The anomalous effect of some ACTH-fragments missing the amino acid sequence 1–10 on the corticosteroidogenesis in purified isolated rat adrenal cells

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The corticosteroidogenicity of ACTH-derived peptides was tested in a purified isolated rat adrenal cell system. The activity was related to the activity of standard ACTH (synthetic $hACTH^{1-39}$; potency fixed at 100). The peptides $ACTH^{11-19}NH_2$ and $ACTH^{11-24}$ showed steroidogenicity at pharmacological doses (potencies: 0.00067 and 0.00032, respectively). None of the peptides tested inhibited or potentiated the ACTH-induced steroidogenesis at low doses (0.5–50 000 pg). The results suggest the presence of a second center within the ACTH molecule capable of inducing steroidogenesis.

ACTH *$ACTH^{11-19}NH_2$* *$ACTH^{11-24}$* *Isolated adrenal cell Corticosteroidogenicity*

1. INTRODUCTION

According to the current concept, 4 functional sites in the ACTH molecule can be identified: (i) the center of ACTH concerning the corticosteroid production which has been localized in the 5–10 amino acid sequence of the molecule [1]; (ii) the basic amino acids in the sequence 15–18 and in position 11 which play a pivotal role in the binding and affinity of the molecule to the receptor [2,3]; (iii) the sequence 1–3 which potentiates the steroidogenic effect [4]; (iv) the acid amino acids localized within the sequence 25–32 which protect the vulnerable amino acid sequence 15–18 against tryptic-like degradation [5,6].

In accordance with its binding function it has been demonstrated that the sequence 11–24 inhibits the complete molecule [7]. As in this latter study a large overdose of $ACTH^{11-24}$ had to be added for the inhibition of the steroidogenesis of the complete ACTH molecule in a crude isolated adrenal cell preparation, we studied in our purified adrenal cell system the effects of $ACTH^{11-24}$ and $ACTH^{11-19}NH_2$ alone and in the presence of $hACTH^{1-39}$. We did not find an inhibitory effect in physiological doses, but with pharmacological doses we found a steroidogenic effect with these fragments indicating the existence of a second steroidogenic center in ACTH.

2. MATERIALS AND METHODS

Synthetic $hACTH^{1-39}$ (revised sequence [8]) with a potency of 188 IU/mg as estimated in [9] was used as standard. This standard, $ACTH^{7-13}NH_2$ and $ACTH^{11-24}$ were generous gifts from Dr W. Rittel and Dr P. Dessaulles (CIBA-Geigy Ltd, Basle). Another $ACTH^{11-24}$ preparation was kindly supplied by Dr H. Greven (Organon, Oss). $ACTH^{11-19}NH_2$ was generously provided by Dr M. Nakamura (Shionogi & Co., Osaka) and $ACTH^{1-4}$ and $ACTH^{5-7}$ by Dr K. Medzihradszky.

In the bioassay isolated rat adrenal cells were used which were purified and pre-incubated before ACTH addition. This methodology has been extensively described [6]. Potencies of the ACTH

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Table 1

Potencies of ACTH-derived peptides

Peptide	Potency	SD	n	Highest dose tested (μg)
hACTH$^{1-39}$	100			
ACTH$^{1-4}$	nd[a]		3	100
ACTH$^{5-7}$	nd		3	100
ACTH$^{7-13}$NH$_2$	nd		5	100
ACTH$^{11-24}$	0.00032	0.00011	10	
ACTH$^{11-19}$NH$_2$	0.00067[b]	0.00025	5	

[a] nd, not detectable

[b] Statistically significant difference between this value and the value of ACTH$^{11-24}$ ($P < 0.01$)

peptides were analyzed as follows. For each peptide complete log dose–response curves were constructed. The potencies of the peptides were expressed as the relation of the reciprocal of their molar ED_{50} to the reciprocal of the molar ED_{50} of the standard. Human ACTH$^{1-39}$ was assigned to the potency of 100

Differences between means were statistically tested using the Mann–Whitney two sample rank test.

3. RESULTS

The steroidogenic potencies of the peptides are

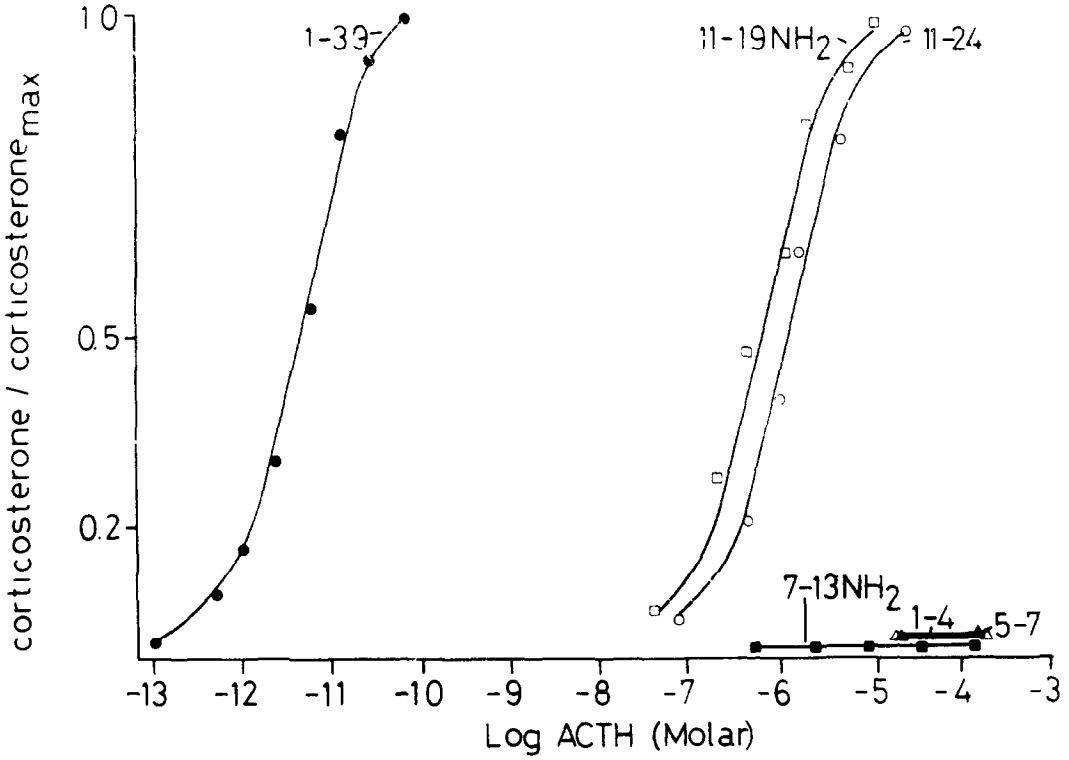

Fig.1. Log dose–response curves for ACTH peptides without the 5–10 amino acid sequence. Symbols represent the means of duplicate estimations.

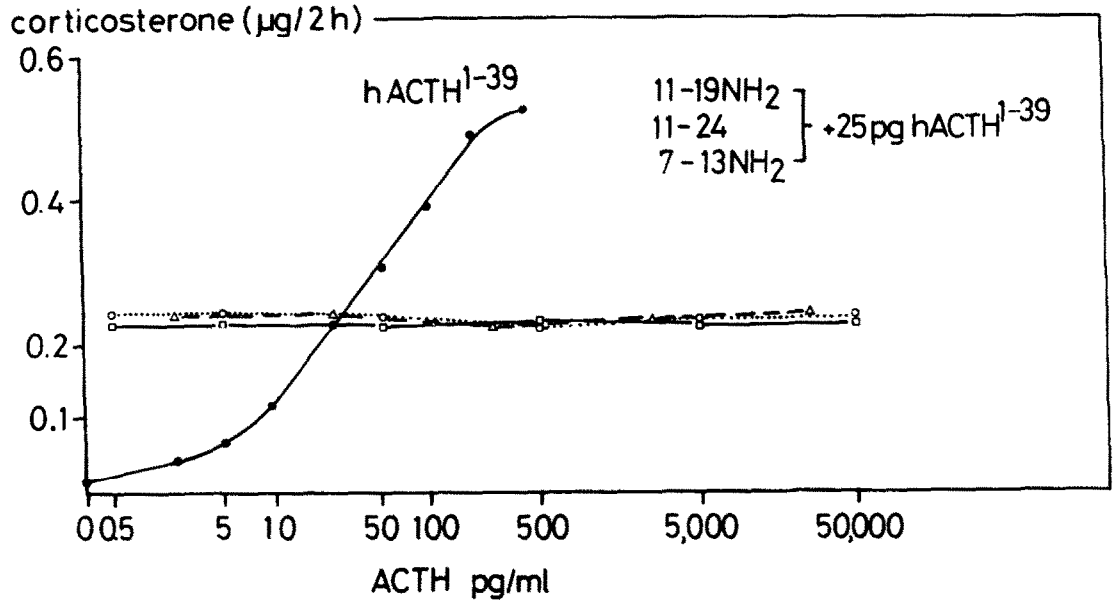

Fig.2. The effect of different doses of $ACTH^{11-19}NH_2$ (○·····○), $ACTH^{11-24}$ (□—□) and $ACTH^{7-13}$ NH_2 (△---△) upon the steroidogenesis induced by 25 pg $hACTH^{1-39}$.

shown in table 1. The peptides $ACTH^{1-4}$, $ACTH^{5-7}$ and $ACTH^{7-13}NH_2$ evoked no steroidogenic response, not even in doses as high as 100 μg. The peptides $ACTH^{11-24}$ and $ACTH^{11-19}NH_2$ did show an albeit low but still measurable biological activity. The potency of $ACTH^{11-19}NH_2$ was observed to be, statistically, significantly higher than the potency of $ACTH^{11-24}$ ($P < 0.01$). No statistically significant difference was found between the potencies of the $ACTH^{11-24}$ peptides obtained from different sources (Organon, 0.00035 ± 0.00012; $n = 6$; CIBA-Geigy, 0.00027 ± 0.00008; $n = 4$). The log dose–response curves of both $ACTH^{11-24}$ and $ACTH^{11-19}NH_2$ were parallel to the curve of the standard (fig.1).

In three experiments the influence of $ACTH^{11-24}$, $ACTH^{11-19}NH_2$ and $ACTH^{7-13}NH_2$ – in doses without steroidogenic activity – upon the ACTH-induced steroidogenesis was studied. Fig.2 shows that none of these peptides inhibited or potentiated the activity of ACTH in the doses tested.

4. DISCUSSION

Our finding that the peptides $ACTH^{1-4}$, $ACTH^{5-7}$ and $ACTH^{7-13}NH_2$ elicited no corticosterone production (table 1) is in agreement with the concept that a complete amino acid sequence 5–10 is responsible for steroid production [1]. However, in contradiction with this concept and with other reports we found an, albeit low, but still measurable steroidogenic activity of the mid-portion peptides $ACTH^{11-24}$ and $ACTH^{11-19}NH_2$ with a potency comparable to that of $ACTH^{1-10}$ [6]. In isolated adrenal cells authors in [7] found that $ACTH^{11-24}$ – in a pharmacological dose – was able to inhibit the steroidogenic activity of human $ACTH^{1-39}$, whereas this peptide did not reveal steroidogenic activity by itself. Remarkably, the author in [10] employing $ACTH^{11-18}NH_2$ observed a potentiating effect of the ACTH-induced steroidogenesis in vitro as well as in vivo, whereas the peptide itself did not show any steroidogenicity. In this respect, it is noteworthy that authors in [11] did find formation of cAMP induced by $ACTH^{11-24}$ in a huge dose of 100 μg – in the presence of the GTP analogue $Gpp(NH)_p$ – in a rat adrenal adenylate cyclase system. In our cell suspensions lower doses up to 50 ng $ACTH^{11-24}$ and $ACTH^{11-19}NH_2$ showed neither inhibition nor potentiation of ACTH (fig.2). These results are discrepant from the reports mentioned earlier but the reason for this is not clear. Maybe

methodological differences in isolation of the adrenal cells – we used collagenase whereas the other authors employed trypsin which has been reported to damage cell receptors in fat cells [12] – or in the quality of the cell suspension – we used purified, preincubated cells, whereas the other authors did not purify or preincubate the cells – could account for these discrepancies.

In addition to the results mentioned above it is of interest that in other biological systems $ACTH^{11-24}$ has been reported to be able to express biological activity. In rat adipocytes lipolysis could be induced by the 11–24 sequence to about the same degree as $ACTH^{1-10}$ [13]. Moreover, $ACTH^{11-24}$ contains a message sequence for activity in the central nervous system [14]. These results together are in favour of the presence of a second steroidogenic center in the ACTH molecule. As is known, the sequence 15–18 which contains the basic amino acids is responsible for the binding and affinity to the receptor [2] and therefore the sequence 11–14 may be of some interest in this respect. In this context it is remarkable that authors in [15] found a second bioactive center in the molecule for α-MSH activity, namely the sequence 11–13.

The finding that $ACTH^{11-19}NH_2$ shows a, statistically, significantly higher potency than $ACTH^{11-24}$ points at a role of the amino acid sequence 20–24 for an optimal steroidogenic activity of the midportion peptides. This is similar to observations made in [16]. These authors found about the same magnesium-accumulating activity in rat adipocyte plasma membranes both for $ACTH^{1-24}$ and $ACTH^{11-24}$. Removal of residues 21–24, however, as in $ACTH^{1-20}$, resulted in a reduction of this activity by almost two-thirds. In addition they found a significantly greater lipolytic activity for $ACTH^{1-20}$ than for $ACTH^{1-24}$ in rat adipocytes. As the magnesium accumulation mentioned involves the mediation of an α-adrenergic receptor [16], it seems worthwhile to further investigate the similarity of the findings in [16] and ours. The influence of α-adrenergic agonists and antagonists on the steroidogenicity of the midportion peptides could further elucidate the characteristics of the second steroidogenic center.

Furthermore, the existence of two separate steroidogenic centers in the ACTH molecule, as we found here, fits very well with the results of the tracer binding study [17], where two different types of receptors were found, each with different binding constants. These two receptor populations have different requirements for stimulating the adrenocortical cell [4]. Because of the fact that the two types of receptor presumably have different effects on the cAMP production [18], it is of interest to study the nature of the new steroidogenic center further by estimating the cAMP production in response to the addition of $ACTH^{11-24}$.

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