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Abstract—Tritium-labeled synthetic fragments of human adrenocorticotropic hormone (ACTH) [3H]ACTH (11–24) and [3H]ACTH (15–18) with a specific activity of 22 and 26 Ci/mmol, respectively, were obtained. It was found that [3H]ACTH-(11–24) binds to membranes of the rat adrenal cortex with high affinity and high specificity (K_i 1.8 ± 0.1 nM). Twenty nine fragments of ACTH (11–24) were synthesized, and their ability to inhibit the specific binding of [3H]ACTH (11–24) to adrenocortical membranes was investigated. The shortest active peptide was found to be an ACTH fragment (15–18) (KKRR) (K_i 2.3 ± 0.2 nM), whose [3H] labeled derivative binds to rat adrenocortical membranes (K_i 2.1 ± 0.1 nM) with a high affinity. The specific binding of [3H]ACTH-(15–18) was inhibited by 100% by unlabeled ACTH (11–24) (K_i 2.0 ± 0.1 nM). ACTH (15–18) in the concentration range of 1–1000 nM did not affect the adenylate cyclase activity of adrenocortical membranes and, therefore, is an antagonist of the ACTH receptor.

Key words: adrenocorticotropic hormone (ACTH), peptides, receptors, adenylate cyclase, adrenal cortex

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INTRODUCTION

The major function of the adrenocorticotropic hormone is to stimulate the synthesis and secretion of glucocorticoids by the cells of the zona fasciculata and zona reticularis of the adrenal cortex. The sensitivity of cortical cells to ACTH depends on the expression and function of the G protein-coupled receptor MC2R, which belongs to the subfamily of melanocortin receptors. The binding of the hormone to MC2R leads to an increase in the adenylate cyclase activity and thus the activation of protein kinase A. Kapas et al. studied the binding of ACTH fragments to the cloned murine hormone receptor expressed in human HeLa cells. According to their data, fragment ACTH (11–24) competes actively with 

RESULTS AND DISCUSSION

ACTH (1–24) is the smallest fragment of ACTH required for the hormone to fully manifest its activity; its sequence without ten C-terminal amino acid residues corresponds to the primary structure of the α-melanocyte-stimulating hormone (α-MSH) (Fig. 1). A comparative analysis of the primary structure of vertebrate ACTH shows that region 15–24 of the molecule is evolutionarily highly conservative. Costa et al. synthesized the analogues of ACTH (1–24) with amino acid substitutions in region 15–24 and estimated their activity in vitro and in vivo. Based on the results obtained, the authors concluded that region 15–18 (KKRR) in the ACTH molecule is necessary for both
the binding to receptor and the subsequent activation of adenylate cyclase. Surprisingly, the analogue in which Ala residues were substituted for five C-terminal amino acid residues 20–24 (VKVYP) was 1.5 times more effective than peptide ACTH (1–24) with the natural sequence in vivo (but not in vitro). In the opinion of Costa et al., region 20–24 of the ACTH molecule is not involved in the binding of the hormone to the receptor but is essential for the formation of the spatial structure, which provides the optimal stability of ACTH in the blood.

According to the data of Kapas et al., ACTH (11–24) acts as an antagonist of the receptor: it competes with $^{125}$I-labeled ACTH for binding to the cloned receptor (IC$_{50} = 1$ nM) but, as distinct from ACTH, does not activate adenylate cyclase [8]. We obtained peptide $[^3]$H$\text{ACTH}$ (11–24) (specific activity 22 Ci/mol) and studied its binding to membranes isolated from the adrenal cortex of the rat. The experiments showed that, under the conditions chosen (see the Experimental section), $[^3]$H$\text{ACTH}$ (11–24) binds specifically to rat adrenocortical membranes (Fig. 3). It follows from the plots of total (I), specific, and nonspecific (3) binding of $[^3]$H$\text{ACTH}$ (11–24) to membranes versus incubation time that the dynamic equilibrium in the system labeled peptide–receptor was established approximately after 1 h and persisted for at least 2 h. Therefore, for determining the equilibrium dissociation constant ($K_d$), the reaction of binding of $[^3]$H$\text{ACTH}$ (11–24) to membranes was carried out for 1 h. The nonspecific binding of $[^3]$H$\text{ACTH}$ (11–24) under these conditions was 6.4 ± 0.8% of the total binding of the peptide.

It follows from the Scatchard plot (I) in Fig. 4 that peptide $[^3]$H$\text{ACTH}$ (11–24) binds to one type of high-affinity receptors on rat adrenocortical membranes ($K_d$ of complex $[^3]$H$\text{ACTH}$ (11–24)–receptor 1.8 ± 0.1 nM and $B_{\text{max}} = 2.8 ± 0.2$ pmol/mg protein). To characterize the specificity of binding, the following unlabeled peptides were tested as potential competitors of $[^3]$H$\text{ACTH}$ (11–24): ACTH (1–24) (positive control), ACTH (4–10), somatostatin, β-endorphin, and [Met$^3$]enkephalin (negative control). The results of the experiments presented in Table 1 indicate that only ACTH (1–24) was capable of inhibiting the binding of $[^3]$H$\text{ACTH}$ (11–24) to membranes ($K_i 1.7 ± 0.1$ nM). The other peptides were inactive. Thus, $[^3]$H$\text{ACTH}$ (11–24) binds with high affinity and high specificity to the ACTH receptor of rat adrenocortical membranes.

To assess the affinity of unlabeled ACTH (11–24) fragments to the ACTH receptor of rat adrenocortical membranes (Table 1), we studied the ability of each fragment to inhibit the specific binding of $[^3]$H$\text{ACTH}$ (11–24) to membranes. It was found that only ACTH (11–24) fragments containing the sequence KKRR (no. 29, fragment 15–18) and tetrapeptide KRRP (no. 26, fragment 16–19) have a high inhibitory activity. The inhibitory activity of the other peptides was very low ($K_i > 1$ µM). Tetrapeptides KKRR and KRRP were the shortest ACTH (11–24) fragments that actively inhibited the binding of $[^3]$H$\text{ACTH}$ (11–24) to adrenocortical membranes ($K_i 2.3 ± 0.2$ and 2.0 ± 0.2 nM) (Table 1).

To characterize the binding of peptide ACTH (15–18) to rat adrenocortical membranes in a direct experiment, labeled $[^3]$H$\text{ACTH}$ (15–18) (specific activity 26 Ci/mol) was obtained (see the Experimental section). The dependence of total (I), specific (2), and nonspecific (3) binding of $[^3]$H$\text{ACTH}$ (15–18) to membranes on incubation time (Fig. 3b) was similar to that for $[^3]$H$\text{ACTH}$ (11–24) (Fig. 3a): the dynamic equilibrium in the system labeled peptide–receptor was established approximately after 1 h and persisted for at least 2 h. Therefore, the reaction of binding of $[^3]$H$\text{ACTH}$ (15–18) to membranes was carried out for 1 h. The nonspecific binding of $[^3]$H$\text{ACTH}$ (15–18) was 6.4 ± 0.6% of its total binding.

An analysis of the specific binding of $[^3]$H$\text{ACTH}$ (15–18) to adrenocortical membranes in the Scatchard coordinates (Fig. 4, 2) indicated that the labeled peptide binds with high affinity to one receptor type ($K_d 2.1 ± 0.1$ nM, $B_{\text{max}} = 2.6 ± 0.2$ pmol/mg protein). A study of binding specificity showed that only ACTH (1–24) and ACTH (11–24) are capable of extruding $[^3]$H$\text{ACTH}$ (15–18) from the complex with receptor ($K_i 1.9 ± 0.1$ and 2.0 ± 0.1 nM, Table 2). Unlabeled ACTH (4–10), somatostatin, β-endorphin, and [Met$^3$]enkephalin,

![Fig. 1. Comparison of amino acid sequences of peptide ACTH (1–24) and human α-MSH. The ACTH (15–24) fragment is designated by boldface type.](image1)

![Fig. 2. Amino acid sequence and the activity (%) of ACTH (1–24) analogues synthesized by Costa et al. (according to the data reported in [9]).](image2)