The Half-Life of Endogenous Immunoreactive ACTH in Rat Plasma[1] (36278)

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There have been no previous estimates of the half-life of endogenous ACTH in the plasma of intact rats. We have recently developed a sensitive radioimmunoassay which will measure ACTH in many species, including the rat (1). The assay was used to determine the rate of disappearance of endogenous ACTH from the plasma of acutely hypophysectomized rats.

Materials and Methods. Male Sprague-Dawley rats weighing approximately 200 g were fed Purina Chow *ad libitum* and maintained in a 24° room with 12 hr of light beginning at 6 AM for several days before performing the experiments. Between 10 and 11:30 AM the rats were hypophysectomized by the parapharyngeal approach under ether anesthesia which was continued until sacrifice. The pituitary was removed by suction within a few seconds of exposing the gland. Beginning 30 sec after removal of the pituitary, blood was withdrawn from the aorta into a heparinized polystyrene syringe for exactly 20 sec. Similar collections of blood were made 2.5, 4.5, 8.5, or 16.5 min after hypophysectomy in other rats. There were 4–8 animals per group and blood was taken from an animal at only one interval. The syringes containing the samples (2–4 ml) were immediately immersed in crushed ice and samples were centrifuged at 5° and 2500 rpm for 20 min. The plasma was transferred to polystyrene tubes, stored at —20° and assayed for ACTH within a few days. The completeness of hypophysectomy was ascertained by examination of the hypophyseal fossa with a 10× dissecting microscope.

ACTH was measured by a radioimmunoassay employing $a_p{}^{1\text{-}39}$ ACTH (III International Working Standard) as a standard and also for the labeled hormone employed in the assay as described elsewhere (1). The antibody employed was produced in the rabbit by immunization with synthetic $a^{1\text{-}24}$ ACTH. It does not cross react with a-MSH or β_p-MSH. The results of the assay are expressed on a gravimetric rather than unit basis. The III International Working Standard of ACTH is supplied in ampules containing 5 International Units (IU) of "approximately 50 μg" purified porcine ACTH. For the purpose of this study, each ampule was considered to contain exactly 50 μg. The results can be converted to the unit scale by the conversion factor of 100 U/mg. The average limit of sensitivity of the assay is approximately 16 pg/sample. If extraction is employed (1) plasma ACTH concentration can be measured reliably in samples if the concentration exceeds approximately 20 pg/ml. A close similarity between bioactivity and immunoreactivity of plasma ACTH in the rat has previously been found with this assay (1).

The ACTH concentration from the plasma of individual rats was determined for the first three intervals of sampling and the plasma from two rats was pooled for each determination at the two later intervals. Similar results were obtained from two separate experiments. The data from both were, therefore, pooled. Statistical evaluation of the ACTH values was made through logit transformation. The half-life of ACTH in plasma was calculated by least-squares analysis.

Results and Discussion. The ACTH concentration of the plasma at the various inter-

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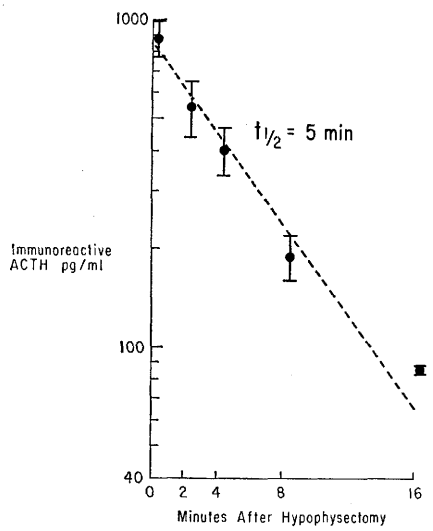

FIG. 1. Disappearance of endogenous immunoreactive ACTH from rat plasma depicted on a semilogarithmic graph. The mean value from at least four rats is plotted as closed circles and the vertical bars indicate the standard errors.

vals selected following hypophysectomy is shown in Fig. 1. A half-life of 5 min was calculated. Sixteen minutes following hypophysectomy, the mean plasma ACTH concentration was less than 100 pg/ml and approached the mean AM value of 23 pg/ml previously found in resting intact animals (1). A single exponential rate of disappearance was found over the 16-min period of the study. It is possible that observations at longer intervals after hypophysectomy would have revealed a decrease in the disappearance rate due to retention in the plasma of immunoreactive fragments of ACTH. However, the concentration of ACTH was very low 16 min after hypophysectomy. Further temporal extension of the study would have required such large amounts of plasma that we did not consider it worthwhile to explore this possibility.

Sydnor and Sayers (2) measured the disappearance of bioactive endogenous plasma ACTH in adrenalectomized rats following hypophysectomy. Their estimate of a half-life of one minute is considerably shorter than our estimate of an immunoreactive half-life of 5 min. There are two major differences between the two studies which might account for the discrepancy. We employed intact rats and measured ACTH immunoreactivity. They studied chronically adrenalectomized rats and measured ACTH bioactivity. It is possible that the half-life of ACTH is shorter in adrenalectomized animals than in intact animals, but such a comparison has not been made to our knowledge.

Previous studies by Besser *et al.* (3) of the half-lives of immunoreactive and bioactive ACTH in man provide a plausible explanation for the discrepancy. Bioactivity disappeared more quickly than immunoreactivity in their study, irrespective of the portion of the ACTH molecule toward which the immunoassay antibody was directed. Presumably, the longer immunoreactive than biological half-life was due to some difference between structural requirements for the two types of activity. If, for example, a longer amino acid sequence is required for bioactivity than immunoreactivity, the former would be expected to disappear more quickly than the latter as the ACTH molecule is metabolically degraded.

It is important to note that Besser *et al.* (3) found a longer immunoreactive half-life of ACTH in man with C-terminally than N-terminally directed ACTH antibodies. The antibody we employed was produced with the N-terminal, biologically active portion of the ACTH molecule. If the studies of Besser *et al.* in man also apply to the rat it might be expected that the half-life we obtained resembles the biological half-life more closely than would be expected with a C-terminally directed antibody.

Despite differences between the immunoreactive and bioactive half-life of ACTH, the short half-life of immunoreactive ACTH in the rat permits studies of rapid changes of ACTH secretion. We have found that 2.5 min of exposure to ether produced a 50-fold rise in plasma ACTH concentration of intact male rats. This concentration decreased to the range obtained in "resting" rats within 40 min after beginning the ether stress (1).

Summary. A radioimmunoassay for ACTH in the rat, which has previously been shown to provide close agreement with bioassay, was used to determine the disappearance rate of

ACTH from the plasma of male rats following hypophysectomy under ether anesthesia. The calculated half-life of the immunoreactive ACTH was 5 min.

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