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Biological ACTH Activity of Lipid Mobilizing Peptide Fractions from Hog Pituitaries*

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S u m m a r y

The ACTH activity of lipid mobilizing fractions from porcine pituitaries was determined using a sensitive bioassay for ACTH. For different lipolytic fractions between 12 and 27 ng ACTH per 100 µg were found. However, no lipolytic response was obtained in the rabbit with approximately 2,5 µg ACTH. It was therefore concluded, that ACTH does not contribute significantly to the lipolytic activity of our lipotropin preparations. The preparations appear however to contain a minimal ACTH contamination, which was lost upon storage of solutions at -16°C with preservation of the lipolytic activity.

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Introduction

ACTH was shown to have considerable intrinsic lipid mobilizing activity in several species tested (*Lebovitz and Engel* 1965). Hence, it is necessary to determine the ACTH activity in all specific lipid mobilizing peptides purified from pituitaries (lipotropins).

The ACTH activity of a lipotropin fraction, peptide A, which was further purified in our laboratories (*Schwandt, Karl, Thüner and Knedel* 1968, *Schwandt, Weisweiler and Lamerz* 1971), was studied by a sensitive bioassay. The results are given in this paper.

Materials and Methods

Purification of lipid mobilizing peptide fractions

Fraction G was extracted from lyophilized hog pituitaries, as published (*Schwandt* et al. 1968). Further purification resulted in fraction J and peptide A (*Schwandt* et al. 1971). The three top fractions of the Sephadex-G-50 rechromatography of peptide A were here defined as A_{peak}.

Biological ACTH activity

The increment of corticosterone output into the adrenal venous blood of acutely hypophysectomized rats was used for bioassay of ACTH activity (*Scriba, Hacker, Dieterle, Kluge, Hochheuser and Schwarz* 1966, *Pister* 1972). Standards (β^{1-23}-corticotropin amide, Acethropan®S, Farbwerke Hoechst) and test solutions were given intraaortally. The sensitivity and reproducibility of the method were improved, chiefly by introduction of a spectrofluorimetric analysis for corticosterone (*Pister* 1972), which is similar to our cortisol method (*Scriba, Gerb, Kluge, Boss and Müller* 1970): Less than 0.005 µg corticosterone per 0.1 ml could be determined. The standard curve for ACTH (Fig. 1) shows a linear relation between dosis ACTH (1 ng = 100 µU) and corticosterone increment. This linearity was found up to doses of 22 ng ACTH. On the other hand 0.05 ng ACTH (= 5 µU) gave a significant rise of corticosterone output (*Pister* 1972).

Lipolytic activity

The lipolytic activity of the various lipotropin fractions and of Acethropan®S was tested in male and female rabbits, 2.5 kg of weight (Grauscheck, single cages, Altromin®-pellets ad libitum). Blood was taken for the determination of FFA (*Dole and Meinertz* 1960) from an ear vein before and 1 hour after subcutaneous injection of 250 µg of fractions G and J, of peptide A and of 2.5 µg ACTH. All test substances dissolved in 0.9% NaCl solution.

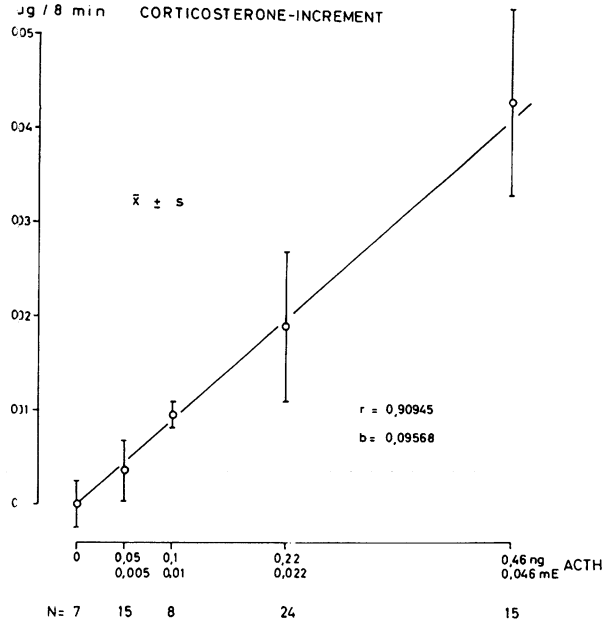

Fig. 1. ACTH standard curve.

N = number of rats, r = correlation, b = regression. For further details see text.

Results

The ACTH activities determined in the different lipid mobilizing fractions varied between 12 and 27 ng ACTH per 100 µg of the lyophilized fractions as for peptide A_{peak} and for fraction J respectively (Table 1). Thus, on a weight basis a ratio of 1:10 000 was calculated for peptide A_{peak}. This result was confirmed for all fractions by determination of the ACTH activity in 10 µg amounts. Further the fraction with the lowest ACTH activity (peptide A_{peak}) was tested for 5 and 1 µg amounts with the result of a dilution curve, the slope of which was very similar to the ACTH standard curve (Table 2). These data had been obtained with lyophilized fractions that had been stored for 2 to 3 weeks at -16°C. No loss of activity was seen when peptide A was stored for 4 months at -16°C: 18.56 ± 1.47 ng ACTH per 100 µg vs. 16.45 ± 1.31 in fresh material (Table 1); However, storage of solutions of the lipid mobilizing fractions for 9 weeks at -16°C resulted in complete loss of the ACTH activity, whereas the lipolytic activity was not diminished.

Discussion

Undoubtedly ACTH activity was demonstrated in all the lipid mobilizing fractions tested: however, ACTH accounts only for 0.1 to 0.3‰ of the amount assayed. Acethropan®-S (β^{1-23}-corticotropin amide) given in 100-fold excess of this did not elevate the FFA (Table 1). The same was shown (*Schwandt* et al. 1968) for equivalent amounts of porcine ACTH (0.4 U). Therefore, the minimal ACTH activity in our lipid mobilizing fractions cannot explain their lipolytic activity.

On the other hand, one cannot definitively answer at present the question, whether the small ACTH activity of the fractions is due to ACTH contamination or intrinsic ACTH activity of the lipotropins. The specificity of the ACTH bioassay (*Schleyer, Evertz, Voigt, Fehm, Faulhaber and Pfeiffer* 1970) and the similar slope of the ACTH standard curve and of the lipotropin dilution curve are both in favor of the ACTH contamination theory, since a non-parallel slope of the dilution curve should have been expected for the case of intrinsic ACTH activity in lipotropins or in fragments of lipotropins. This view is further supported by the observation, that with purification of the lipid mobilizing fractions the ACTH activity per µg injected substance decreases with increasing lipolytic activity. Finally, the observed loss of ACTH activity with preservation of the lipolytic activity in solutions kept at -16°C is clearly in favor of the assumption that we are dealing with ACTH contaminations. The disappearance of the biological ACTH-activity in solution at -16°C was demonstrated in own experiments (*Pister* 1972) and has also been shown in pilot experiments

Table 1. Protein content as % weight (N = number of tested preparations), lipolytic and ACTH activity (N = number of the animals tested) of the lipid mobilizing fractions.

Δ FFA = increase of the free fatty acids.

Fraction	% Protein (Lowry-method) mean ± SD	Δ FFA mval/l mean ± SD	ng ACTH (10 ng = 1 mE) per 100 µg injected substance mean ± SD	per 10 µg injected substance mean ± SD
G	58.7 ± 2.2 (N=6)	1.51 ± 0.24 (N=11)	13.98 ± 0.51 (N=3)	1.34 ± 0.08 (N=5)
J	77.7 ± 2.2 (N=6)	2.99 ± 0.20 (N=6)	27.21 ± 7.31 (N=6)	1.85 ± 0.13 (N=2)
A	99.4 ± 2.0 (N=6)	3.28 ± 0.09 (N=6)	16.45 ± 1.31 (N=3)	1.71 ± 0.15 (N=3)
A_{peak}	–	–	12.35 ± 2.79 (N=3)	1.10 ± 0.13 (N=3)
ACTH (Acethropan®S	–	0.05 ± 0.15 (from -0.11 to +0.26) (N=5)	–	–

Table 2. ACTH activity in different dilutions of the lipolytic peptide A_{peak}

The decrease of the ACTH activity parallels the diminition of the amount of the added lipolytic material.

lipolytic peptide A_{peak} µg	ACTH activity ng (10 ng = 1 mE) single values determined	mean ± SD
100	15.46 12.89 8.70	12.35 ± 2.79
10	1.23 1.14 0.92	1.10 ± 0.13
5	0.72 0.71 0.69	0.71 ± 0.01
1	0.21 0.12	0.16 ± 0.04

by former investigators (*Scriba* et al. 1966). The basis of this observation remains unclear.

The ACTH activity varies in the different preparations of lipotropins reported. ACTH accounted for 0.2 to 0.3‰ of the preparations from porcine and human pituitaries of *Rudman, Seidman and Reid* (1960), of *Graf and Cseh* (1968) and of *Cseh, Graf and Goth* (1968). *Schleyer* et al. (1970) reported 0.1‰ ACTH in a fraction of weak lipolytic activity (P-LF I) and 1‰ ACTH in fractions P-LF II and P-LF III of higher lipolytic activity. Similar ACTH activities have been found in lipolytic preparations by *Ryschka and Chochlow* (1965). *Trygstad* (1968) reported 2‰ ACTH for LMF VI from human pituitaries and *Lohmar and Li* (1968) 10‰ for β-LPH from ovine pituitaries.

All these authors agree, however, that the lipolytic activity of their lipid mobilizing fraction was not caused by ACTH contamination only. However, a definite discrimination of the lipotropins against all other lipolytic pituitary hormones has not been achieved so far.

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