SYNTHESIS OF ADRENOCORTICOTROPIN IN THE PITUITARY OF NORMAL AND IRRADIATED RATS IN VITRO

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In most investigations the state of the hypothalamus — pituitary — adrenal cortex system after irradiation has been judged indirectly (from the content of cholesterol and ascorbic acid in the adrenals, of corticosteroids in the blood, and of their metabolic products in the urine, the ACTH concentration in the blood, etc.), or from the results of morphological investigations [1, 2, 5, 6, 8, 9, 11].

Mateyko and Edelman [7] found that after total irradiation of rats in a dose of 1000 R the ACTH content in the pituitary rose sharply during the first hour and fell slightly below the normal level 6 h after irradiation.

In other experimental conditions M. A. Larina [3] found no statistically significant change in the ACTH content in the pituitary of rats 3 h after irradiation in a dose of 700 R. However, determination of the ACTH level in the pituitary alone is insufficient for describing its reaction to irradiation. Bearing in mind that the pituitary hormones are proteins or peptides, O. V. Molotkov [4], investigated protein metabolism in the pituitary of irradiated animals by means of labeled amino acids and discovered statistically significant changes at various times after irradiation. In this connection it was interesting to examine whether the rate of synthesis of the individual pituitary hormones and, in particular, of ACTH is modified in the irradiated animal.

In the present investigation ACTH synthesis in the pituitary of irradiated animals was studied in experiments in vitro as shown by the incorporation of labeled amino acids into this polypeptide.

EXPERIMENTAL METHOD

Experiments were carried out on 252 noninbred male rats weighing 200-300 g. Whole-body irradiation was given on the RUM-11 x-ray therapy apparatus in a dose of 800 R. The conditions of irradiation were: voltage 180 kV, current 15 mA, filters Al 1 mm, Cu 0.5 mm, skin-focus distance 40 cm, dose rate 25 r/min. The rats were killed by decapitation. The anterior lobes of the pituitaries were placed in the small container (4-5 adenohypotheses per container) of a Warburg's apparatus containing 1 ml of basic Krebs-Ringer solution and 2 μCi of labeled amino acid. For most of the experiment methionine-S^{35} was used. Parallel experiments were carried out with glycine-C^{14} and tyrosine-1-C^{14}. The effect of the incubation medium on the velocity of incorporation of methione-S^{35} into ACTH was first studied. For this purpose each adenohypothesis was divided into halves, some halves were incubated in basic Krebs-Ringer solution, and the other halves were incubated in the same conditions in Kreb's-Ringer phosphate (pH 7.2) with glucose (200 mg%). The degree of incorporation of the label was closely similar in both cases.

Incubation was carried out in an atmosphere of 95% O_2 and 5% CO_2 (37.5°) with 130-150 oscillations per minute for 3 h. The ACTH was isolated from the contents of the small container by a slight modification of the method [12] used to determine incorporation of phenylalanine-C^{14} into ACTH in adrenalectomized and normal rats. An ACTH preparation from the Leningrad Medical Preparations Factory was used as carrier. Centrifugation after precipitation with NaCl and acetone was replaced by passage through a Schott No. 3 filter. The hydroxycellulose was washed on a Büchner funnel.

The radioactivity was measured with a T-25 BFL end-type counter in a thin layer. The counting period guaranteed an error of not more than 5%.

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TABLE 1. Radioactivity of ACTH (in pulses/min/100 mg pituitary tissue) Isolated after Incubation of Pituitaries with Labeled Amino Acids (2 μCi per container), M±m

Amino acid	Normal	Time after irradiation					Adrenalectomy
		immediately	3 days	1 day	3 days	5 days	
Methionine-S^{35}	1234±96	1191±93	1227±79	1062±101	1053±146	1320±74	2101±108
P	—	*P*>0,5	*P*>0,5	*P*>0,2	*P*>0,2	*P*>0,5	<0,001
Tyrosine-C^{14}	442±37		482±39		359±20		
P			*P*>0,5		*P*>0,2		

To show that the radioactivity of the isolated ACTH was associated with it and not with nonspecific contamination, 3 experiments were carried out with incubation of muscle tissue. The weighed sample corresponded roughly to the samples of the pituitaries and the tissue was cut into pieces of approximately the same size as the pituitaries. Incubation and isolation of the ACTH fraction on the carrier were carried out as described above. In these experiments the "ACTH fractions" showed no radioactivity.

The reproducibility of this method of isolation of ACTH was also checked. For this purpose 25 pituitaries were incubated in 3 containers, after which a total homogenate was prepared from them by means of a manual glass homogenizer, and ACTH was isolated from six equal parts of the homogenate by the method described above. The radioactivity of these samples (1 ml of the hydrochloric extract was placed on the target in each case) varied within very narrow limits (from 50 to 59 pulses/min).

EXPERIMENTAL RESULTS

The results expressed as radioactivity of ACTH in pulses/min/100 mg fresh pituitary tissue are given in Table 1. As Table 1 shows, at none of the times studied (3 h, 1, 3, and 5 days, and immediately after irradiation) were significant changes observed in the rate of incorporation of methionine-S^{35} into ACTH compared with normal. This was also true of tyrosine-1-C^{14}.

There was a sharp difference in the level of incorporation of the various labeled amino acids used into ACTH. The incorporation of methionine-S^{35} was the greatest of all. In the case of glycine-1-C^{14}, five experiments were carried out on normal rats and three experiments on irradiated (3rd day after irradiation) animals. In no case was incorporation of the label observed into the isolated ACTH fraction. This phenomenon is difficult to account for, for the amino acid composition of rat ACTH has not been studied. The suggestion of loss of label as the result of decarboxylation of glycine-1-C^{14} was invalid, because glycine was incorporated into the protein fraction remaining in solution in 0.1 N acetic acid after adsorption of the ACTH onto hydroxycellulose even to a slightly greater degree than methionine-S^{35}.

A statistically significant increase in the rate of synthesis was observed in these experiments and also by other authors [12], in adrenalectomized rats 7-10 days after the operation. This intensification of ACTH synthesis may be associated with the formation of actively synthesizing cells after adrenalectomy [10]. Irradiation, however, judging by the results obtained, causes no significant disturbances in ACTH biosynthesis in vitro. This conclusion is valid for the experiments in which the pituitary was extracted from the animals at various times after irradiation. It is possible, of course, that in the intact organism, ACTH biosynthesis follows a different course from that in vitro, but the experiments on adrenalectomized animals shows conclusively that the changes arising after the operation are reflected in the activity of the enzyme system when determined in vitro.

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