Interactions of Adrenocorticotropic Hormone with Its Adrenal Receptors

DEGRADATION OF ACTH₁⁻₂₄ AND ACTH₁₁⁻₂₄

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SUMMARY

Crude membranes (20,000 x g pellet) prepared from human, rat, and ovine adrenals bind ¹²⁵I-corticotropin-(1-24)-tetracosapeptide (¹²⁵I-NPS-ACTH₁⁻₂₄) and degrade unbound hormone. The degradation is dependent on temperature and the concentration of membrane proteins.

The degradation of ¹²⁵I-[9-tryptophan(o-nitrophenylsulfenyl)]-corticotropin-(1-24)-tetracosapeptide (¹²⁵I-NPS-ACTH₁⁻₂₄) is similar to ¹²⁵I-ACTH₁⁻₉, but that of ¹²⁵I-corticotropin-(11-24)-tetradecapeptide (¹²⁵I-ACTH₁₁⁻₂₄) is larger.

The degradation of ¹²⁵I-ACTH₁⁻₂₄ is inhibited by ACTH₁⁻₂₄ and corticotropin-(1-10)-decapeptide (ACTH₁⁻₁₀), but ACTH₁⁻₂₄ and ACTH₁₁⁻₂₄ at the same molar concentration have no effect. On the other hand, the degradation of ¹²⁵I-ACTH₁₁⁻₂₄ is protected by ACTH₁⁻₁₀ and ACTH₁⁻₂₄, but not by ACTH₁⁻₉. This suggests two systems of degradation, one will have the NH₂-terminal sequence of ACTH₁⁻₂₄ as substrate, and the other the 11-24 COOH-terminal sequence.

Recently it has been shown that at the time of the inter-reaction of certain polypeptide hormone with membranes from target tissues, there are two simultaneous but independent processes. One is the binding of the hormone with its receptor; the other is the inactivation of the hormone (1-5). It has been a few years since the existence of ACTH inactivation by plasma and the homogenates of several organs has been shown (6-8). More recently ACTH has been proven to bind to adrenal preparations from various species (9-13), but the relationship between the two phenomena has not yet been studied.

In this study we have examined the kinetics of the degradation of ACTH₁⁻₂₄ in the presence of adrenal preparations. The independence of the binding and degradation has been demonstrated and the sequence specificity of the degradation process studied.

EXPERIMENTAL PROCEDURE

Materials—ACTH₁⁻₂₄ was provided by CIBA. ACTH₁⁻⁻₂₄, ACTH₁⁻⁻₁₀, corticotropin-(1-10)-decapeptide; NPS-ACTH₁⁻⁻₂₄, (9-tryptophan(o-nitrophenylsulfenyl))-corticotropin-(1-24)-tetracosapeptide; NPS-ACTH₁⁻⁻₁₀ from the carboxymethylcellulose column are shown in Fig. 1. The iodinated ACTH₁⁻₉ was separated almost completely from non-iodinated ACTH₁⁻₉ (Fig. 2). The peak of iodinated ACTH₁⁻₉ obtained in experiments similar to Fig. 2 stimulated the adenylate cyclase activity of subcellular preparations of adrenals. However, to obtain the same stimulation about twice as much iodinated ACTH₁⁻₉ as non-iodinated ACTH₁⁻₉ was required. Comparable results were reported by Letkowitz et al. (9).

The elution profiles of the three labeled ACTHs (ACTH₁⁻₂₄, ACTH₁⁻⁻₁₀, and NPS-ACTH₁⁻⁻₁₀) from the carboxymethylcellulose column are shown in Fig. 1. The iodinated ACTH₁⁻₉ was separated almost completely from non-iodinated ACTH₁⁻₉ (Fig. 2). The peak of iodinated ACTH₁⁻₉ obtained in experiments similar to Fig. 2 stimulated the adenylate cyclase activity of subcellular preparations of adrenals. However, to obtain the same stimulation about twice as much iodinated ACTH₁⁻₉ as non-iodinated ACTH₁⁻₉ was required. Comparable results were reported by Letkowitz et al. (9).

Owing to insufficient quantities of unlabeled hormone, we were unable to check whether iodinated and non-iodinated NPS-ACTH₁⁻⁻₁₀ and ACTH₁⁻⁻₁₀ were separated on a carboxymethylcellulose column. ACTH₁⁻⁻₁₀ was separated on a carboxymethylcellulose column.
lulose column. However, the following observation suggests that they were separated. After purification on a column and in the presence of 0.1 mg of membrane protein, the percentage of binding of iodinated ACTH, 11-24, and iodinated NPS-ACTH, 1-14, is similar to that of radioactivity was bound to the membranes. In addition, when the specific activities of the three iodinated ACTHs were measured by an antiserum that reacts specifically with the COOH-terminal sequence of ACTH, 1-24 (The Radiocmpial Centre, Ams, England), the activities were similar (300 to 400 μCi per μg).

**Measurement of Degradation of ACTH**—The membrane was incubated in 1 ml of 20 mM Tri-HCl (pH 7.4) containing 1% albumin and labeled ACTH at temperatures and times stated in figures and tables. At the end of incubation the medium was layered over 2.5 ml of 20 mM Tri-HCl (pH 7.4), 0.25 M sucrose, and 2% albumin, and centrifuged at 50,000 x g for 20 min at 0°. Only the bound fraction was aspirated and kept (unbound fraction). The pellet was washed once with 2 ml of the same buffer and centrifuged at 50,000 x g for 20 min. The supernatant was discarded and its radioactivity was measured in a Beckman micro-centrifuge. In these instances, only the degradation of the unbound fraction was studied.

The degradation of the hormone, in the bound and unbound fractions, was studied by the following methods: (a) electrophoresis in the system n-butyl alcohol-acetic acid-water (4:1:5) by gel filtration on a Sephadex G-50 column and by paper chromatography in the system n-butyl alcohol-acetic acid-water (4:1:5) (16).

Adenylate cyclase activity was measured as described elsewhere (14).

**RESULTS**

Degradation of iodinated ACTH, 1-14 by Ovine, Rat, and Human Adrenal Preparations—iodinated ACTH specifically bound to the crude adrenal membranes of the three species (Table I). Moreover the adenylate cyclase activity of these preparations was stimulated by ACTH (14). These results testify to the presence of ACTH receptors in those adrenal preparations.

After incubating iodinated ACTH, 1-14 for 30 min with those fractions the four methods used showed a degradation of unbound iodinated ACTH, 1-14 (Table II). The binding to fresh membranes gave the highest values of degradation. The degradation at 37° was greater than that observed at 4°. In contrast to the unbound

| Experiment | Rat | Human | Sheep |
|------------|-----|-------|-------|
| Iodinated ACTH, 1-14 bound | 16 | 30 | 21 |

TABLE I

Membrane proteins were incubated with 1 X 10^-7 M iodinated ACTH, 1-14 at 4° for 30 min. Specific binding was determined as described under "Experimental Procedure". Nonspecific binding represented 1 to 3% of total binding.
ACTH, the bound $^{125}$I-ACTH$_{1-24}$ was not degraded. After dissociation of $^{125}$I-ACTH$_{1-24}$ from its receptors, purity estimated by binding to fresh membranes was higher than the initial $^{125}$I-ACTH$_{1-24}$ (Table II).

ACTH is degraded by all of the subcellular fractions of the adrenal (Table III). However, the most powerful degrading action was in the 20,000 $\times g$ pellet (crude membranes). Membranes purified by the method of Finn et al. (11) had a specific activity for degradation, less than that of crude membranes, whereas their specific activity for binding was increased. These results suggest that the binding and degradation occur at different sites.

Due to the difficulties of obtaining sufficient quantity of highly purified human and rat adrenal membranes, the degradation studies were all done with crude membranes.

**Time Course of Degradation of $^{125}$I-ACTH$_{1-24}$**—The degradation of $^{125}$I-ACTH$_{1-24}$ by adrenal crude membranes is time- and temperature-dependent (Fig. 3). After 10 min of incubation, 5 and 30% of the hormone is degraded at 4 and 37°, respectively, as measured by adsorption to Quso and talc.

**Effects of Membrane and Substrate Concentrations**—The degradation of $^{125}$I-ACTH$_{1-24}$ depends on the concentration of membrane proteins (Fig. 4). At concentrations as low as 40 $\mu$g per ml, after 30 min of incubation 5 and 30% of the hormone were degraded at 4 and 37°, respectively. Under the same conditions, 20 and 9% of the ACTH were specifically bound to the membranes. Given that the binding at 4° is greater than at 37° (13) and that the bound ACTH is protected from degradation, the total quantity of ACTH degraded at 37° is several times greater than at 4°.

The degradation of $^{125}$I-ACTH$_{1-24}$ as a function of increasing concentrations of ACTH$_{1-24}$ (Fig. 5) suggests the existence at 4 and 37° of two sites with an apparent $K_m$ of about $6 \times 10^{-8}$ and $5 \times 10^{-8}$, respectively. The $V_{max}$ varies as a function of

### Table II

**Degradation of $^{125}$I-ACTH$_{1-24}$ by crude adrenal plasma membranes**

|                  | Unbound | Bound |
|------------------|---------|-------|
|                  | Talc    | Quso  | Electro-phoresis | binding | Talc    | Quso  | Electro-phoresis | Binding |
| Sheep            | 82      | 75    | 65     | 52     | 98      | 100   | 99              | 111     |
| 4°               | 45      | 37    | 32     | 21     | 99      | 98    | 99              | 100     |
| 37°              | 88      | 76    | 68     | 56     | 97      | 100   | 100             | 110     |
| Rat              | 48      | 40    | 30     | 27     | 98      | 98    | 99              | 110     |
| 4°               | 67      | 61    | 63     | 40     | 99      | 99    | 100             | 110     |
| 37°              | 40      | 34    | 26     | 14     | 99      | 98    | 97              | 107     |
| Human            |         |       |        |        |         |       |                 |         |
| 4°               |         |       |        |        |         |       |                 |         |
| 37°              |         |       |        |        |         |       |                 |         |

### Table III

**Distribution of adenylyl cyclase, ACTH binding, and ACTH degradation activities in different subcellular fractions of sheep adrenal**

|                  | Basal | ACTH ($10^{-8}$ M) | NaF (12 mm) | Binding | Per cent of hormone remaining intact |
|------------------|-------|--------------------|-------------|---------|-------------------------------------|
|                  | pmol cAMP/20 min/mg protein | pmol/mg protein |             |         |                                     |
| Whole homogenate | 193 ± 8 | 301 ± 13          | 352 ± 22    | 12 ± 1.1 | 26                                  |
| 800 $\times g$.  | 192 ± 7 | 253 ± 11          | 545 ± 20    | 8 ± 0.9  | 35                                  |
| 20,000 $\times g$. | 274 ± 10 | 452 ± 14          | 1102 ± 40   | 23 ± 1.4 | 16                                  |
| 105,000 $\times g$. | 156 ± 6 | 177 ± 8           | 949 ± 39    | 6 ± 0.8  | 40                                  |
| Supernatant (105,000 $\times g$) | 47 ± 1 | 24 ± 2           | 42 ± 4      | 85       |                                     |
| Purified membranes | 364 ± 12 | 600 ± 15         | 4749 ± 60   | 30 ± 1.6 | 25                                  |
| Purified mitochondria | 7 ± 1 | 5 ± 1           | 16.7 ± 3    | 0.4 ± 0.2 | 90                                  |

* Specific binding was determined at 4° for 30 min in the presence of $3 \times 10^{-8}$ M $^{125}$I-ACTH$_{1-24}$ as described under "Experimental Procedure."

† Degradation was studied at 37° for 40 min in the presence of $3 \times 10^{-8}$ M $^{125}$I-ACTH$_{1-24}$. The percent of hormone remained in the supernatant was determined by the ability to bind to fresh membranes.

Mean ± 1 S.D. of six measurements.

Prepared by the method of Finn et al. (11).
**TABLE IV**

Degradation of ACTH analogues by sheep adrenal crude membranes

| Membrane proteins (0.5 mg per ml) were incubated separately with 4.2 × 10⁻⁹ M \(^{125}\text{I}-\text{ACTH}_{1-24}\), \(^{125}\text{I}-\text{NPS-ACTH}_{1-24}\), or \(^{125}\text{I}-\text{ACTH}_{1-14}\) for 30 min. Degradation was measured as described under "Experimental Procedure." |
|---------------------------------|-----------------|-----------------|-----------------|
| Per cent of hormone remaining intact | Quso | Electrophoresis | Binding |
| \(^{125}\text{I}-\text{ACTH}_{1-24}\) | 4° | 37° | 4° | 37° |
| 60 | 58 | 48 | 37 | 30 | 23 |
| \(^{125}\text{I}-\text{NPS-ACTH}_{1-24}\) | 4° | 37° | 54 | 36 | 28 | 24 |
| \(^{125}\text{I}-\text{ACTH}_{1-14}\) | 4° | 37° | 71 | 17 | 51 | 34 |
| | | | | | | 2 |

**TABLE V**

Effect of ACTH analogues on degradation of \(^{125}\text{I}-\text{ACTH}_{1-24}\) and \(^{125}\text{I}-\text{ACTH}_{11-24}\) by human crude membranes

| Protein (0.8 mg per ml) was incubated 45 min at 4° with 2 × 10⁻⁹ M \(^{125}\text{I}-\text{ACTH}_{1-24}\) or 1.8 × 10⁻⁹ M \(^{125}\text{I}-\text{ACTH}_{11-24}\). Degradation was measured as described under "Experimental Procedure." |
|---------------------------------|-----------------|-----------------|-----------------|
| Per cent of hormone remaining intact | Quso | Talc | Electrophoresis | Quso | Talc | Electrophoresis |
| \(^{125}\text{I}-\text{ACTH}_{1-24}\) | | | | | | |
| Control | 70 | 72 | 64 | 52 | 49 | 41 |
| + \(^{125}\text{I}-\text{ACTH}_{1-24}\) (2.6 × 10⁻⁹ M) | 95 | 97 | 99 | 97 | 94 | 98 |
| + NPS-ACTH₁₋₂₄ | 98 | 100 | 99 | 99 | 100 | 99 |
| + \(^{125}\text{I}-\text{ACTH}_{11-24}\) (2.6 × 10⁻⁹ M) | 72 | 70 | 65 | 95 | 96 | 98 |
| + \(^{125}\text{I}-\text{ACTH}_{11-24}\) (2.6 × 10⁻⁹ M) | 92 | 94 | 90 | 56 | 52 | 42 |

**Fig. 4.** Degradation of \(^{125}\text{I}-\text{ACTH}_{1-24}\) as a function of membrane protein concentration. Crude sheep adrenal membranes were incubated 30 min at 4° or 37° with \(^{125}\text{I}-\text{ACTH}_{1-24}\) (4 × 10⁻⁹ M). Degradation of unbound \(^{125}\text{I}-\text{ACTH}_{1-24}\) at 4° or 37° was measured by adsorption to Quso (△) and by binding to fresh membranes (●), respectively.

**Fig. 5.** Sheep adrenal membranes (0.5 mg per ml) were incubated for 30 min at 4° or 37° with \(^{125}\text{I}-\text{ACTH}_{1-24}\) (4 × 10⁻⁹ M) mixed with increasing amounts of unlabeled \(^{125}\text{I}-\text{ACTH}_{1-24}\). The amount of \(^{125}\text{I}-\text{ACTH}_{1-24}\) degraded (per cent of degraded \(^{125}\text{I}-\text{ACTH}_{1-24}\) measured by adsorption to Quso times amount of substrate) is plotted against the substrate concentration.

Degradation of ACTH Analogues—A comparative study of the degradation of the three labeled ACTHs (Table IV) shows that at both temperatures, 4° and 37°, the degradation of the NPS-ACTH₁₋₂₄ is similar to that of the ACTH₁₋₂₄. In contrast the degradation of ACTH₁₁₋₂₄ is much greater.

Effects of ACTH Analogues on Degradation of \(^{125}\text{I}-\text{ACTH}_{1-24}\)—Degradation of \(^{125}\text{I}-\text{ACTH}_{1-24}\) at 4° has been studied in the presence of NPS-ACTH₁₋₂₄, ACTH₁₋₂₄, ACTH₁₁₋₂₄, and ACTH₁₋₁₀ (2.6 × 10⁻⁹ M). ACTH₁₋₂₄ and NPS-ACTH₁₋₂₄ inhibited the degradation of \(^{125}\text{I}-\text{ACTH}_{1-24}\) in a similar fashion, but ACTH₁₁₋₂₄ was without effect. By contrast, ACTH₁₋₁₀ is almost as effective as the first two analogues (Table V). On the other hand, the binding of \(^{125}\text{I}-\text{ACTH}_{1-24}\) is inhibited in a similar fashion by ACTH₁₋₂₄ and NPS-ACTH₁₋₂₄, but the displacement produced by ACTH₁₁₋₂₄ and ACTH₁₋₁₀ at the same molarity is respectively about 6 and 15 times less than that produced by ACTH₁₋₂₄ (Fig. 6).

These results suggest that the sequences of ACTH which inhibit the fixation and degradation of \(^{125}\text{I}-\text{ACTH}_{1-24}\) are different. ACTH₁₋₂₄ inhibits the degradation almost as well as ACTH₁₋₂₄, but its power of displacing the binding of \(^{125}\text{I}-\text{ACTH}_{1-24}\) is about 15 times less than ACTH₁₋₂₄. On the other hand, ACTH₁₋₁₀ is much more effective than ACTH₁₋₂₄ for inhibiting the binding of \(^{125}\text{I}-\text{ACTH}_{1-24}\) and has a negligible effect on degradation.

Effects of ACTH Analogues on Degradation of \(^{125}\text{I}-\text{ACTH}_{11-24}\)(Table V)—The degradation of \(^{125}\text{I}-\text{ACTH}_{11-24}\) was virtually completely inhibited by ACTH₁₋₂₄, NPS-ACTH₁₋₂₄, and ACTH₁₁₋₂₄, but ACTH₁₋₁₀ was without effect. On the other hand the binding of \(^{125}\text{I}-\text{ACTH}_{11-24}\) was displaced by ACTH₁₋₂₄ and NPS-ACTH₁₋₂₄, but the displacement produced by these two peptides was greater than that produced by ACTH₁₋₂₄ while ACTH₁₋₁₀ at high concentrations (5 × 10⁻⁴ M) was without effect (Fig. 7).
FIG. 6. Displacement of \(^{125}\)I-ACTH\(_{1-24}\) bound to crude sheep adrenal plasma membranes by ACTH analogues. Membranes were incubated 30 min at \(4^\circ\) in 0.25 ml of 20 mM Tris-HCl (pH 7.4) containing 1% albumin and \(4 \times 10^{-10}\) M \(^{125}\)I-ACTH\(_{1-24}\), and the indicated concentrations of unlabeled ACTH analogues: NPS-ACTH\(_{1-24}\) (○), ACTH\(_{1-24}\) (◇), ACTH\(_{11-24}\) (△), and ACTH\(_{11-19}\) (▲). In these experiments the total binding was 71%.

Degradation Products of \(^{125}\)I-ACTH\(_{1-24}\) and \(^{125}\)I-ACTH\(_{11-24}\)—The degradation products of these two derivatives of ACTH were analyzed by filtration on Sephadex G-50 and paper chromatography. After gel filtration, the main radioactive degradation product is eluted with the same elution volume as \([^{125}\text{I}]\)-monoiodotyrosine (data not shown). Paper chromatography (Fig. 8) confirmed this result and clearly demonstrated the absence of diiodotyrosine as a degradation product. With some adrenal preparations, a small peak of radioactivity, less polar than diiodotyrosine, which could not be identified represented 10 to 15% of the total radioactivity. Fig. 8 shows that the degradation of \(^{125}\)I-ACTH\(_{11-24}\) measured by the quantity of monoiodotyrosine liberated is greater than that of \(^{125}\)I-ACTH\(_{1-24}\).

Comparative Study of the Degradation of \(^{125}\)I-ACTH\(_{1-24}\) and \(^{125}\)I-ACTH\(_{11-24}\) by Leucine Aminopeptidase and Adrenal Preparations (Table VI)—After 6 hours of incubation of the two iodinated ACTHs with leucine aminopeptidase, about 80% of the radioactivity was in the form of monoiodotyrosine. As this compound is the main degradation product of \(^{125}\)I-ACTH\(_{1-24}\) and \(^{125}\)I-ACTH\(_{11-24}\) when incubated with adrenal preparations, we have made a comparative study of these two systems of degradation. Adrenal crude membranes liberate monoiodotyrosine more rapidly from ACTH\(_{11-24}\) than ACTH\(_{1-24}\). The opposite was seen with leucine aminopeptidase. In fact, after 30 min of

\[\text{Degradation of } ^{125}\text{I-ACTH}_{1-24} \text{ and } ^{125}\text{I-ACTH}_{11-24} \]

Labelled ACTH (\(4 \times 10^{-8}\) M) was incubated at \(37^\circ\) with either \(^{125}\)I-ACTH\(_{1-24}\) (left), or \(^{125}\)I-ACTH\(_{11-24}\) (right), at \(4^\circ\) (top), or \(37^\circ\) (bottom). After centrifugation a 0.2-ml aliquot of the supernatant was applied to Whatman No. 3MM paper and run in n-butyl alcohol-acetic acid-water system. Labeled ACTHs remain at the origin of the chromatogram. In the same experiments, the percentage of hormone remaining intact, estimated by binding to fresh crude membranes at 4 and 37°, was 30 and 3%, for \(^{125}\)I-ACTH\(_{1-24}\), and 11 and 0%, for \(^{125}\)I-ACTH\(_{11-24}\).

\[\text{Table VI} \]

\begin{tabular}{|c|c|c|c|c|c|}
\hline
 & Leucine aminopeptidase & Crude membranes & \\
 & Quan\(^a\) & Binding\(^b\) & [\(\text{MIT}\)] & Quan\(^a\) & Binding\(^b\) & [\(\text{MIT}\)] \\
\hline
\(^{125}\)I-ACTH\(_{1-24}\) & & & & & & \\
30 min & 49 & 24 & 46 & 30 & 14 & 50 \\
60 min & 29 & 10 & 78 & 12 & 3 & 80 \\
6 hours & 0 & 90 & & & & \\
\hline
\(^{125}\)I-ACTH\(_{11-24}\) & & & & & & \\
30 min & 80 & 2 & 7 & 12 & 2 & 75 \\
60 min & 74 & 0 & 18 & 5 & 0 & 87 \\
6 hours & 0 & 80 & & & & \\
\hline
\end{tabular}

\(^a\) Per cent of hormone remaining intact.

\(^b\) The \([^{125}\text{I}]\)-monoiodotyrosine ([\(\text{MIT}\)]) was isolated by paper chromatography. The results are expressed as per cent of the total radioactivity recovered in the paper.
incubation with leucine aminopeptidase, $^{125}$I-ACTH$_{1-24}$ completely lost its ability to bind to adrenal fresh membranes while only 7% of monoiodotyrosine was liberated. This suggests that the enzyme attacks the peptide sequence required for binding, probably lysine 11, before liberating tyrosine 23.

**Comparative Study of the Binding and Degradation of $^{125}$I-ACTH$_{1-24}$**—The objective was to obtain new arguments which would enable the binding and degradation to be separated. These two processes have been measured using the same adrenal preparation; the results are given in Table VII. Calcium and pancreatic trypsin inhibitor in the concentrations indicated completely inhibited the binding without altering the degradation. Glucagon, however, reduced the degradation but had no effect on the binding.

### TABLE VII

|       | Degradation | Binding |
|-------|-------------|---------|
| Control, 4°   | 100   | 100     |
| Control, 37°  | 250   | 40      |
| CaCl$_2$, 24 mM | 100   | 100     |
| Glucose, 1.4 X 10$^{-4}$ M | 81    | 100     |
| Insulin, 1.4 × 10$^{-6}$ M | 100   | 100     |
| Pancreatic trypsin inhibitor, 3 × 10$^{-5}$ M | 100   | 2       |
| N-Ethylmaleimide, 0.76 mM | 18    | 80      |
| p-Chloromercuribenzoic acid, 0.2 mM | 11    | 75      |

**Discussion**

In three species, our results testify to ACTH degradation in subcellular adrenal preparations containing specific ACTH receptors whose existence was proved by $^{125}$I-ACTH binding and adenylyl cyclase activity.

However, several arguments suggest that ACTH binding and degradation are independent processes: a) the maximum specific activity of the degradation system is in the 20,000 × g pellet and the maximum binding is to purified membranes; b) calcium and pancreatic trypsin inhibitor completely inhibit the binding at concentrations that do not affect the degradation; c) ACTH$_{1-10}$ displaces the binding of $^{125}$I-ACTH$_{1-24}$ but has no effect on its degradation, although ACTH$_{1-10}$ has only a weak effect on the binding, inhibits the degradation. This type of independence of the phenomena of binding and degradation has been described previously for insulin (2), glucagon (1, 3, 5), and calcitonin (4).

The degradation system of adrenal fraction seems to be different and more complex than leucine aminopeptidase, though monoiodotyrosine is the main radioactive product of both labeled ACTH. The degradation of $^{125}$I-ACTH$_{1-24}$ produced by crude membranes (measured by its ability to bind to fresh membranes) is accompanied by a liberation of monoiodotyrosine that is more rapid and greater than the degradation produced by leucine aminopeptidase. Given that tyrosine in position 23 does not take any part in the binding (10), the degrading system should attack ACTH$_{1-24}$ either at several points on the molecule or at the NH$_2$-terminal. In the latter case, the degrading system should liberate the amino acids between lysine 11 and tyrosine 23 more rapidly than leucine aminopeptidase.

The kinetic study of the degradation of both ACTHs by leucine aminopeptidase is the hydrolysis of serine 1, once the serine is removed, tyrosine 2 is liberated rapidly (17). Study of the degradation of $^{125}$I-ACTH$_{1-24}$ and $^{125}$I-ACTH$_{1-14}$ shows that for ACTH$_{1-24}$ there is a relationship between the diminution of ability to bind and the liberation of labeled monoiodotyrosine; by contrast, for ACTH$_{1-14}$ the first process is far more marked than the second. This strongly suggests that the monoiodotyrosine in these two ACTH analogues is in different positions. In ACTH$_{1-24}$, monoiodotyrosine is obligatory in position 22; in ACTH$_{1-24}$, the iodination is mainly in position 2.

Studies of ACTH structure-function relationship made with several analogues (10, 11, 18-22) have shown that peptide sequence necessary for the binding is located in the COOH-terminal fragment—11-24. Our study agrees with these findings but also suggests that the sequence 1-10 NH$_2$-terminal, in addition to being the active biological site of the hormone, could contribute to binding since the affinity of ACTH$_{1-24}$ (Figs. 6 and 7) for the adrenal receptor is much weaker than that of ACTH$_{1-24}$. In addition, ACTH$_{1-10}$ at high concentrations is able to displace the bound $^{125}$I-ACTH$_{1-24}$.

Using various ACTH analogues when studying ACTH degradation, we have found data suggesting the existence of at least two enzymatic degradation systems. The degradation of $^{125}$I-ACTH$_{1-24}$ produced by adrenal particulate fractions is protected by ACTH$_{1-24}$ and ACTH$_{1-14}$, but not by ACTH$_{1-10}$. On the other hand, the degradation of $^{125}$I-ACTH$_{1-24}$ is inhibited by ACTH$_{1-14}$ and ACTH$_{1-24}$, whereas ACTH$_{1-10}$ is without effect. The first system should have the NH$_2$-terminal sequence of ACTH$_{1-24}$ as substrate; the second the sequence 11-24. This second enzyme system could degrade ACTH$_{1-24}$ but either it attacks the peptide molecule after the first system or its affinity for ACTH$_{1-24}$ is very low.

The physiological significance of the results is unknown. However, we must take ACTH degradation into account to interpret data of ACTH binding and adenylyl cyclase stimulation. Moreover, ACTH degradation might also explain that isolated adrenal cells' response to ACTH led to cyclic adenosine 3':5'-monophosphate and corticosterone productions of which the Hill coefficient is above unity (23) since ACTH is also degraded in rat and sheep isolated adrenal cells preparation.

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