-C^{14} with a specific activity of 34.8 mc/mM were used. After stopping the incuba-

tion by three washings with ice-cold saline containing "cold" uridine (1 mM) or casein hydrolysate (1%), the ganglia were precipitated with cold 7% perchloric acid, in the presence of 2.5 mg of serum albumin. The precipitate was processed following the scheme of Flexner et al (1962) but the hot perchloric and the hot ethanol steps were omitted when uridine-H^3 incorporation was measured. The final precipitate was dissolved with 1 ml of formamide (20 hrs at 140°), mixed with ethanol (8.6 ml) and with 10.4 ml of liquid scintillator (NE213, Nuclear Enterprises, G.B., Ltd.). Radioactivity was measured with a low-background automatic apparatus (Liquid scintillation System 725, Nuclear, Chicago).

RESULTS

RNA labeling: Fig. 1 shows that a higher incorporation of uridine-H^3 in the presence of NGF is found after 3 to 4 hrs incubation; this difference was found consistently in all the experiments and increased markedly at 6 and 9 hrs incubation. Results of short-time labeling (60 to 90 min) are shown in Fig. 2: uridine-H^3 was added after 4 to 5 hrs preincubation. Under these conditions a 100% increase of incorporation

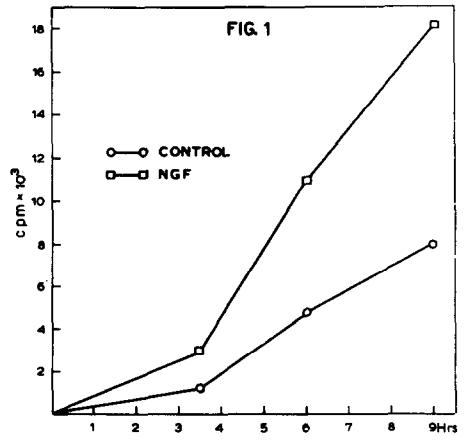
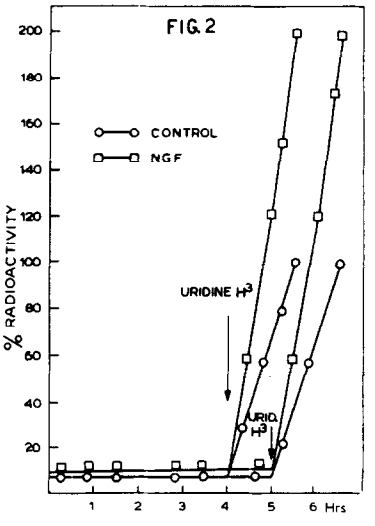


FIGURE 1.
Sensory ganglia from chick embryo, incubated in Eagle's basal medium with and without NGF (0.5 μg/ml), in the presence of uridine-H^3 (20 μc/ml).

was regularly observed in the experimental ganglia. A preliminary characterization of this 60-min-labeled RNA, extracted by hot phenol and examined by chromatographic and ultracentrifugal procedures,

has shown it to be mainly a material with high molecular weight (from 26 to 45 S). In order to correlate the observed effect of NGF on the RNA labeling with its effect on the amino acid incorporation, an experiment was carried out, in which, after 4 hrs preincubation, some ganglia were exposed to uridine-H^3 and the others to leucine-C^{14}. Results, illustrated in Fig. 3, clearly indicate that the stimulation of RNA synthesis preceeds the effect on protein synthesis. In fact the RNA labeling with NGF is double when the effect on protein labeling is barely detectable.



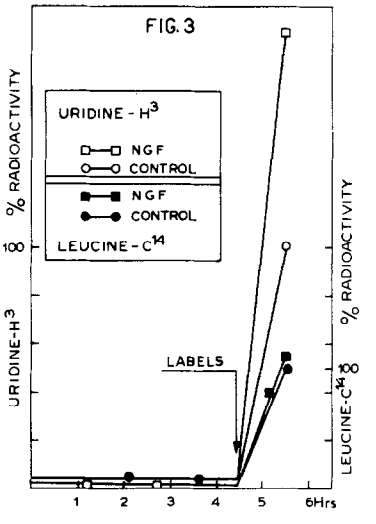
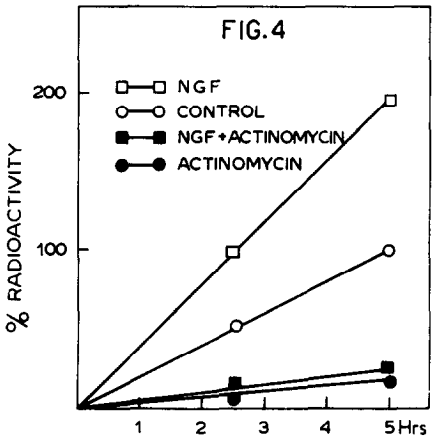


FIG. 2: Sensory ganglia incubated in Eagle's basal medium with and without NGF. Uridine-H^3 added at arrows (4th to 5th hr); incubation stopped after 60 to 90 min labeling. Results expressed as percent of the control values.

FIG. 3: Sensory ganglia incubated in Eagle's basal medium with and without NGF. Uridine-H^3 or Leucine-C^{14} (2 μc/ml) added at arrows (4hr 30 min); incubation stopped after 60 min labeling. Results expressed as in Fig. 2 .

Effects of inhibitors: Fig. 4 shows that Actinomycin-D (1 μg/ml) after 2, 30 and 5 hrs incubation cuts RNA labeling down to 10 to 15%, both in the control and the experimental ganglia, and suppresses any significant difference between them. This same level of Actinomycin has been found to suppress the effect of NGF on the amino acid incor-

poration by ganglia incubated with leucine-C^{14} for 6 hrs (Angeletti et al). On the other hand, when Actinomycin was added after 4 hrs preincubation with NGF, the effect of the latter on the amino acid incorporation in the next 3 hrs was still present. This effect can be accounted for by the stimulation of RNA synthesis during preincubation.



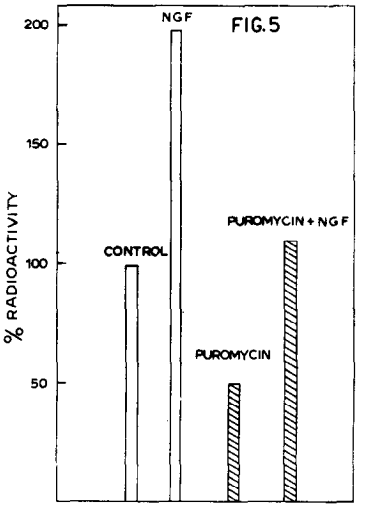


FIG. 4: Effect of Actinomycin-D (1 μg/ml) on uridine-H^3 incorporation by sensory ganglia, incubated in Eagle's basal medium with and without NGF, in the presence of uridine-H^3. Results expressed as in Fig. 2.

FIG. 5: Effect of Puromycin (20 μg/ml) on uridine-H^3 incorporation by sensory ganglia incubated in Eagle's basal medium with and without NGF for 5 hrs. Uridine-H^3 added at the 4th hr. Results expressed as in Fig. 2.

As shown in Fig. 5, the presence of Puromycin (20 μg/ml) in the medium reduces the RNA labeling, both in control and experimental ganglia, but does not suppress the effect of NGF. Under the same experimental conditions, Puromycin was found to cut down to 5 to 10% the amino acid incorporation (Angeletti et al).

The results presented here demonstrate that the early effect of NGF on RNA metabolism is a prominent feature of its action on the responsive nerve cells. The short-time labeling is likely to involve

mainly nuclear RNA (Graham and Rake, 1963). A further characterization of this NGF-stimulated RNA is in progress. These experiments also give evidence that, in the sequence of the metabolic events provoked by the NGF, the stimulation of RNA synthesis ("RNA effect") preceeds that of protein synthesis ("protein effect"). Furthermore, the experiments with specific inhibitors indicate that the "RNA effect", within certain limits, is not dependent on the "protein effect", whereas the latter is suppressed by the inhibition of the former.

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