HORMONE–RECEPTOR INTERACTIONS. ADRENOCORTICOTROPHIN-(7–24)-OCTADECAPEPTIDE STIMULATES ADIPOCYTE MEMBRANE ADENYLATE CYCLASE WITHOUT CAUSING LIPOLYSIS IN FAT CELLS

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1. Introduction

A widely used method for the study of polypeptide hormone–receptor interactions is the chemical synthesis of hormone analogues and the comparison of their biological properties [1]. As a result, it has become clear that ACTH is a molecule with sychnological organization, meaning that discrete sequences of adjacent amino acids ('continuate words') are responsible for different components of its total biological activity [2]. Thus, ACTH-(4–10)-heptapeptide ($ACTH_{4-10}$) is a message sequence ('active site') responsible for stimulating the receptors, and ACTH-(11–24)-tetradecapeptide ($ACTH_{11-24}$) an address sequence supplying specific affinity for the occupation of rat fat cell and adrenal cell receptors (for references and a more thorough discussion see [3,4]).

As in our studies on α-MSH [3,4], we were interested in the question whether or not the message sequence can further be subdivided into stretches with different biological information. To this end, ACTH fragments containing the address sequence and adjacent parts of the message were prepared by conventional synthetic methods and tested for their ability to enhance lipolysis in isolated rat fat cells and to activate adenylate cyclase (cAMP production by rat adipocyte membrane preparations, 'ghosts'). Human ACTH and the peptides used in this study have the following amino acid sequences (message in italics):

Abbreviations of amino acids and peptides according to the recommendations of the 'IUPAC–IUB Joint Commission on Biochemical Nomenclature', e.g. Eur. J. Biochemistry 1, 375 (1976). The one-letter notation used here correlates as follows: Ala = A, Arg = R, Asn = N, Asp = D, Cys = C, Gln = Q, Glu = E, Gly = G, His = H, Ile = I, Leu = L, Lys = K, Met = M, Phe = F, Pro = P, Ser = S, Thr = T, Trp = W, Tyr = Y, Val = V. Other abbreviations are: ACTH=adrenocorticotrophic hormone, cAMP = cyclic 3′,5′-AMP, α-MSH = alpha melanocyte stimulating hormone.

SYS*MEHFRWG*KPVGKKRRPVKVYPNGAEAESAEAFPLEF	ACTH
SYS*MEHFRWG*KPVGKKRRPVKVYP	$ACTH_{1-24}$
*EHFRWG*KPVGKKRRPVKVYP	$ACTH_{5-24}$
*FRWG*KPVGKKRRPVKVYP	$ACTH_{7-24}$

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It was found that the longer fragment, $ACTH_{5-24}$, and its $N(\alpha)$-benzyloxycarbonyl derivative, $ZACTH_{5-24}$, can (like ACTH and $ACTH_{1-24}$) stimulate both lipolysis and cAMP production. The shorter peptides, $ACTH_{7-24}$ and $ZACTH_{7-24}$, stimulate adenylate cyclase to practically the same extent as $ACTH_{5-24}$, but do not enhance lipolysis. Other hormone analogues (which we are preparing) will be necessary to describe the specific contributions to such a differential activity by the individual amino acid residues 5(Glu), 6(His), 7(Phe), 8(Arg), and 9(Trp).

This type of differential behaviour has – to our knowledge – not been previously published, except for the observation that glucagon stimulates rabbit adenylate cyclase, but fails to stimulate lipolysis [5].

No extrapolations to other hormones, actions, or cell-types can be made at the moment. However, preliminary experiments by W. Schlegel in this laboratory suggest an analogous behaviour of our compounds towards bovine adrenal cortex cells and purified cell membrane vesicles.

2. Experimental

$ACTH_{1-24}$ was prepared according to [6], $ACTH_{5-24}$, $ACTH_{7-24}$, and their $N(\alpha)$-benzyloxycarbonyl derivatives, $ZACTH_{5-24}$ and $ZACTH_{7-24}$, by appropriate modifications of the procedure. All compounds were analytically pure (to be published in Helv. Chim. Acta).

Adipocytes and fat cell ghosts were prepared from epididymal adipose tissue of male rats weighing 180–200 g according to the procedures of Rodbell [7,8]. Lipolysis rates were measured as nanomoles of glycerol produced by about 7800 cells (= 1 mg cell dry weight, cdw) in 30 min [9–11].

Adenylate cyclase activity was measured by the methods of Ramachandran [12] and Salomon et al. [13] and expressed as nano- or picomoles of cAMP produced in 15 min per 1 mg of cell ghost protein.

3. Results

Stimulation of lipolysis and adenylate cyclase activity by the ACTH fragments in adipocytes and adipocyte ghosts, respectively, is displayed as log dose–response (LDR) curves in fig.1; cAMP production and lipolytic rates were calculated as the difference between stimulated and control values.

$ACTH_{5-24}$ behaves as a partial agonist [14] for lipolysis (intrinsic activity relative to $ACTH_{1-24}$, $\alpha = 0.6$) with a potency about 275 times less than that of $ACTH_{1-24}$. $ZACTH_{5-24}$ is a full agonist and about 20 times more potent than $ACTH_{5-24}$ (however, the LDR curve is less steep and could be interpreted as consisting of two superposed curves with different potency and intrinsic activity parameters). Both

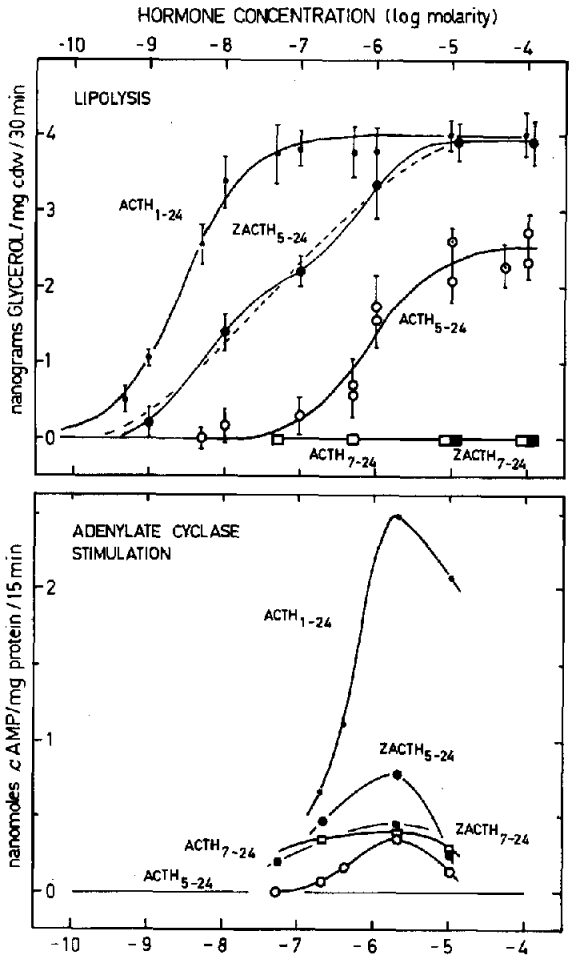

Fig.1. Log dose–response curves of the ACTH analogues $ACTH_{1-24}$, $ACTH_{5-24}$, $ZACTH_{5-24}$, $ACTH_{7-24}$, and $ZACTH_{7-24}$ for lipolytic rates with isolated rat fat cells and for adenylate cyclase activation (rates of cAMP production) with rat adipocyte ghosts.

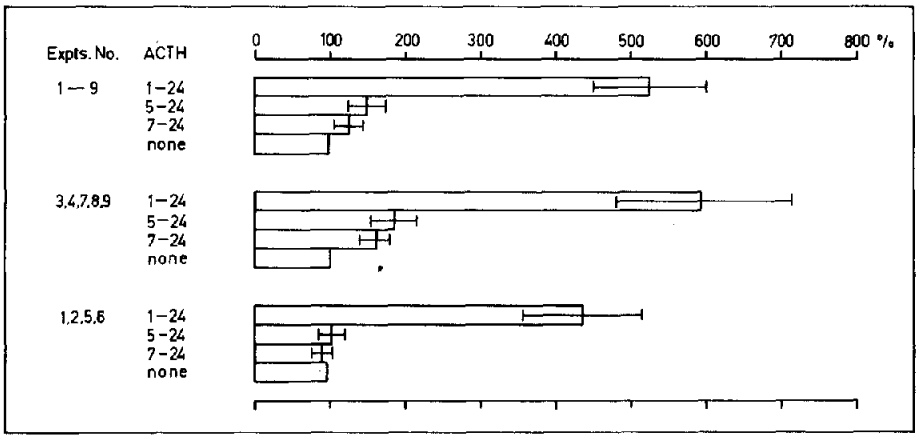

Fig.2. Mean maximal stimulation of adenylate cyclase activity in rat adipocyte ghosts by 3×10^{-6} M ACTH analogues in percent of the normalized, unstimulated control activity. Deviations as 95% confidence limits. Experiments 1–9: $ACTH_{1-24}$ = 524 ± 74% ($P < 0.001$), $ACTH_{5-24}$ = 150 ± 25% ($P < 0.001$), $ACTH_{7-24}$ = 126 ± 19% ($P < 0.01$). Experiments 3, 4, 7, 8, 9: $ACTH_{1-24}$ = 593 ± 112% ($P < 0.001$), $ACTH_{5-24}$ = 186 ± 30% ($P < 0.001$), $ACTH_{7-24}$ = 161 ± 21% ($P < 0.001$).

compounds are partial agonists for adenylate cyclase activation ($\alpha = 0.1–0.2$) with potencies comparable to that of $ACTH_{1-24}$. The LDR curves all reach a maximum, not a plateau, probably indicative of adenylate cyclase inhibition by high hormone dosis.

$ACTH_{7-24}$ and $ZACTH_{7-24}$ do not stimulate lipolysis, in fact, they act as competitive inhibitors for $ACTH_{1-24}$ (K_i about 10^{-4}). However, they are also partial agonists for adenylate cyclase stimulation ($\alpha = 0.1$).

Because of this unexpected finding, over 30 comparative experiments with 9 different ghost preparations were performed, using the improved cAMP assay [13]. The results were statistically evaluated in 3 groups: (a) all ghost preparations, nos. 1–9, (b) those in which $ACTH_{5-24}$ was active, nos. 3, 4, 7, 8, 9, and (c) those which were not stimulated by $ACTH_{5-24}$, nos. 1, 2, 5, 6. $ACTH_{7-24}$ was found also to be active in the first two groups and its activity to be indistinguishable from that of $ACTH_{5-24}$ on the 5% level ($P > 0.05$), see fig.2.

4. Discussion

Three conclusions can readily be drawn from these results: (1) The octadecapeptide $ACTH_{7-24}$ with N-terminal Phe–Arg–Trp–Gly– suffices to stimulate adipocyte ghost adenylate cyclase; however, additional amino acids in positions 6 and perhaps 5 (Glu–His–) are required for producing lipolysis in isolated fat cells. (2) The $N(\alpha)$-benzyloxycarbonyl residue can cause the partial agonist $ACTH_{5-24}$ to become a full lipolytic agonist, it also enhances its intrinsic activity on ghost adenylate cyclase to some extent. (3) The stimulation of ghost adenylate cyclase by $ACTH_{7-24}$ is an insufficient condition for evoking lipolysis in whole cells.

The first conclusion fits well into the general picture of the one-dimensional organization of pleiotropic information in molecules of the ACTH-MSH-lipotropin family [3,4], where different sequences of adjacent amino acid residues serve different biological functions. It suggests that the receptor on the adipocyte surface that eventually produces lipolysis has different requirements for stimulation than the adipocyte ghost receptor that activates adenylate cyclase.

In the second conclusion, the role of the $N(\alpha)$-benzyloxycarbonyl residue can best be compared to that of methionine-(4), which it replaces. This residue probably acts as a lipophilic contact to the receptor [4,15–17], producing additional binding forces

(enhanced potency and intrinsic activity. It might well be that the slight increase of cAMP production caused by $ZACTH_{5-24}$ over that of $ACTH_{5-24}$ (fig.1, this has not yet been verified statistically) could be the cause for restoring full lipolytic agonism.

The third conclusion is, of course, very unexpected. Usually – in adipocytes and other cells – hormonal adenylate cyclase activation is found to correlate well with hormonal effects on whole cells [18–21], much better than cAMP accumulation does [9,10,22–24]. The observation that $ACTH_{7-24}$ stimulates ghost adenylate cyclase to almost the same extent as $ACTH_{5-24}$, but fails to enhance lipolysis in whole cells might be explained in three ways: (1) $ACTH_{7-24}$ fails to stimulate adenylate cyclase in whole cells. In this case, the ACTH receptors in ghosts would differ from those in whole cells by recognizing for some unknown and unusual, specific reason $ACTH_{7-24}$ as a stimulus. (2) $ACTH_{7-24}$ enhances cAMP production in whole cells, but this cAMP finds itself in the wrong 'compartment' (e.g. in one in which it is very rapidly destroyed). This is tantamount to saying that discrete hormonal effects depending on amino acid residues His-(6) and, perhaps, Glu-(5) are indispensable for 'guiding' [25] cAMP into stimulation of the lipolytic response. (3) Other receptors than adenylate cyclase receptors, e.g. Ca^{2+}-activating receptors [26,27], have to be stimulated in order to produce the observed immediate lipolytic response. Such receptors would not be stimulated by $ACTH_{7-24}$, but only by $ACTH_{5-24}$ and $ZACTH_{5-24}$. (Arguments 2 and 3 are, of course, logically very closely connected.) Much more work will be needed to decide the issue.

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