ISOLATED ADRENAL CELLS: $ACTH_{11-24}$, A COMPETITIVE ANTAGONIST OF $ACTH_{1-39}$ AND $ACTH_{1-10}$

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1. Introduction

$ACTH_{4-10}$ * has been demonstrated to be a weak agonist in the isolated adrenal cell system [1]. Although low in potency, this fragment of the ACTH molecule, when given in sufficiently large doses induces the same maximum rate of corticosterone production (B_{max}) as that characteristic of a strong agonist ($ACTH_{1-39}$ or $ACTH_{1-24}$). It appears that the full complement of amino acids involved in activation of receptor is in the region 4 to 10 of the ACTH molecule; amino acids in the region 11–24 are not involved in activation but rather provide affinity of ACTH for receptor. In support of this suggestion are the observations of this report to the effect that $ACTH_{11-24}$ is a competitive antagonist of $ACTH_{1-39}$.

2. Materials and methods

A crude preparation of porcine ACTH, kindly supplied by Dr. Sam Epstein of the Upjohn Company, was purified by carboxymethyl cellulose chromatography [2]. The purified porcine $ACTH_{1-39}$ exhibited a potency of 100 International Units per mg when assayed against the Third International Standard (1.5 intravenous units per ampoule) by the isolated adrenal technic.

$ACTH_{1-10}$ and $ACTH_{11-24}$ were prepared according to Schwyzer and Kappeler [3] and [4] (and literature quoted therein), respectively, and generously provided by Dr. Rittel, CIBA-Geigy AG, Basel, Switzerland. Protective groups were cleaved with trifluoroacetic acid, and the peptides isolated as pure acetates after ion-exchange chromatography and gel filtration.

Methodology has been described in the preceding paper [1].

3. Results and discussion

$ACTH_{1-39}$ and $ACTH_{1-10}$ increased production of corticosterone (B) when added alone to aliquots of a suspension of isolated adrenal cells. Log dose response (LDR) curves for B production are displayed as solid lines in fig. 1. $ACTH_{1-39}$ and $ACTH_{1-10}$ exhibited the same B_{max}, estimated by computer to be 1.4 μg per 60 min. Doses required to induce ½ B_{max} (A_{50}'s) were markedly different and equalled 170 pg and 101 μg for $ACTH_{1-39}$ and $ACTH_{1-10}$, respectively. $ACTH_{11-24}$ when added alone in doses of 1, 100 and 540 μg failed to increase B production. The possibility existed that $ACTH_{11-24}$ interacted with the receptor but failed to activate it.

In order to test this possibility, $ACTH_{11-24}$ was added in combination with various doses of

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* 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; $ACTH_{1-10}$ = corticotrophin-(1–10)-dekapeptide; $ACTH_{4-10}$ = corticotrophin-(4–10)-heptapeptide; $ACTH_{11-24}$ = corticotrophin-(11–24)-tetradekapeptide; [Lys(DNS)^{21}] $ACTH_{1-24}$ = [21-Lysine (dansyl)]-corticotrophin-(1–24)-tetracosipeptide.

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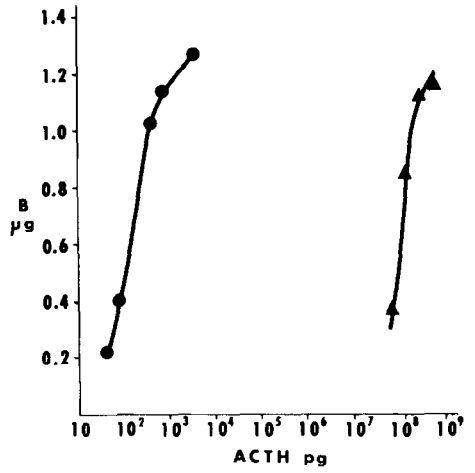

Fig. 1. B production by aliquots of a suspension of isolated adrenal cells in response to $ACTH_{1-39}$ alone, •; and $ACTH_{1-10}$ alone, ▲. The points are averages of B analyses on duplicate incubates of cell suspension. The values are net, i. e., quantities in aliquots to which ACTH was added minus quantities in aliquots to which vehicle only was added. Aliquots incubated 60 min at 37° in 95% O_2 – 5% CO_2. B blanks incubated 60 min equalled 0.07 μg.

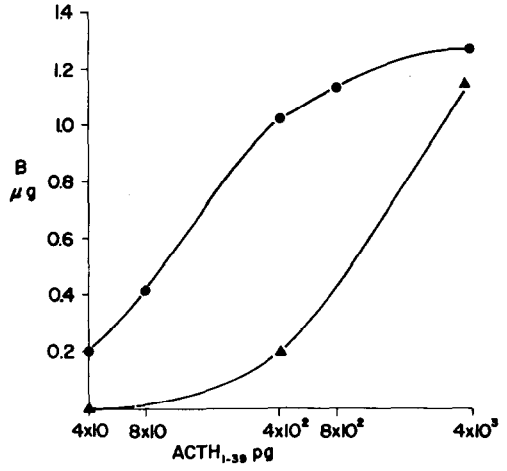

Fig. 2. B production by aliquots of a suspension of isolated adrenal cells in response to $ACTH_{1-39}$ alone, •; and in combination with 100 μg $ACTH_{11-24}$, ▲. Other conditions are the same as those provided in legend to fig. 1.

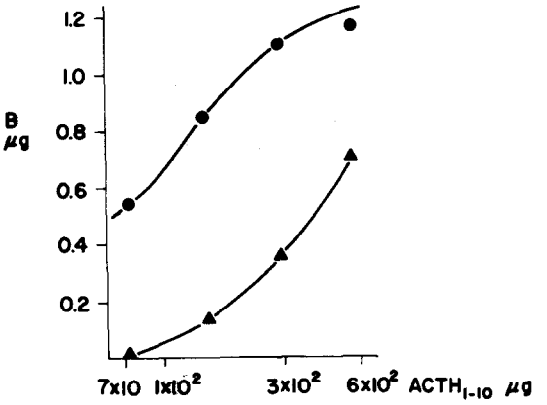

Fig. 3. B production by aliquots of a suspension of isolated adrenal cells in response to $ACTH_{1-10}$ alone, •; and in combination with 100 μg $ACTH_{11-24}$, ▲. Other conditions are the same as those provided in legend to fig. 1.

$ACTH_{1-39}$. A dose of 1 μg of $ACTH_{11-24}$ had no significant effect on B production induced by $ACTH_{1-39}$. At a dose of 100 μg, $ACTH_{11-24}$ markedly inhibited the response to 40 and 400 pg of $ACTH_{1-39}$ (fig. 2). A dose of 4000 pg of $ACTH_{1-39}$ almost completely reversed the inhibitory action of $ACTH_{11-24}$.

$ACTH_{11-24}$ was also added in combination with various doses of $ACTH_{1-10}$. As in the case of $ACTH_{1-39}$, 1 μg of $ACTH_{11-24}$ had no significant effect on B production. However, at a dose of 100 μg, $ACTH_{11-24}$ markedly inhibited the steroidogenic action of all doses (74, 148, 295 and 590 μg) of $ACTH_{1-10}$ (fig. 3).

$ACTH_{11-24}$ tetradekapeptide fulfills three criteria for a competitive antagonist. First, $ACTH_{11-24}$ does not have the capacity to stimulate B production. Second, $ACTH_{11-24}$ inhibited in a dose-dependent fashion the action of $ACTH_{1-39}$ and $ACTH_{1-10}$. Third, this inhibition was reversed by increasing doses of $ACTH_{1-39}$ and $ACTH_{1-10}$. These observations suggest that the amino acids in position 11 to 24 are to a major degree, if not entirely, involved in modifying the affinity of the molecule for the receptor. This does not exclude the possibility that amino acids in the region 1–10 are involved in modifying affinity. Certain amino acids in the region 1–10 may play a dual role, excitation of, and attraction for the receptor.

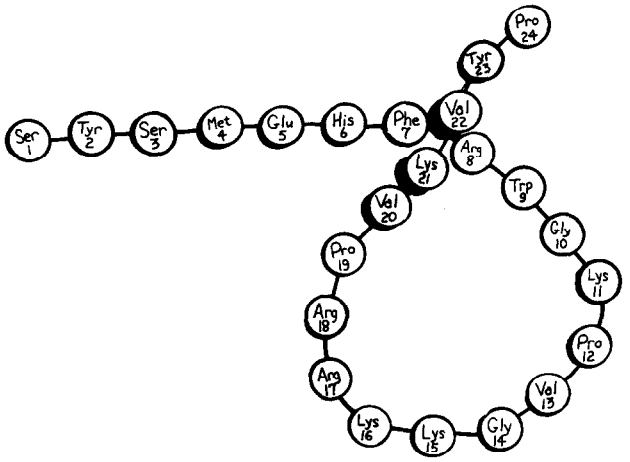

Fig. 4. Possible conformation of $ACTH_{1-24}$ at its receptor site. "Active center" is located in the region 4–10. Note suggested loop arrangement with Lys^{21} Val^{22} crossing over Phe^{7} Arg^{8} Trp^{9}.

Perhaps unexpected is the fact that $ACTH_{11-24}$ is a competitive antagonist of $ACTH_{1-10}$. On one hand, the interaction of the free carboxyl on Gly^{10} of $ACTH_{1-10}$ and the free α-amino of Lys^{11} on $ACTH_{11-24}$ may account for the antagonism. On the other hand, this antagonism may provide insight into the conformation of ACTH at the instant it excites the receptor. Let us assume that $ACTH_{1-24}$ forms a loop such that Lys^{21} Val^{22} cross over the region Phe^{7}, Arg^{8} and Trp^{9} which lies deep in the receptor (see fig. 4). Furthermore, a negatively charged area of the receptor attracts the positive charges of Arg^{8} and of Lys^{21}. This arrangement would pertain for the intact structure $ACTH_{1-24}$. When the fragments $ACTH_{1-10}$ and $ACTH_{11-24}$ are added in combination, the "binding" of Val^{20}, Lys^{21}, Val^{22} to the receptor may be expected to hinder the "binding" of Arg^{8} and associated amino acids of the fragment $ACTH_{1-10}$. This model is not excluded by the findings of Schwyzer and Schiller ([5], and literature quoted therein) that the biologically active [$Lys(DNS)^{21}$] $ACTH_{1-24}$ is present in solutions as an equilibrium population of different conformers with a mean distance of about 20 Å from Trp^{9} to $Lys(DNS)^{21}$. A special "fitting" conformation with a smaller distance might well be imposed upon the hormone by a receptor molecule (see also Schwyzer [6]). These results were obtained by fluorescence techniques, especially excitation energy transfer from Trp^{9} to $Lys(DNS)^{21}$.

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