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RAPID COMMUNICATIONS

REDUCED ABILITY OF OLD MALE RATS TO RELEASE ACTH AND CORTICOSTERONE IN RESPONSE TO CRF ADMINISTRATION

HYLKA, V.W., SONNTAG, W.E., AND MEITES, J.[1]

Department of Physiology and Biophysics
Michigan State University, East Lansing, Michigan 48824

A single injection of oCRF at either 2 or 15 ug/kg BW was given to old (19-21 mo) and young (4 mo) male rats, and plasma ACTH and corticosterone (CORT) were measured. Both doses of CRF caused a significant increase in plasma ACTH by 5 min in both age groups ($p<0.01$), but young rats had significantly greater ACTH levels by 25 min after injection ($p<0.05$) compared with old. Plasma CORT in both age groups was significantly elevated ($p<0.01$) above preinjection values by 5 min after either dose of CRF. However at 5, 15, and 25 min, young animals had significantly greater levels of CORT ($p<0.05$) compared with old. These results indicate that old male rats have a diminished capacity to secrete ACTH in response to oCRF; this decreased sensitivity may be responsible for decreased release of ACTH and CORT observed in old rats in response to stress.

Introduction

As rodents age, they are less capable of secreting hormones in response to stress. Old rats have been shown to exhibit significantly reduced release of immunoreactive β-endorphin (1), adrenocorticotropin (ACTH) (2), and corticosterone (CORT) (3,4) in response to ether stress when compared with young rats. The ACTH-CORT response to stress in rats is partly regulated by corticotropin-releasing factor (CRF) from the hypothalamus. The decreased response of the hypothalamo-pituitary system in old rats to stress could be due to reduced release of CRF or to diminished response of the pituitary to CRF stimulation. The aim of the present study was to compare the ability of synthetic ovine CRF to release ACTH and CORT in old and young rats.

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Materials and Methods

Young (4 mo) and old (19-21 mo) male Sprague-Dawley rats were kept on a 14L:10D cycle, and received food and water ad libitum.

Three days prior to blood sampling, a silastic indwelling atrial cannula was implanted into each rat through the right jugular vein. Animals were then placed into individual cages.

A stock solution of ovine CRF (oCRF; Penninsula Laboratories, Belmont, CA) in 0.1 M acetic acid was initially prepared. This was diluted in a solution containing 1 mg ascorbic and 10 mg BSA/ml in 0.9% NaCl. Diluted oCRF was injected i.v. via cannula. BSA-ascorbic acid-saline solution was used as a control.

Animals were allowed free access to food and water throughout the experiment. After a 2.5 h adaptation, 1-2 ml blood was collected and immediately replaced with an equal volume of pooled erythrocytes obtained from young animals (pooled erythrocytes were washed 5 times in sterile isotonic saline). Blood was collected on ice in poly-

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propylene tubes containing 100 ul (per ml of blood) of a solution of 0.01 M phosphate-buffered saline (PBS; pH 7.6) containing 0.25 M EDTA, 500 KIU aprotinin (Sigma), 2% mercaptoethanol (for ACTH), or into glass tubes (for CORT). Tubes were immediately centrifuged at 2,000 x g for 5 min (4°C), and plasma was decanted and frozen (-40°C). Plasma ACTH was extracted by a procedure modified from Orth (5). One ml of silicic acid suspension (200 mg/ml PBS) was added (for every 1.1 ml of plasma-PBS), shaken for 30 min (4°C) to absorb ACTH, and then centrifuged at 4,000 x g (4°C) for 5 min, and supernatant discarded. The pellet was washed twice (3 ml PBS, 4,000 x g, 4°C, 5 min), discarding the supernatant each time. ACTH was extracted twice from the silicic acid by shaking for 30 min (25°C) with 1.5 ml of acetic acid:acetone:water (2:20:80), and centrifuging at 4,000 x g (4°C; 10 min). Supernatants were decanted into glass tubes pre-coated with 1% gel, frozen on dry ice, lyophilized, and reconstituted in PBS (containing 0.25% human serum albumin and 0.1% gel) for radioimmunoassay (RIA).

Immunoreactive ACTH (IR-ACTH) was assayed using a heterologous double antibody RIA. Porcine ACTH (#10690;Serva Biochemicals, Heidelberg) was iodinated using chloramine-T, purified on quso microsilica quartz, and eluted with acetic acid:acetone: water (2:40:58). Rabbit anti-porcine ACTH (#851, NIAMDDK) was used at a dilution of 1:6,000. Porcine ACTH was also used as standard. Second antibody was sheep anti-rabbit serum #435, a generous gift from Dr. John Lu.

Displacement of ^{125}I-ACTH by rat pituitary homogenates, extracted rat plasma, and porcine ACTH were parallel and dose-related. Sensitivity of the assay was 7 pg/tube, with 50% displacement of tracer at 220 pg. Percent recovery of ACTH was 75-85% (estimated by the recovery of 400 or 1000 pg porcine ACTH added to rat serum). Intra- and inter-assay coefficients of variation were 3.0% and 12.2%, respectively.

The CORT assay was that of Gomez-Sanchez et al. (7), using anti-CORT provided by Dr. Gordon Niswender (Colorado State University). Intra- and inter-assay coefficients of variation were 3.99% and 6.55%, respectively.

Repeated measures analysis of variance followed by the Newman-Keuls' procedure, were used for statistical comparisons.

Results

Plasma IR-ACTH in old and young male rats after injection of two doses of oCRF are shown in Fig. 1. Resting

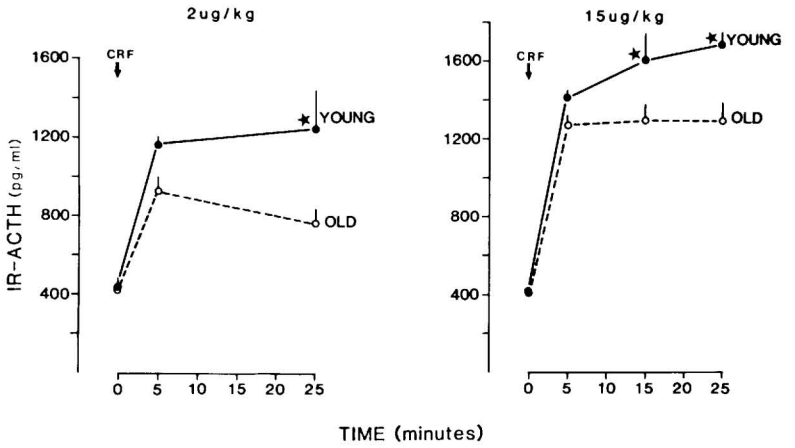

Fig. 1. Effects of two doses of oCRF on plasma IR-ACTH in young (4 mo) and old (19-21 mo) male rats. Values presented are the mean ± standard error (verticle line) of 6-7 animals. Star = $p<0.05$ compared with old. Experiment was performed twice with similar results.

levels of IR-ACTH were not different in old versus young rats. Ovine CRF significantly increased plasma IR-ACTH in both young and old male rats by 5 minutes ($p<0.01$); this increase was significantly greater in the young compared with old rats with both doses of CRF ($p<0.05$). Vehicle-injected controls showed no significant change in plasma IR-ACTH from basal levels (data not shown).

The effects of oCRF on plasma CORT in young and old male rats are shown in Fig. 2. Both doses of CRF produced significant increases in CORT by 5 minutes in old and young male rats ($p<0.01$), but the rise in plasma CORT was significantly greater in young rats ($p<0.05$) at all times after administration.

Discussion

These results show that old male rats release less IR-ACTH and CORT than young male rats in response to oCRF. Reasons for decreased ACTH and CORT response to CRF in old rats are not clear at present, but may involve a change in pituitary CRF receptor number and/or affinity on the corticotropes. Alternatively, the decreased response of old rats to CRF may involve altered cellular functions at some point past the receptor interaction. It is also possible that oCRF may be differentially metabolized in the two age groups. Our results do not preclude the possibility that during stress in old rats, the ability of the hypothalamus to secrete and release CRF may be impaired.

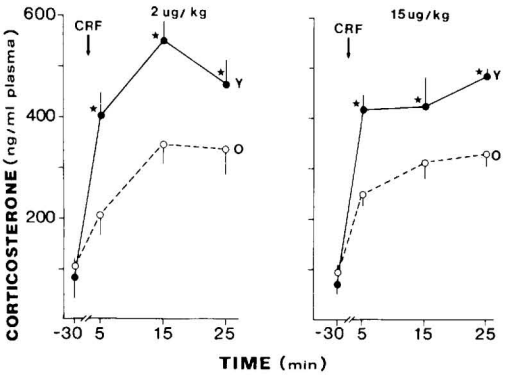

Fig. 2. Effects of two doses of oCRF on plasma corticosterone in old (19-21 mo) and young (4 mo) male rats. Values presented are mean ± standard error of 6-7 animals. Y = young; O = old; star = $p<0.05$ compared with old. Experiment was performed twice with similar results.

The lower CORT response to CRF in old rats may be due to reduced output of ACTH by the pituitary, but also to lower adrenal cortical response to ACTH. The work of Rivier et al. (7) suggests that the amount of IR-ACTH released in our experiment by old male rats in response to CRF was more than sufficient to stimulate maximum CORT output. Rivier et al. (7) showed that increasing doses of oCRF caused dose-related increases in ACTH, but increases in CORT that were identical. Similar results were obtained in our experiment for both old and young rats. Thus, decreased capacity of old rats to release CORT may be due to a reduced ability of old adrenals to respond to ACTH. Several investigators have reported that the adrenals of old rats released less CORT in response to ACTH administration in vivo (2,4) and in vitro (8,9). However, these results are controversial (10). Another possibility is that IR-ACTH secreted from the pituitaries of old male rats may be less active biologically than that secreted by pituitaries of young males. Although there are no data at present to support such a possibility for ACTH, altered biological activity of TSH (11) and LH (12) has been reported in aging rats.

Our results show that basal levels of plasma ACTH and CORT were not different in the two age groups. The observations on CORT are in agreement with others (2,4) who also found no differences in resting levels of CORT between old and young rats. However, some investigators have reported differences in basal secretion of ACTH (2) and CORT (3).

Our results may explain, in part, the reports that the ACTH-CORT response to stress is reduced in old rats. The attenuated rise in ACTH and β-endorphin during stress in old rats (1,2,3) could be due to alterations that develop in hypothalamic-pituitary function. The present results suggest that part of the decreased ability of old rats to respond to stress with increased ACTH-CORT output is due to reduced pituitary response to CRF.

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