CONCENTRATION OF ACTH IN THE HYPOPHYSIS OF RATS AT DIFFERENT AGES

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The work of Selye has shown that the system of the hypophysis and adrenal cortex plays an important role in adaptation phenomena [8]. Experimental evidence is available suggesting that the adaptive powers of the body decrease with age [1]. It is perfectly logical to suggest that these changes are associated, on the one hand, with changes in the adrenocorticotropic function of the hypophysis or of the mechanisms which regulate it, and on the other hand, with a relaxation or alteration of the reaction of the adrenals to ACTH. Fortier [3] found no changes in the ACTH concentration in the adenohypophysis of rats between the 30th and 150th days of life.

We were interested to study the ACTH concentration in the hypophysis of rats throughout the postnatal period, and to determine the character of the age changes in this link of the general adaptation syndrome.

EXPERIMENTAL METHOD

We determined the ACTH concentration in the hypophysis of rats aged 1, 3, 12, and 24 months by the method suggested by Saffran and Schally [6]. Various writers [5, 10] have stated that this method has advantages over the method of determining the ACTH concentration by the fall in the ascorbic acid content of the adrenals of hypophysectomized rats, suggested by Sayers and co-workers [7]. The method of Saffran and Schally is more accurate, less cumbersome, and requires the use of fewer test animals.

The adrenals of test animals (2-month old male albino rats) were incubated at 37.5° in Krebs-Ringer bicarbonate solution (pH 7.4) containing 200 mg% glucose and saturated with a mixture of 95% oxygen with 5% carbon dioxide. After preincubation for 30 min, the incubation medium was discarded to remove substances which would interfere with the subsequent spectrophotometry of the corticosteroids, and replaced with fresh.

Extracts for analysis were prepared from the hypophysis of the experimental rats in hydrochloric acid [4, 9], and the extracts of 3-4 hypophyses from rats of the same age were usually pooled. Quantities of extract corresponding to 0.01, 0.02, and 0.04 of a hypophysis (in the case of 24-month old rats—0.005, 0.01, and 0.02, of a hypophysis on account of the larger size of the hypophyses of old animals) were added to the incubation vessels. A standard ACTH preparation was added in an amount of 1.25, 2.5, 5 and 10 mU*. All dilutions were made with Krebs-Ringer bicarbonate solution. The final volume of the incubation medium in the vessel was always 1.5 ml. Incubation continued for 2 h 30 min. At the end of incubation known volumes of incubation medium were extracted with small volumes of methylene chloride.

The concentration of corticosteroids in the methylene-chloride extract was deduced from the difference in the optical densities at 240 and 255 mμ with a type SF-4 spectrophotometer. As might have been expected, all the extracts had a maximum of absorption at 240 mμ. The difference between the optical densities was multiplied by the dilution, and the result was regarded as a measure of the secretion of corticosteroids in the corresponding vessel. The value of the secretion in conventional units was calculated per 1 g fresh adrenal tissue.

In each experiment the secretion of corticosteroids was determined without the addition (control) and with the addition of different volumes of test extracts and different doses of ACTH. Two parallel estimations were made for each dose. In the experiment from 3 to 7 tests animals were used, their adrenals being cut up into several parts and placed in the incubation vessels in such a way that part of an adrenal from each animal was found in each vessel. The ACTH concentration in the added extract was estimated from the difference between the secretion of corticosteroids after addition of the extract and in the control tests. By making simultaneous determinations of the secretion

*Milliunits—Publisher's note.

of corticosteroids by the adrenals after the addition of known doses of ACTH, it was possible to express the concentration of the hormone in the hypophyses of the experimental rats in milliunits. The numerical results obtained were treated by statistical methods.

EXPERIMENTAL RESULTS

After incubation of the adrenals of the test animals with known doses of ACTH, the secretion of corticosteroids increased along with the dose (Table 1). In the dose range which we used, a linear relationship was observed between the logarithm of the dose and the increase in the secretion of corticosteroids.

TABLE 1. Secretion of Corticosteroids by the Adrenals of Test Animals after the Addition of ACTH to the Incubation Medium (in conventional units per 1 g fresh weight of adrenal)

Dose of ACTH (in milliunits)	No. of expts.	Magnitude of secretion (mean and standard error of mean)	Increase in secretion after addition of ACTH (difference between experiment and control)	Significance of difference (Student's "t")
Control	20	7.03 ± 0.624	–	–
1.25	4	9.17 ± 1.338	2.14	1.41
2.5	15	12.41 ± 1.148	5.38	4.5
5	13	15.80 ± 1.939	8.73	6.97
10	2	19.78 ± 4.21	12.75	5.65

TABLE 2. Secretion of Corticosteroids by the Adrenals of Test Animals after Addition of Extracts of Hypophyses of Experimental Rats to the Incubation Medium (in conventional units per 1 g fresh weight of adrenal)

Age of experimental rats (in months)	Amounts of extract added (in fractions of hypophysis)	No. of expts.	Magnitude of secretion (mean and standard error of mean)	Increase in secretion after addition of ACTH (difference between experiment and control)	Significance of difference (Student's "t")
	Control	6	4.96 ± 0.750	–	–
	0.01	5	7.42 ± 0.199	2.46	2.40
1	0.02	5	10.25 ± 1.063	5.37	4.28
	0.04	6	11.34 ± 0.808	6.38	5.75
	Control	7	6.01 ± 0.538	–	–
	0.01	5	8.82 ± 0.299	2.81	4.07
3	0.02	7	11.00 ± 0.576	4.99	6.35
	0.04	4	16.18 ± 2.966	10.17	5.03
	Control	7	7.22 ± 0.943	–	–
	0.01	5	11.04 ± 1.104	3.82	2.62
12	0.02	7	15.44 ± 1.162	8.22	5.48
	0.04	4	24.89 ± 3.080	17.67	6.87
	Control	4	8.30 ± 1.341	–	–
	0.005	4	12.05 ± 1.233	3.75	2.10
24	0.01	4	14.14 ± 1.044	5.84	3.50
	0.02	4	19.90 ± 1.926	11.60	5.00

After incubation of the adrenals of the test animals with different amounts of hypophyseal extracts, an increase in the secretion of corticosteroids was also observed, corresponding to the amount of extract added (Table 2). The character of the relationship between response and dose was the same for extracts of the hypophyses of the rats of all ages and for a standard solution of hormone. By means of a calibration curve constructed from the data given in Table 1, the ACTH concentration in the added volumes of extract was calculated in milliunits.

By correcting for the dilutions, the content of ACTH in the whole hypophysis was obtained. It was mentioned above that for the analysis we used pooled extracts of several hypophyses, so that when the calculation of the result per unit weight of hypophysis was made, the content of the hormone in the whole hypophysis was divided by the mean weight of the hypophysis. The results are given in Table 3.

It will be clear from Table 3 that with advancing age there was a progressive increase in the total content of ACTH in the hypophysis of the rats, and also that this increase followed a course parallel to the increase in the weight of the hypophysis, so that the concentration of the hormone in unit weight of the hypophysis remained constant. The ACTH concentration in the hypophysis of the rats, according to our findings, was 24.5-29.8 mU/mg fresh hypophyseal tissue. Fortier [2], who determined the ACTH content of the adenohypophyses of rats by the same

TABLE 3. Content and Concentration of ACTH in the Hypophysis of Rats of Different Ages

Age of rats (in months)	ACTH content in a whole hypophysis in mU (mean and standard error of mean)	Mean wt. of hypophysis (in mg)	Concentration of ACTH in mU/g fresh tissue of hypophysis (mean and standard error of mean)
1	99.50 ± 10.53	4	24.90 ± 2.64
3	147.00 ± 29.27	6	24.50 ± 4.92
12	230.20 ± 33.46	8	24.90 ± 3.51
24	356.45 ± 49.59	12	29.80 ± 4.13

method, found that the hormone concentration was 64.6 ± 1.8 mU/mg adenohypophysis tissue. Guillemin and coworkers [5] obtained a value of 62 ± 7.2 mU/mg adenohypophysis tissue. Our values were close to those cited by other writers (it must be remembered that we calculated the concentration per unit weight of the whole hypophysis and not of the adenohypophysis alone). I. A. Eskin and N. V. Mikhailova [1] reported an equal decrease in the ascorbic acid content of the adrenals of new-born rats after administration of hydrochloric acid extracts of the hypophyses of young and old rats, which indicated that the content of ACTH in the hypophysis of these animals was identical. Our findings demonstrated that the ACTH concentration in the hypophyseal tissue of rats of different ages was constant.

TABLE 4. Content of ACTH of Hypophysis per Gram Body Weight of Rats

Age of rats (in months)	Mean weight of animal (in g)	Content of ACTH per gram body wt. (in milliunits)
1	56.20	1.77
3	171.00	0.86
12	301.75	0.76
24	420.57	0.84

It will be clear from Table 4 that the ACTH content of the hypophysis, when calculated per unit body weight, decreased to the age of 3 months, and thereafter was maintained at a constant level.

These results demonstrate that the decrease with advancing age in the adaptive powers of the organism (at least after 3 months of age) is not associated with changes in the ACTH concentration in the hypophysis. The reaction of the adrenals to ACTH evidently changes with age, or changes take place in the mechanisms regulating the secretion of ACTH so that the possible increase in its synthesis in young animals or in those approaching maturity corresponds to a more intensive secretion of the hormone into the blood stream.

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SUMMARY

A study was made of the ACTH content in the hypophyses of rats aged 1, 3, 12, and 24 months. Determinations were conducted on the basis of increased production of corticosteroids by the adrenal glands of test animals in vitro on addition to the incubation medium of the extracts of hypophyses obtained from experimental animals. As established, the content of ACTH shows a constant increase with advancing age in the whole hypophysis. However, the content of ACTH, as calculated per unit of body weight, remains unchanged (at least beginning from the age of

3 months). The concentration ACTH, as calculated per unit of raw weight of hypophysis tissue, does not change with the progress of age.

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