ISOLATED ADRENAL CELLS: LOG DOSE RESPONSE CURVES FOR STEROIDOGENESIS INDUCED BY $ACTH_{1-24}$, $ACTH_{1-10}$, $ACTH_{4-10}$ AND $ACTH_{5-10}$

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1. Introduction

$ACTH_{1-39}$ * increases the production of corticosterone when added to suspensions of isolated rat adrenal cells [1]. Response and dose are related by the expression $B/B_{max} = A/(A + A_{50})$ where B is the rate of corticosterone production, B_{max} is the maximum rate of corticosterone production, A is the dose of ACTH and A_{50} is the dose required to induce $\frac{1}{2}$ B_{max} [2]. According to the model proposed by Ariens [3], A_{50}, an apparent dissociation constant, is the reciprocal of the *affinity* of the hormone for its receptor, and B_{max} is a measure of the effectiveness with which the hormone interacts with its receptor ("*intrinsic activity*"). In contrast to conventional methods of assay [4–6], the isolated adrenal cell technique provides measures of both *affinity* and "*intrinsic activity*", information of some importance to the understanding of the relation of structure to biological action among members of a series of polypeptides related to ACTH.

An analysis of log dose response (LDR) curves constructed on the basis of the increased production of corticosterone which follows addition of $ACTH_{1-24}$, $ACTH_{1-10}$ and $ACTH_{4-10}$ to suspensions of isolated adrenal cells indicates that these polypeptides vary widely in potency (variations in A_{50}'s) but exhibit the same maximum biological response (B_{max}) as the natural agonist, $ACTH_{1-39}$. It appears that the full complement of amino acids ("active center") involved in activation of receptor resides in the region 4–10 of the ACTH molecule.

At the highest dose tested, $ACTH_{5-10}$ significantly increased corticosterone production.

2. Materials and methods

$ACTH_{1-24}$ was synthesized according to Schwyzer and Kappeler [7] and generously provided as Synacthen by Dr. Rittel, CIBA-Geigy AG, Basel, Switzerland, and as Cortrosyn by Dr. H. Strade, Organon Ltd., West Orange, New Jersey. $ACTH_{1-10}$, $ACTH_{4-10}$ and $ACTH_{5-10}$ were synthesized according to Schwyzer and Kappeler [8], Kappeler and Schwyzer [9] and Kappeler [10] and were generously provided by Dr. W. Rittel. The protective groups were cleaved with trifluoroacetic acid, and the peptides isolated as pure acetates after ion-exchange chromatography and gel filtration. Insulin and glucagon were

* The following abbreviations of amino acids and peptides are used (IUPAC-IUB Commission on Biochemical Nomenclature, European J. Biochem. 1 (1967) 375): ACTH = $ACTH_{1-39}$ = porcine adrenocorticotrophic hormone; $ACTH_{1-24}$ = corticotrophin-(1–24)-tetracosipeptide; $ACTH_{1-10}$ = corticotrophin-(1–10)-dekapeptide; $ACTH_{4-10}$ = corticotrophin-(4–10)-heptapeptide; $ACTH_{5-10}$ = corticotrophin-(5–10)-hexapeptide; $ACTH_{11-24}$ = corticotrophin-(11–24)-tetradecapeptide.

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Suspensions of cells of the rat adrenal cortex were prepared by the trypsin method of Sayers et al. [1]. The pellet of harvested cells was washed twice with Krebs-Ringer bicarbonate buffer (KRB) containing glucose, 2%, lima bean trypsin inhibitor, 0.1%, and bovine serum albumin (BSA), 0.5% to remove trypsin. The washed pellet was resuspended in 60 ml KRB containing calcium, 7.65 mM, glucose, 0.2%, and BSA, 0.5%. Aliquots of the suspension, 0.9 ml in volume, together with 0.1 ml of vehicle or with 0.1 ml of vehicle to which had been added ACTH, were incubated in an atmosphere of 95% O_2–5% CO_2 at 37°. At the end of incubation, methylene chloride was added and an aliquot of the methylene chloride phase analyzed for corticosterone [11].

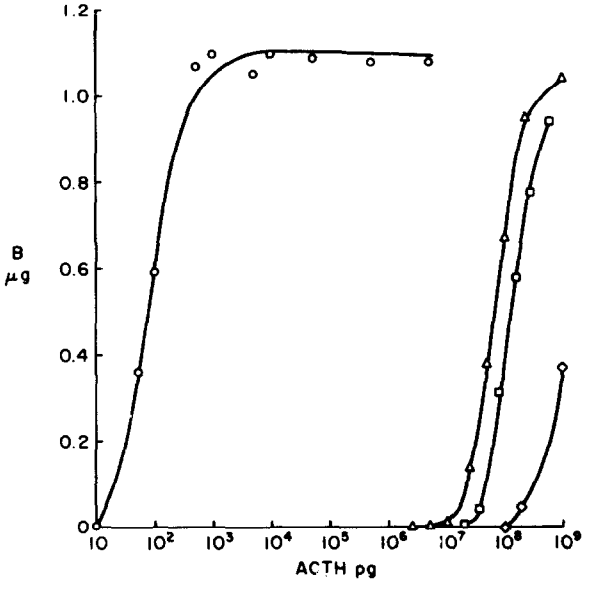

Fig. 1. B production by aliquots of a suspension of isolated adrenal cells in response to $ACTH_{1-24}$, ○; $ACTH_{1-10}$, □; $ACTH_{4-10}$, △; and $ACTH_{5-10}$, ◇. The points are averages of B analyses on duplicate incubates of cell suspension. The values are net, B in aliquots to which ACTH was added minus B in aliquots to which vehicle only was added. Aliquots incubated 60 min at 37° in 95% O_2–5% CO_2. B in blanks incubated 60 min equaled 0.05 and 0.05 μg.

Table 1
Molar potencies of ACTH polypeptides assayed by steroidogenesis.

Polypeptide	Potency*
$ACTH_{1-39}$	100
$ACTH_{1-24}$	140+
$ACTH_{1-10}$	$.37 \times 10^{-4}$
$ACTH_{4-10}$	$.75 \times 10^{-4}$
$ACTH_{5-10}$	$.22 \times 10^{-5}$**

Molar potencies of synthetic polypeptides expressed as reciprocals of their A_{50}'s relative to $ACTH_{1-39}$ which is assigned a value of 100.

$$* \text{ Potency} = \frac{1}{A_{50} \text{ in moles}} \times 100$$

+ Responses to various doses of $ACTH_{1-39}$ and of $ACTH_{1-24}$ added to aliquots of a single suspension of isolated adrenal cells, have been determined and complete LDR curves constructed and analyzed. The two polypeptides have the same B_{max}; $ACTH_{1-24}$ is estimated to be 40% more active than $ACTH_{1-39}$ on a molar basis.

** Based on response at highest dose tested (1 mg) and on the *assumption* that B_{max} equals that of ACTH.

3. Results and discussion

$ACTH_{1-39}$ and $ACTH_{1-24}$ have about the same potency and exhibit the same B_{max} when added to suspensions of isolated adrenal cells (see table 1). LDR curves for $ACTH_{1-24}$, $ACTH_{1-10}$ and $ACTH_{4-10}$ are displayed in fig. 1. These compounds varied widely in potency but exhibited the same B_{max}, estimated by computer to be 1.1 μg B/60 min. At the highest dose tested (1 mg), $ACTH_{5-10}$ induced a response approximately one-third the B_{max} of $ACTH_{1-24}$. Relative molar potencies are presented in table 1. The fact that $ACTH_{4-10}$ appears to be about twice as active as $ACTH_{1-10}$ on a molar basis is interesting and we offer no explanation at this time.

To date, $ACTH_{1-10}$ is the smallest fragment of the ACTH molecule demonstrated to exhibit biological activity [12]. The isolated adrenal cell technique reveals that $ACTH_{1-10}$ and in addition, $ACTH_{4-10}$ and $ACTH_{5-10}$ possess steroidogenic activity. That the responses are non-specific is hardly likely since 1 mg of insulin, 1 mg of glucagon or 1 mg of a mixture of amino acids 1–13 of the N-terminal of ACTH (in the molar ratios characteristic of the hormone) fail to

stimulate B production when added to suspensions of isolated adrenal cells.

Biological activity is determined by both the affinity of the hormone for the receptor, as reflected in displacement of the LDR curves along the abscissa, and the capacity of the hormone to activate the receptor ("efficacy" [13], "intrinsic activity" [3]), as reflected in the value of B_{max} (for discussion of these concepts as applied to oxytocin and analogues, see [14]). In this connection [Trp(Nps)^9] $ACTH_{1-39}$, an o-nitrophenyl sulfenyl derivative of ACTH, exhibits a B_{max} approx. 0.6 that of $ACTH_{1-39}$ (Seelig, Kumar and Sayers, unpublished observation) and is classified as a partial agonist. Furthermore, since $ACTH_{4-10}$ is an agonist (same B_{max} as $ACTH_{1-39}$), we suggest that the "active center" of the ACTH molecule resides in the region 4–10. Fujino et al. [15] report that $ACTH_{5-23}$ amide exhibits weak steroidogenic activity when tested *in vivo*. This information, together with the data presented in this report, indicates that the amino acids in the region 5–10 are capable of at least partial activation of the receptor.

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