Electrophoretic fractionation of proteins of the subesophageal ganglionic complex of Helix pomatia showed that the relative electrophoretic mobility of most proteins is 0.2-0.6. On incubation of the subesophageal ganglionic complex with L-leucine-4,5-SH for 3, 6, and 24 h the mean relative radioactivity of the neurospecific proteins increased. Dibutyryl-cyclic AMP (2 • 10^-6 M) was shown to inhibit the processing of low-molecular-weight neurospecific proteins.

KEY WORDS: Dibutyryl-cyclic AMP; specific proteins of neurons; processing.

It has been shown in the last decade that the nerve tissue of vertebrates and invertebrates contains a number of unique proteins that are not found in the other organs of these animals [5, 9]. In particular, Peng Loh and Gainer [6, 7] found such proteins in the mollusk Aplysia californica. The specific low-molecular-weight proteins of the neurons of this animal were found to be split into fragments of lower molecular weight [7] during transport along the axon.

It was decided to study how dibutyryl-cyclic AMP affects the processing of the neurospecific low-molecular-weight proteins of the mollusk Helix pomatia.

EXPERIMENTAL METHOD

Snails active for two weeks were chosen for the experiment and killed; the subesophageal ganglionic complex was removed, its connective-tissue membrane opened, than the ganglia were kept for 1 h in physiological medium [3]. The ganglia were then transferred to 1 ml of this medium with 100 μCi L-leucine-4,5-3H and 2 • 10^-5 M dibutyryl-cyclic AMP. Incubation continued for 4, 6 or 24 h at 20-22°C. After the end of incubation the following ganglia were excised from the complex, the right and left pleural, the right and left parietal, and the visceral [3]. All ganglia were homogenized in 0.2 ml of 0.9 M acetic acid and 10 M urea (pH 2.4). The homogenate was applied to disks of 10.5% polyacrylamide gel (PAG) and subjected to electrophoresis. Pyronine was used as the reference substance. The disks of gel were then cut into 3-mm fractions, 0.12 ml 30% H2O2 was added, and the samples were kept at 4-5 h at 40°C, after which 10 ml toluene-based scintillator was added. The
Fig. 1. Electrophoresis of proteins of sub-esophageal ganglionic complex of Helix pomatia in 10.5% PAG with 0.9 M acetic acid and 10 M urea (pH 2.4).

radioactivity of the fractions was measured on a Multimat spectrometer with an efficiency of 38%. The radioactivity of the fractions in these experiments before conversion to a percentage was 500-5000 cpm. The radioactivity of the individual fractions was expressed as the ratio of their radioactivity to the total radioactivity of the whole spectrum of fractions in PAG.

Statistical analysis was carried out by the use of the U-criterion [1].

**EXPERIMENTAL RESULTS**

Peng Loh and Gainer [7] studied the processing of low-molecular-weight proteins under experimental conditions by keeping the cells for different time intervals after incubation with labeled leucine in medium without the label.

The aims of the present experiments were to study: 1) changes in incorporation of labeled leucine into neuron proteins under the influence of dibutyryl-cyclic AMP and 2) changes in the radioactivity of the fraction of low-molecular-weight proteins at different times after the beginning of incubation.

The profile of relative radioactivity of a typical electrophoretic "distillate" and the electrophoretic distribution of the fractions are illustrated in Figs. 1 and 2. Most proteins were found to have relative electrophoretic mobility (Rf) within the range from 0.2 to 0.6. Proteins with a high Rf value (0.75-1.0), according to several investigations, are neurosecretory proteins [6, 8]. The good quality of separation of low-molecular-weight proteins from other proteins in this gel system (pH 2.7) must be emphasized. Staining the PAG disks and determination of the radioactivity of the start strip showed that this region contained only 4-5% of the total synthesized protein, whereas the quantity of labeled leucine-3H in the fraction with maximal radioactivity was 20-30%. The content of radioactivity in the fraction of neurospecific proteins is given in Table 1. It will be noted that these data are semiquantitative in character. The reason is partly that on extraction of the protein from PAG by means of 30% H2O2 oxygenation and depolymerization took place not only of the gel, but also of the protein, with the result that 25-30% loss of label could not be accounted for [2]. It was therefore decided to use control and experimental values of the U-criterion for comparison, a procedure that is recommended for the comparison of independent groups of data of semiquantitative character. Radioactivity in each of the fractions was expressed as the ratio of the radioactivity of that fraction to the total radioactivity of all fractions. This enabled the radioactivities of the individual experiment to be compared. It will be clear from Table 1 that the