Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 82 MARCH, 1953 No. 3

SECTION MEETINGS

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Adrenal Repair and Chromatophore Expanding Activities of Corticotropin-B.* (20118)

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Recent reports have presented evidence for the possible existence of separate adrenocorticotropic factors concerned with adrenal ascorbic acid depletion (A.A.F) and with adrenal weight (A.W.F.)(1-4). However, the routes of administration, number of injections, the time required for the hormone to act, and the nature of the response elicited are different for the two types of assays. Therefore, such factors as rate of absorption and rate of inactivation within the animal body may affect the apparent A.W.F./A.A.F. ratio obtained for a given preparation. It is possible that preparations from different animal sources may be handled differently by the body. Contamination with other pituitary hormones may also influence the result; pituitary extracts rich in somatotropin are reported to give particularly high A.W.F./A.A.F. ratios(3). One group of investigators believes that A.A.F. and A.W.F. responses are due to a single substance(5).

A highly active product from pepsin digests of corticotropin has recently been isolated and designated as corticotropin-B(6). This product had an A.A.F. activity as high as 300 International Units per mg, and behaved as a pure substance in countercurrent distribution studies. It was of interest to determine the A.W.F. and chromatophore expanding activities of corticotropin-B in comparison with less highly purified materials having lower A.A.F. activity.

Materials and methods. The adrenocortico-

* The term "corticotropin-B" as used in this paper designates a product obtained by countercurrent distribution after fractionation of pepsin digests of corticotropin with oxycellulose. The designation "corticotropin-B" has been given to this substance because its properties are different from those of corticotropin(6), particularly with regard to its solubility behavior. Corticotropin-B shows no evidence of inhomogeneity by countercurrent distribution studies. Its method of preparation has been described(6).

TABLE I. Comparison of Adrenal Weight Activity of Preparations. A (alkaline extract of beef pituitaries) and E (corticotropin-B).

Preparation	Total amt inj., mg	No. of animals	Mean values: Body wt, g — Initial	Body wt, g — Final	Thymus, mg	Adrenal wt, mg ± S.E.
A	1.05	8	112	115	217	13.69 ± .68
A	15.00	3	120	129	—	19.03
E	1.05	10	108	114	127	18.68 ± .61
None	—	11	106	109	210	13.56 ± .58

tropic preparations used in this study were as follows: A, an alkaline extract of beef pituitary glands having an A.A.F. activity of less than 1 U/mg; B, Armour Acthar-A precipitated at pH 4.6, activity about 5 U/mg; C, Armour Acthar-A hydrolyzed for several hours in boiling 0.001 N HCl, and precipitated at pH 4.6, activity about 3 U/mg; D, partially purified corticotropin-B, activity about 175 U/mg; E, corticotropin-B, purified by counter-current distribution, activity about 250 U/mg. The animals used in this study were Sprague-Dawley male rats of 100 to 120 g body weight, hypophysectomized about a week prior to the test by the Hormone Assay Laboratories, Chicago, Ill. Injections of corticotropin in aqueous solution were administered subcutaneously.

Results. Exp. 1. In this experiment, the A.W.F. activity of preparations A and E were compared. Injections were continued for one week; the daily dose was divided into two injections except on Saturday and Sunday, when the usual daily amount was given in a single injection. The total number of injections was therefore 12. The rest of the details of the experiment are evident from inspection of Table I.

It is clear that 1.05 mg of preparation A produced no effect on adrenal size. 1.05 mg of corticotropin-B (preparation E) produced a moderate increase in adrenal weight which was highly significant ($P < 0.01$). The very small thymus glands found in the animals treated with preparation E provide further evidence of the adrenal-stimulating activity of this substance. The increment in adrenal weight in this group of animals was about the same as in those receiving 15 mg of preparation A. It does not necessarily follow, however, that preparation E is 15 times more potent in A.W.F. than is A, since this experiment gives us no information as to the dose-response relationship of various effective doses of either preparation.

Exp. 2. In this experiment, a more complete study was made, including dose-response curves for preparations B, C, and D. One hundred hypophysectomized rats were divided into 10 groups of 10 animals each, as shown in Table II. Injections were made twice daily over a 7-day period (total of 14 injections), and solutions were freshly prepared before each injection. The injection schedule was therefore slightly different from that of Exp. 1, so direct comparisons of activity of these preparations with those used in Exp. 1 would not be valid. The adrenal weight data in Table II may be plotted to yield straight lines with the following regression equations: B, $y = 19.31 + 9.03X$; C, $y = 16.42 + 3.84X$; D, $y = 24.60 + 9.03X$, where y = adrenal weight in mg, and X = log total dose. The amounts of hormones required to produce a 50% increase over control adrenal weight are calculated from these equations to be as follows: D 0.54 mg, B 2.09 mg, and C 32.00 mg. A direct comparison of potency on this basis

TABLE II. Dose-Response Data of Adrenal Weights in Hypophysectomized Rats Treated with Preparations B (pH 4.6 Precipitate of Armour Acthar-A), C (pH 4.6 Precipitate of Acid Hydrolysate of Armour Acthar-A), and D (Partially Purified Corticotropin-B).

Preparation	Total amt inj., mg	Body wt, g — Initial	Body wt, g — Final	Adrenal wt, mg
B	1.0	112	116	18.2
B	4.0	113	121	26.2
B	16.0	106	112	29.9
C	1.0	112	115	16.6
C	4.0	117	117	18.4
C	16.0	115	114	21.2
D	1.0	110	108	25.0
D	4.0	110	111	30.1
D	16.0	113	102	35.0
Controls	—	115	113	14.8

between preparation C and the other two preparations, however, must be subject to some reservations, since the data for C have a significantly different slope, and the highest dose of C gave somewhat less than 50% increment in adrenal weight; also, preparation D may be more potent in comparison to B than the data indicate, since the lowest dose of D gave more than 50% increase in adrenal weight.

Exp. 3. In addition to these observations on adrenal weight, a preliminary experiment was performed to determine whether corticotropin-B also possesses a higher degree of chromatophore-expanding activity than a cruder preparation. This was of interest in view of the recent claim that corticotropin and the chromatophorotropic hormone are identical(7,8), although this has been denied (9,10). We have tested the action of corticotropin-B on frog skin *in vitro*. Pieces of skin of *Rana pipiens* about 1.5 cm square were immersed in frog Ringer's solution containing hormone in concentrations of 0.01, 0.1, 1.0 and 10.0 μg/ml. Two hormone preparations were used: a relatively crude corticotropin acid hydrolysate (about 1.2 U per mg) and highly purified corticotropin-B.

There was a gradation in the degree of darkening produced. The skin exposed to 10.0 μg/ml of corticotropin-B was by far the darkest, while 1.0 μg/ml of corticotropin-B and 10.0 μg/ml of the cruder preparation produced moderate darkening of about equal degree. Concentrations of 0.1 μg/ml of corticotropin-B and 1 μg/ml of the cruder preparation produced slight darkening. No noticeable effect was produced by 0.1 μg/ml of the cruder preparation or by 0.01 μg/ml of either preparation. Corticotropin-B, therefore, appeared to have melanophorotropic activity on the order of 10 times that of the cruder corticotropin, but this ratio must be accepted with reservations, since the method used was at best no more than semi-quantitative.

Discussion. Activity in the adrenal ascorbic acid depletion test as high as 300 times that of Armour Standard LalA ACTH has been reported for corticotropin-B(6). This is not necessarily the highest activity for corticotropin-B, since partial inactivation may occur in the process of working up the pure substance in the peak plates of the distribution. This partial loss of activity has been frequently observed, and is most marked in the most highly purified preparations. Corticotropin-B is obtained from pepsin digest of corticotropin derived from swine pituitary glands. The application of countercurrent distribution which produced through 450 transfers corticotropin-B, behaving as a pure substance, is a very critical criterion of purity. It is therefore of considerable interest that this substance not only possesses all three biological activities tested (adrenal ascorbic acid depletion, adrenal weight increase, and chromatophore expansion) but possesses them to a higher degree than do cruder preparations.

In the adrenal weight test, the dose-response curve for the acid-hydrolyzed material, preparation C, had a different slope than the curves for B or D. It is therefore possible that the nature of the A.W.F. is changed by the acid treatment. This change may be either in the way the active principle affects the adrenal, or in the way the body handles the injected material. Not only is it impossible to obtain a valid ratio of a single activity between two substances showing such a different slope of response, but comparison of A.W.F./A.A.F. ratios between such materials would be equally meaningless. Furthermore, the errors inherent in the assays make it difficult to determine such ratios with a practicable degree of precision. For these reasons, as well as because of the considerations outlined in the introductory paragraph, we have not attempted to calculate A.W.F./A.A.F. ratios for any of our preparations.

The method of preparation of corticotropin-B and the apparent high degree of purity of this substance(6) make it seem unlikely that the very high A.W.F. and chromatophorotropic activities are due to contaminants.

A reappraisal of certain controversial issues can be made in the light of these data on the hormonal activities of corticotropin-B: for example, are A.A.F. and A.W.F. activities due to the same or to different chemical entities? We now have an apparently pure substance exhibiting both activities, but this does not

preclude the possibility that some other substance present in ACTH-concentrates might have only one or the other activity. The characterization of one or more active substances other than corticotropin-B is required for satisfactory appraisal of the issue, and in some instances it might be necessary to prove the absence of corticotropin-B in certain concentrates before drawing conclusions about the presence of different hormonal entities in a given concentrate.

Corticotropin-B is from swine pituitaries. Any comparison of activities of various corticotropin preparations should take into account the species from which the glands were secured. The importance of this consideration has been established for certain other protein hormones. For example, vasopressin from beef posterior pituitary glands has been revealed to be chemically different from that derived from swine posterior lobes. The hormone from beef contains an arginine moiety, while that from swine contains a lysine moiety(11). The insulins from beef, swine, and sheep glands have been found to be chemically different with respect to six amino acids, although physical and biological differences are slight(12). It is not unreasonable to anticipate that similar differences may be found in corticotropin and other anterior lobe hormones of diverse animal origins. It is possible that differences in biological activity may accompany chemical diversities. A preparation recently reported by Hess and Carpenter(13) had A.A.F. activity comparable to that of corticotropin-B. Since both the animal source material and method of preparation are different in the two preparations, it is not unlikely that they may show a different spectrum of biological activities.

A second controversial issue is concerned with the question of whether adrenal ascorbic acid depletion and chromatophore expansion are due to the same or to different chemical entities. Since corticotropin-B also shows potent chromatophorotropic activity, the same reservations must be made about species differences and about the possibility of the existence of substances other than corticotropin-B which may possess predominantly either one or the other type of activity. It is entirely possible that when other substances are isolated from ACTH-concentrates, they may show spectra of biological activity different from that of corticotropin-B.

Summary. A product isolated from a pepsin digest of swine corticotropin and behaving in countercurrent distribution studies like a pure substance has been designated corticotropin-B. This product not only has very high adrenal ascorbic acid reducing activity, but is also extremely active in restoring adrenal weight in hypophysectomized rats, and has a high degree of chromatophorotropic activity.

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Received November 18, 1952. P.S.E.B.M., 1953, v82.