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EFFECT OF $N^6O^{2'}$-DIBUTYRYL-CYCLIC ADENOSINE-3',5'-MONOPHOSPHATE ON PROTEIN SYNTHESIS IN THE SUBESOPHAGEAL GANGLIONIC COMPLEX OF Helix pomatia

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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-^{3}H for 3, 6, and 24 h the mean relative radioactivity of the neurospecific proteins increased. Dibutyryl-cyclic AMP ($2 \cdot 10^{-6}$ 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-^{3}H and $2 \cdot 10^{-6}$ 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% H_2O_2 was added, and the samples were kept at 4-5 h at 40°C, after which 10 ml toluene-based scintillator was added. The

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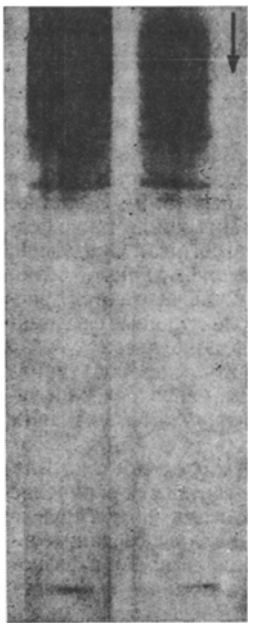

Fig. 1. Electrophoresis of proteins of subesophageal ganglionic complex of Helix pomatia in 10.5% PAG with 0.9 M acetic acid and 10 M urea (pH 2.4).

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 neutron 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 (R_f) within the range from 0.2 to 0.6. Proteins with a high R_f 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 luecine-^{3}H 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% H_2O_2 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

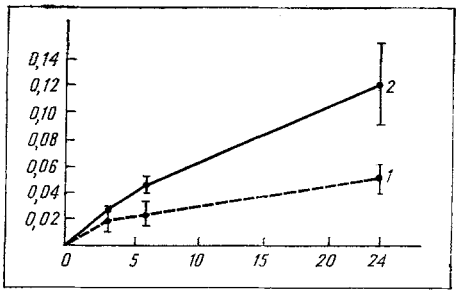

Fig. 2. Changes in radioactivity of low-molecular-weight protein fraction of subesophageal ganglionic complex of Helix pomatia at different times after beginning of incubation. 1) Experiment; 2) control. Abscissa, time (in h); ordinate, mean relative radioactivity.

TABLE 1. Relative Radioactivity of Low-Molecular-Weight Fraction of Ganglionic Complex of Helix pomatia (M±m)

Experiment no.	Incubation time, h	Relative radioactivity		Mean relative radioactivity	
		control	experiment	control	experiment
1	3	0,0054±0,0005	0,0029±0,0003	0,0276±0,009	0,0196±0,007
2		0,0082±0,0006	0,0047±0,0004		
3		0,0481±0,0003	0,0310±0,0002		
4		0,0488±0,002	0,0399±0,002		
5	6	0,0093±0,0002	0,0063±0,0002	0,0464±0,015	0,0262±0,014
6		0,0575±0,0005	0,0300±0,0004		
7		0,0724±0,0005	0,0422±0,0002		
8	24	0,0442±0,003	0,0107±0,0008	0,1178±0,030	0,0525±0,014
9		0,3015±0,0006	0,0582±0,0003		
10		0,1029±0,0002	0,0596±0,0002		
11		0,0699±0,0004	0,0666±0,0004		
12		0,0705±0,0002	0,0675±0,0003		

mean values of the relative radioactivities of the neurospecific fractions differed after 3, 6 and 24 h; whereas after 3 and 6 h the differences between the radioactivities of the neurospecific fractions in the control and experimental series were not significant, after 24 h they became significant; it can thus be deduced that dibutyryl-cyclic AMP, at least under the experimental conditions used, inhibits the synthesis of neurospecific proteins. The existence of significant differences not only between the control and experimental values after 24 h, but also as regards the relative radioactivities of the control with each other and the experimental groups with each other after 3 and 24 h will be noted. This suggests that the radioactivity of the fractions of neurospecific proteins 24 h after the beginning of incubation had increased significantly for all the experimental groups given in Table 1. Consequently, the results agree with those obtained by Peng Loh and Gainer, who showed that during transport of neurospecific proteins with a molecular weight of 12,000 daltons these proteins are degraded into proteins of lower molecular weight, which are evidently secreted by the cells but are not decomposed.

The authors cited found no change in the content of label in proteins with $R_f = 0.8$. In many experiments simultaneously with inhibition of the synthesis of neurospecific proteins, we observed that dibutyryl-cyclic AMP caused increased incorporation of labeled leucine into the high-molecular-weight fractions of proteins with R_f = 0.25-0.3. This suggests that the fraction of high-molecular-weight protein is synthesized de novo under the influence of dibutyryl-cyclic AMP. However, this phenomenon was not always observed; more precisely, the picture of increase of radioactivity in the fractions of high-molecular-weight proteins was so complex that no definite conclusion can be drawn on the effect of dibutyryl-cyclic AMP on the synthesis of these proteins. On the other hand, inhibition of neurospecific proteins was reproduced from one experiment to another. Most probably, besides inhibiting the de novo synthesis of neurospecific proteins, dibutyryl-cyclic AMP most probably affects the synthesis of many other proteins in neurons also, including those of high molecular weight. This hypothesis is not in conflict with Peng Loh and Gainer's findings, for their experimental conditions and ours differed. Mean-

while the results of both our and their experiments indicate that low-molecular-weight proteins are not degraded, at least during the period chosen for these experiments, but accumulate, for, as the present experiments showed, the relative radioactivity of neurospecific proteins increased after 6 and 24 h. Had degradation of the proteins taken place, this radioactivity would either have decreased or would have remained unchanged if the rates of synthesis and degradation of the protein had been equal. Since the relative radioactivity of the fraction with R_f=1.0 increased, this could have arisen through an increase of radioactivity as a result of the accumulation of neurospecific low-molecular-weight proteins.

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EFFECT OF RADIOPROTECTORS ON CYCLIC AMP-DEPENDENT PROTEIN PHOSPHORYLATION

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The ability of radioprotectors (serotonin, aminoethylisothiouronium) in radioprotective doses to stimulate cyclic AMP-dependent phosphorylation of mouse liver cytosol and nuclear and spleen cytosol proteins in vivo was demonstrated. In experiments in vitro, the radioprotectors had no direct action on protein kinase activity or its stimulation by cyclic AMP. It is postulated on the basis of these results and those of previous investigations that activation of cyclic AMP-dependent phosphorylation is due to an increase in the intracellular cyclic AMP concentration under the influence of the radioprotectors.

KEY WORDS: Radioprotectors; cyclic AMP system; protein kinases; phosphorylation of proteins.

The authors showed previously that radioprotectors directly or indirectly activate adenylate cyclase and increase the intracellular concentration of cyclic AMP in the tissues of the body [2, 4, 5]. Elevation of the cyclic AMP level is known to cause activation of cyclic AMP-stimulated protein kinases which, by phosphorylating various protein substrates, modify the course of a wide spectrum of biochemical processes [11]. The action of radioprotectors on cyclic AMP-dependent protein phosphorylation could lead to substantial changes in cell metabolism and could cause biochemical structural changes that could lead to increased radioresistance under conditions of chemical protection [1, 3, 7].

In the investigation described below the effect of radioprotective agents – serotonin and aminoethylisothiouronium (AEP) – on cyclic AMP-dependent protein phosphorylation and on protein kinase activity was investigated in animal tissues.

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