EVIDENCE FOR ANOTHER FACTOR IN THE REGULATION OF CORTICOSTERONE BIOSYNTHESIS BY ACTH

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Received June 10, 1971

Summary The capacity of rat adrenals to produce corticosterone, as determined by in vitro superfusion with ACTH, has been shown to decay after hypophysectomy with a half-life period of approximately 6-7 hours. The decay cannot be attributed to any of the known factors under ACTH regulation. It is postulated that this new factor may be a m-RNA.

Introduction

Adrenocorticotropic hormone (ACTH) regulates the biogenesis of the glucocorticoids mainly by the Zona fasciculata and reticularis of the adrenal cortex (1, 2). The effect of ACTH on this gland is manifested at several levels: (i) The hormone is required for the normal maintenance of the gland both in terms of weight and fine structure (3). (ii) It is required for the maintenance of normal levels of enzyme activities. Specifically, the cholesterol side chain desmolase complex declines after hypophysectomy (4, 5) showing a half-life of 3-4 days. (iii) It stimulates a rapid output of glucocorticoids. This effect was shown to be mediated via the translation of a pre-formed m-RNA to a labile protein with an unknown but essential function in glucocorticoid biosynthesis (6). The lability of this protein with a half-life of approximately 10 minutes explains the observation that the basal rate of glucocorticoid output by superfused glands (superfused without ACTH) drops very rapidly after hypophysectomy (7).

We wish to report here that there is another level of ACTH regulation. This regulation determines the "capacity" of the gland for glucocorticoid output, as determined by superfusion in the presence of ACTH. This regulation appears to involve a factor with a half-life period of approximately 7 hours which could indicate an RNA species.

Materials and Methods

Normal and hypophysectomized (operated on at 8-9 a.m.) male, Sprague-Dawley rats weighing about 200 g. were purchased from the Hormone Assay Laboratories, Chicago. ACTH (Acthar, Armour, Co.) was used at a final concentration of 0.1 U/ml. Corticosterone standard, tetramethylammonium hydroxide and tetrazolium blue used in the assay were purchased from Sigma. ^{3}H-Corticosterone obtained from New England Nuclear was purified by TLC. Unless otherwise specified, all other chemicals used were reagent grade. Whole, trimmed, intact adrenals from groups of 4-6 rats were used in duplicate superfusion runs. The superfusion medium was essentially Kreb-Ringer bicarbonate, pH 7.4, modified to contain 5 mM Ca^{++} and 0.2% glucose. The superfusion technique was an adaptation of the method of Tait et al (9) with modifications mainly to allow a continuous flow of 95% O_2/5% CO_2 gas mixture through the superfusion chamber containing the glands*. Experiments were carried out in a water bath of 38°C. The rate of superfusion was 24 ml per hour and perfusate fractions were collected at 30 to 60 minute intervals. Corticoids were extracted by established methods (10,11) using freshly redistilled dichloromethane. Corticosterone was separated by TLC on silica gel plates according to Lisboa (12) and assayed by Blue Tetrazolium assay method of Elliott et al (13) as modified by Peron (10). Losses occuring during extraction and purification were estimated and corrected for by using purified ^{3}H-corticosterone as an internal indicator.

Results and Discussion

The rate of corticosterone output, (μg corticosterone per rat per hour) as a function of time after hypophysectomy are given in Table I. The kinetics of ACTH stimulated output was similar to that reported by Schulster et al (14) with maximum output observed either in the 30-60 min. collections or the 60-90 min. collections. In all cases, the values obtained in the two periods of collection of highest output are used for computation of the rate of ACTH stimulated output. The basal rates of corticosterone output (superfused

* The authors would be glad to provide detailed information per request.

TABLE I

The maximal rate of corticosterone output (μg/rat/hr.) by whole adrenals of normal, and hyophysectomized rats at different time intervals following hypophysectomy. Adrenals from groups of 4 to 6 male rats were superfused and corticosterone output was determined as described in methods.

Time of Sacrifice after hypophysectomy	Corticosterone output (μg/rat/hr) Unstimulated	ACTH stimulated
0	6.5	8.3
3 hrs.*	0.5	6.3
6	1.2	4.2
9	0.9	3.75
12	0.7	2.8
32	0.55	0.9
42	0.5	0.95
4 1/2 days	0.5	0.8
7 1/2 days	0.5	0.8

* The 3 hour values are taken from published data by Schulster et al (14).

without ACTH) were fairly constant for several hours in the case of hypophysectomized animals and the values given in this table represent this low but steady rate of output. In the case of normal animals the glands superfused in the absence of ACTH showed a fairly high rate in the first half hour followed by a rapid decline. The value shown in this table is therefore the rate of corticosterone production in the first half hour of superfusion. The values shown for animals 3 hours post-hypophysectomy

were taken from the reports of Schulster et al (14) which were obtained with decapsulated glands from a different strain of rats.

The observed rapid decline of basal output of corticosterone after hyophysectomy confirms earlier reports of Tait et al (7) and can be explained by the decay of the labile protein postulated by Garren (6). The relatively slower decline of the capacity of the gland for corticosterone output, measured experimentally as ACTH-stimulated output, on the other hand, cannot be explained by any of the known factors under ACTH control. It is too slow to be accounted for by the decay of the labile protein and is too rapid to be accounted for by the decay of the cholesterol side chain desmolase complex (4, 5) or by gross anatomical changes (3). It appears therefore, that there is another factor, with a half-life period of 6-7 hours, which is under ACTH control.

Preliminary experiments on the "regeneration" of this factor indicate that it is in fact "regenerated" by ACTH administration, in vivo, and that this regeneration process requires several hours to be complete. Thus, when animals were given ACTH 6 hours post-hypophysectomy and sacrificed 20 minutes or 3 hours after ACTH administration, the gland's capacity to secrete corticosterone during ACTH superfusion was significantly restored (6.4 μg/rat/hr.) after 3 hours of ACTH treatment but was unchanged (3.7 μg/rat/hr.) after 20 minutes of in vivo ACTH treatment.

The nature of this factor is not yet known. However, its rates of decay and "regeneration" would be in accord with an RNA species and it is tempting to speculate that this may be the m-RNA coding for Garren's labile protein. Further work is currently in progress.

ACKNOWLEDGEMENT

This work was supported by Public Health Service Grants AM 12734 and AM 05384.

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