Pharmacology Biochemistry and Behavior, Vol. 46, pp. 101–109, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Neuroendocrine and Neurochemical Responses to Novelty Stress in Young and Old Male F344 Rats: Effects of *d*-Fenfluramine Treatment

ROBERT J. HANDA,*[1] MOLLY K. CROSS,* MAGDELENA GEORGE,† BEN H. GORDON,‡
LLOYD H. BURGESS,* THERESA M. CABRERA,† NORIO HATA,†
D. BRUCE CAMPBELL‡ AND STANLEY A. LORENS†

*Departments of *Cell Biology, Neurobiology and Anatomy and †Pharmacology, Loyola University, Chicago, Stritch School of Medicine, 2160 South First Avenue, Maywood, IL 60153*
‡Servier Research and Development, Fulmer Hall, Windmill Road, Slough SL3 6HH, UK

Received 20 October 1992

HANDA, R. J., M. K. CROSS, M. GEORGE, B. H. GORDON, L. H. BURGESS, T. M. CABRERA, N. HATA, D. B. CAMPBELL AND S. A. LORENS. *Neuroendocrine and neurochemical responses to novelty stress in young and old male F344 rats: Effects of* d*-fenfluramine treatment.* PHARMACOL BIOCHEM BEHAV **46**(1) 101–109, 1993.—To understand some of the mechanisms underlying the neuroendocrine and neurochemical changes associated with aging, we administered the serotonin [5-hydroxytryptamine (5-HT)] releaser and reuptake inhibitor *d*-fenfluramine (*d*-FEN; 0.0, 0.2, or 0.6 mg/kg/day, p.o) for 30–38 days to young (4 months) and old (22 months) F344 male rats. Rats were stressed by placement into a novel open field (OF) for 20 min before sacrifice. Control animals were sacrificed immediately upon removal from their home cage (HC). Old rats exhibited less ($p < 0.05$) exploratory behavior than young rats, which was not altered by treatment with *d*-FEN. Old HC rats also had higher ($p < 0.05$) basal plasma levels of adrenocorticotropic hormone (ACTH) and prolactin (PRL) than young HC rats. Old OF rats showed higher ($p < 0.05$) levels of ACTH and corticosterone (CORT) than young OF animals. A stress-induced increase in PRL secretion was not observed in old rats. Subchronic low-dose *d*-FEN normalized the enhanced ACTH and CORT responses of old animals to novelty. In addition to these endocrine changes, stress-induced increases in medial frontal cortex (MFC) dopamine (DA) and norepinephrine (NE) turnover also were observed. The increase in NE turnover was greater ($p < 0.01$) in old than in young rats. *d*-FEN treatment blocked the stress-induced increase in MFC NE but not MFC DA turnover in both young and old rats. These data support a role for 5-HT and/or NE in some age-related neuroendocrine perturbations and suggest that increased 5-HT neurotransmission can normalize the hyperactivation of the hypothalamo-pituitary-adrenal axis of old male rats.

Aging ACTH Corticosterone Dopamine Exploratory behavior *d*-fenfluramine
Medial frontal cortex Male F344 rat Norepinephrine Prolactin Serotonin Stress

AGE-RELATED changes in anterior pituitary hormone secretion are well documented in the rat (3,4,8–10,15–17,20, 24,27,32–34). These changes include, but are not restricted to, altered basal secretion of adrenocorticotropic hormone [ACTH; (32,34)], corticosterone [CORT; (15)], and prolactin [PRL; (9,10,20)] and increased secretion of CORT following stress (3,19,25). The elevated levels of CORT in aging male rats have been suggested as causing a variety of pathologic conditions including decreases in muscle mass, decreased glucose tolerance (17,37), and hippocampal neuronal death (8, 15–17,28,29).

Some factors responsible for the age-related changes in hypothalamo-pituitary-adrenal (HPA) axis function have been described. One major deficit leading to hyperactivation of the HPA axis is an age-related loss of hippocampal CORT receptors, thus resulting in an inability to feedback regulate anterior pituitary ACTH secretion (27).

Recent studies have also shown changes in 5-hydroxytryptamine (5-HT) content and turnover within certain brain regions of aged male rats (13,19,31,35). 5-HT has been shown to be a central factor involved in regulating anterior pituitary ACTH and PRL release (1,5,11,36). Acute administration of 5-HT-releasing agents (30,36) or receptor agonists (20) will cause a dose-dependent increase in plasma ACTH and CORT, suggesting a stimulatory role for 5-HT in HPA function. Together, these reports suggest that central 5-HT changes may be responsible for alterations in the HPA axis during senescence. However, functional studies implicating the involvement of

[1] To whom requests for reprints should be addressed.

5-HT in age-related changes in HPA function have yet to be reported.

In addition, we recently reported that subchronic in vivo administration of low doses (0.6–1.8 mg/kg/day, p.o) of the 5-HT releaser and reuptake inhibitor *d*-fenfluramine (*d*-FEN) can increase some parameters of immune function when measured in vitro (7,23). Because CORT, ACTH, and PRL (2,22) have all been demonstrated as having immunomodulatory properties, we examined the effects of *d*-FEN on hormone secretion in young and old male rats in hopes of determining if endocrine alterations were potentially responsible for the observed changes in immune function. Anterior pituitary hormone secretion was stimulated using a mild psychological stressor, placement into a novel environment. This stressor has previously been used to activate the HPA axis (3,21).

METHOD

Animals

Male Fischer 344 (F344) rats were obtained from the NIA colony at Harlan-Sprague-Dawley (Indianapolis, IN). Animals arrived at 3.5 (n = 54) or 21.5 (n = 54) months of age. Animals were housed individually and allowed to adapt to animal facilities at Loyola University for 2 weeks. Animals were maintained on a 12 L : 12 D cycle (light on at 0700 h) in a temperature (20–22°C)- and humidity (50–55%)-controlled environment according to the guidelines of the American Association for the Accreditation of Laboratory Animal Care.

Procedure

Animals received *d*-FEN HCl (0.2 or 0.6 mg/kg body weight/day, p.o.; Servier, France) in their drinking water for 30–38 days prior to sacrifice. These doses were chosen because we have previously shown that they will alter some immune parameters in F344 rats (7). Control animals had ad lib access to unadulterated deionized water. All rats had ad lib access to Purina Rat Chow. Body weights were recorded every 3 days and 24-h fluid intake was determined daily. The concentration of *d*-FEN in the drinking water was adjusted accordingly.

On the day of sacrifice, animals were rapidly decapitated between 0900 and 1200 h either immediately upon removal from the home cage (HC) or 20 min after being placed in a novel environment (open field, OF; novelty stress). Trunk blood was collected into polypropylene tubes containing 0.3 ml 3 M EDTA and 1,000 KIU trasylol (Sigma Chemical Co., St. Louis, MO). Whole blood was centrifuged at 1,500 × g for 15 min and plasma was removed and frozen at −70°C until assayed for hormone levels by radioimmunoassay (RIA). Brains were removed from the skull and dissected over ice. The medial frontal cortex (MFC) and rostral 4.0 mm of the suprarhinal dorsolateral frontal cortex (DLFC) were obtained, frozen on dry ice, and stored at −70°C until assayed, respectively, for monoamines and their metabolites and *d*-FEN and *d*-norfenfluramine (*d*-norFEN) levels. Carcasses were examined for any gross pathologic changes and any animals with evidence of pathology were dropped from the study.

Behavioral Analysis

Prior to sacrifice, some animals were placed in a novel environment and their behavior quantitated for 20 min. The novel environment apparatus measured 100 × 100 × 40 cm. The floor was painted flat white and divided into nine squares (20 cm^2) by thin black lines. Four equidistantly spaced holes (3.5 cm diam.) were located in the four corner squares of the central nine squares. The open field was located in a sound-attenuated dark room and illuminated by a 40-W bulb positioned 100 cm over the center of the chamber as well as a 20-W fluorescent bulb located under the elevated floor of the chamber. Animals were placed in the middle of the chamber and allowed to roam free for 20 min prior to sacrifice. Behavior in the open field was videotaped for later analysis. The following parameters were analyzed: a) number of wall and center squares entered; b) number of nose pokes (rat inserts its snout into one of the holes); c) number of rears (rat stands on hind legs and sniffs); and d) number of fecal boli excreted.

Hormone Assays

Plasma levels of ACTH, PRL, and CORT were determined in plasma samples by RIA using previously described procedures (6,19). For all hormone RIAs, all samples were run in a single assay to prevent interassay variance from biasing the data. Intraassay variance was 6% for ACTH, 3% for CORT, and 4% for PRL.

d-FEN Levels

The DLFC concentrations of *d*-FEN and its active metabolite, *d*-norFEN, were assayed as detailed by Richards et al. (25).

Neurochemical Levels

The concentrations of dopamine (DA), dihydroxyphenylacetic acid (DOPAC), norepinephrine (NE), 3-methoxy-4-hydroxyphenylglycol (MHPG), homovanillic acid (HVA), 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) were measured in the MFC using high-performance liquid chromatography with electrochemical detection according to previously described methods (19).

Data Analysis

Data reduction and statistical analysis were performed using the PC analysis of variance (ANOVA) statistical package (Version 1.0; Human Systems Dynamics, Northridge, CA). The hormonal and neurochemical data were analyzed by a three-way ANOVA (age × drug × stress) followed when appropriate by a Newman-Keuls multiple-range test for posthoc comparisons (38). The drug concentrations and open-field behavioral data were analyzed by a two-way ANOVA (age × drug) and the body weight and fluid intake measures by two-way ANOVA (age × drug) with repeated measures across time.

RESULTS

Animals

Upon arrival, all animals appeared healthy. Autopsy of young animals revealed no gross pathology. In contrast, pituitary, trunk, or intestinal masses were found at necropsy in 7 of 54 old rats (13%). These animals were eliminated from the study.

Significant age, $F(1, 94) = 70.0$, $p < 0.0001$, time, $F(9, 846) = 6.2$, $p < 0.0001$, and age × time, $F(9, 846) = 101.2$, $p < 0.0001$, effects on body weight were found. No significant effects of *d*-FEN were noted. Over the course of these studies, young animals, which weighed less than old animals at the outset, gained weight whereas old animals lost weight (Table 1). Consequently, by the end of these experiments there

TABLE 1

EFFECTS OF SUBCHRONIC *d*-FEN ON MEAN (±SEM) BODY WEIGHTS AND FLUID INTAKES OF YOUNG (5 months) AND OLD (23 months) MALE F344 RATS

Group Age/Dose (mg/kg)	*n*	Body Weight (g)		Fluid Intake (ml/24 h)	
		Day 1	Day 30	Day 1	Day30
Young					
0.0	18	352 ± 6	382 ± 5	29 ± 2	30 ± 1
0.2	18	356 ± 6	383 ± 6	30 ± 1	30 ± 1
0.6	18	355 ± 5	383 ± 5	28 ± 1	30 ± 1
Old					
0.0	15	415 ± 6	400 ± 5	24 ± 2	29 ± 1
0.2	15	420 ± 5	403 ± 4	24 ± 1	26 ± 1
0.6	17	411 ± 4	394 ± 6	25 ± 1	31 ± 2

was no difference in body weight between young and old animals.

Significant age, $F(1, 95) = 12.9$, $p < 0.001$, time, $F(9, 855) = 11.3$, $p < 0.0001$, and age × time, $F(9, 855) = 2.2$, $p < 0.02$, effects on fluid intake were observed. No significant effects of *d*-FEN were seen. The fluid intakes of young rats remained constant during the drug treatment phase (Table 1). Although old rats drank significantly less fluid than young animals at the start of the study, they increased their fluid intakes during the drug treatment phase such that no age differences were apparent by the time of sacrifice (Table 1).

Drug Concentrations

Significant age, $F(1, 64) = 33.1$, $p < 0.001$, dose, $F(1, 64) = 107.7$, $p < 0.001$, and age × dose, $F(1, 64) = 33.1$, $p < 0.0001$, effects were found for frontal cortical levels of the bioactive metabolite of *d*-FEN, *d*-norFEN. Posthoc analysis revealed that the neocortical levels of *d*-norFEN were dose dependent and higher in old rats (Fig. 1). Importantly, the concentrations of *d*-norFEN in young rats receiving the 0.6-mg/kg dose of *d*-FEN were equivalent to those in old rats administered the 0.2-mg/kg dose. Brain levels of the parent compound, *d*-FEN, were not detectable.

Open-Field Behavior

Significant decreases in exploratory behavior were detected in old animals (Table 2). Significant age effects, $F(1, 44) = 10.3$–13.0, $p < 0.001$, were seen in the number of squares entered, nose pokes, and fecal boli. No significant drug or drug × age interaction effects were noted. No significant effects on rearing were observed. Only data from young and old vehicle groups are presented in Table 2 for the sake of clarity.

Hormone Measurements

ACTH. Significant age, $F(1, 83) = 12.0$, $p < 0.001$, stress, $F(1, 83) = 29.4$, $p < 0.0001$, drug, $F(2, 83) = 4.1$, $p < 0.02$, and age × drug interaction, $F(2, 83) = 3.6$, $p < 0.03$, effects on plasma ACTH were detected. ACTH levels in old HC rats were significantly greater than in young HC rats (Fig. 2). *d*-FEN treatment had no effect on HC values. Exposure (20 min) to the OF led to significant increases in plasma ACTH in young and old vehicle-treated rats, with the ACTH titers in old rats being significantly greater than in young animals. *d*-FEN treatment did not alter the ACTH levels of young OF males but normalized the hypersecretion of ACTH in old OF males to that of young OF males.

Corticosterone. Significant stress, $F(1, 84) = 345.3$, $p < 0.0001$, effects on plasma CORT were observed. No age, drug, or interaction effects were found. There were no treatment group differences in HC CORT levels. OF stress led to a substantial increase in plasma CORT levels that was greatest in the old vehicle-treated group (Fig. 3). *d*-FEN treatment did not alter the CORT response to stress in young animals but significantly ($p < 0.05$) decreased the CORT response in old rats.

Prolactin. Significant, $F(1, 79) = 10.7$–54.9, $p < 0.002$, age, stress, and age × stress effects on plasma PRL levels were observed. Posthoc analysis revealed that basal PRL lev-

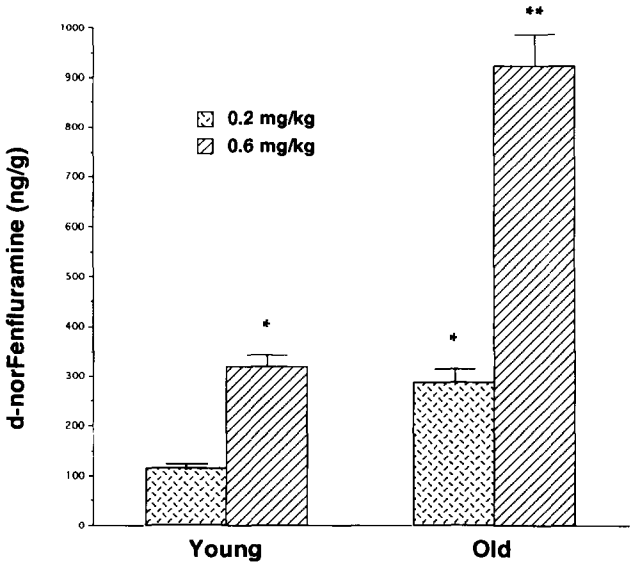

FIG. 1. *d*-Norfenfluramine levels (mean ± SEM ng/g, $n = 15$–18/group) in the dorsolateral frontal cortex of young (5 months) and old (23 months) male F344 rats administered *d*-fenfluramine in their drinking water (0.2 or 0.6 mg/kg/day) for 30–38 days. *Significantly ($p < 0.05$) greater than young 0.2-mg/kg/day group. **Significantly ($p < 0.01$) greater than the other three groups.

TABLE 2

DIFFERENCES IN THE EXPLORATORY BEHAVIOR OF YOUNG (5 months) AND OLD (23 months) F344 MALE RATS

Group Age	*n*	Total Squares	Rears	Nose Pokes	Fecal Boli
Young	9	40* ± 14	19 ± 5	4* ± 1	1* ± 1
Old	8	12 ± 10	13 ± 2	2 ± 1	4 ± 1

Data presented as group mean ± SEM.

*Significant ($p < 0.05$) group differences (ANOVA followed by Newman-Keuls' test).

els were higher in old animals and that novelty stress increased PRL levels in young but not in old rats (Fig. 4). No significant effects of *d*-FEN were observed.

Neurochemistry

Serotonin. No significant age, drug, treatment, or interaction effects were observed on MFC 5-HT and 5-HIAA levels or on 5-HIAA/5-HT ratios, an estimate of 5-HT turnover. The MFC 5-HT and 5-HIAA concentrations (mean ± SEM ng/g) of vehicle-treated HC animals were: young, 5-HT = 1,123 + 53, 5-HIAA = 967 + 62; old, 5-HT = 1,132 + 11, 5-HIAA = 1,083 + 43.

Norepinephrine. Significant drug, $F(2, 65) = 9.1$–28.0, $p < 0.0006$, stress, $F(1, 65) = 23.0$–69.3, $p < 0.0001$, and drug × stress, $F(2, 65) = 6.3$–20.9, $p < 0.003$, effects on NE and MHPG levels as well as on MHPG/NE ratios were found. In addition, a significant age effect was observed on MHPG concentrations, $F(1, 65) = 10.0$, $p < 0.003$, and MHPG/NE ratios, $F(1, 65) = 4.9$, $p < 0.03$, as well as a significant age × drug × stress interaction for NE levels, $F(2, 65) = 3.6$, $p < 0.03$. Posthoc analysis showed that all of these effects could be ascribed to significant differences between vehicle- and drug-treated stressed rats. In addition, the MFC MHPG/NE ratio for old vehicle OF rats was significantly ($p < 0.01$) greater (23%) than in young vehicle OF rats (Fig. 5).

The marginal means (ng/g) for vehicle HC and vehicle OF young and old groups were: HC-NE = 331, MHPG = 251; OF-NE = 252, MHPG = 313. Thus, both young and old

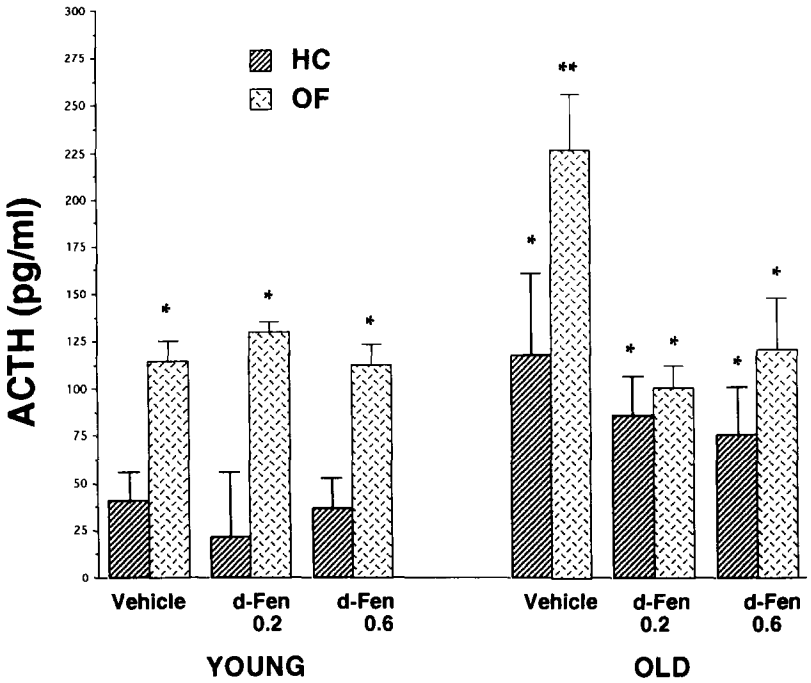

FIG. 2. Plasma adrenocorticotropic hormone (ACTH) titers (mean ± SEM pg/ml, $n = 6$–9/group) in young (5 months) and old (23 months) male F344 rats administered vehicle (deionized water) and either 0.2 or 0.6 mg/kg/day *d*-fenfluramine (*d*-FEN) in their drinking water for 30–38 days. Animals were killed and blood samples taken either immediately upon removal from the home cage (HC; hatched bars) or 20 min following exposure to a novel open field (OF; stippled bars). *Significantly different from young HC groups ($p < 0.05$). **Significantly different from all other groups ($p < 0.05$).

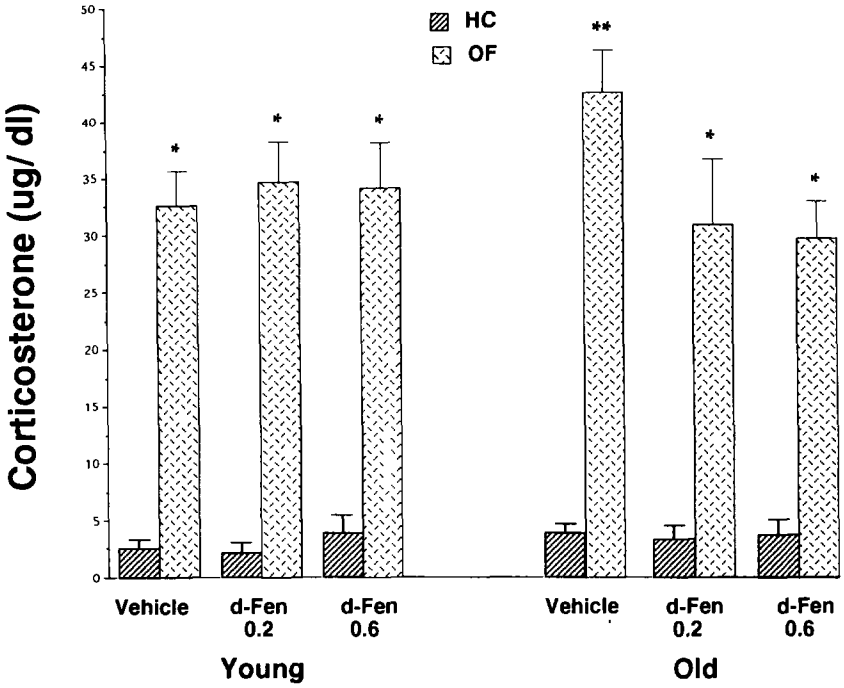

FIG. 3. Plasma corticosterone titers (mean ± SEM μg/dl, n = 6–9/group) in young (5 months) and old (23 months) male F344 rats. For details, see Fig. 2.

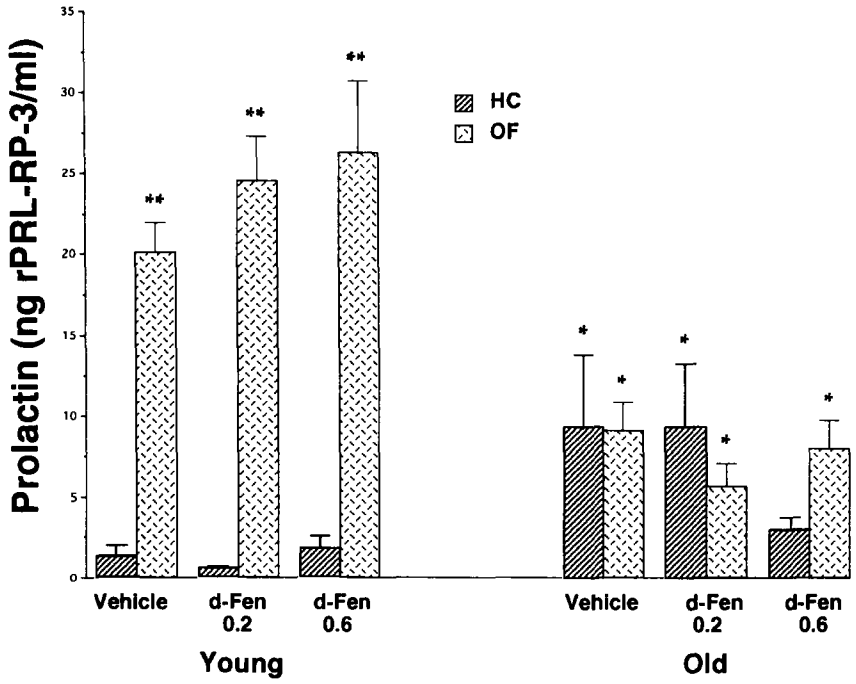

FIG. 4. Plasma prolactin titers (mean ± SEM ng/ml, n = 6–9/group) in young (5 months) and old (23 months) male F344 rats. For details, see Fig. 2.

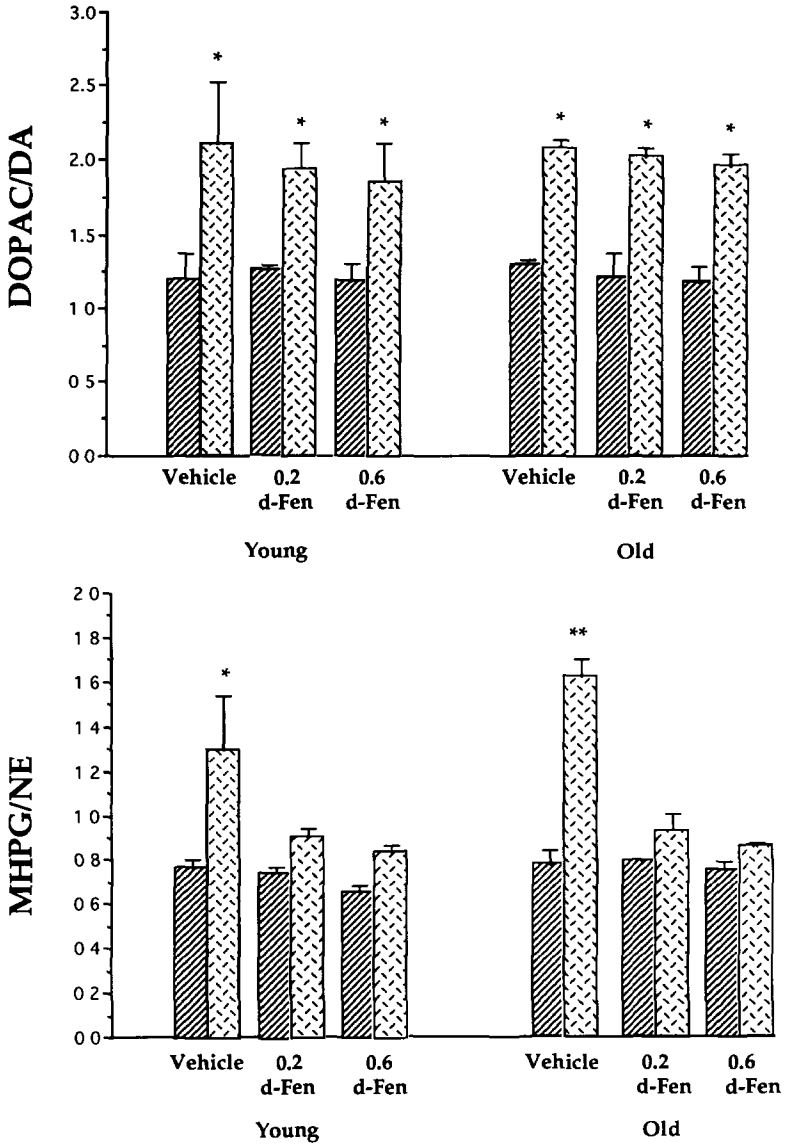

FIG. 5. Medial frontal cortex dihydroxyphenylacetic acid (DOPAC)/dopamine (DA) and MHPG/norepinephrine (NE) ratios (mean ± SEM, n = 6-9/group) in home cage (HC) control (hatched bars) and stressed (stippled bars) young (5 months) and old (23 months) male F344 rats administered *d*-fenfluramine (*d*-FEN; 0.0, 0.2, or 0.6 mg/kg, p.o.) in their drinking water for 30-38 days. *Significantly ($p < 0.01$) different from all HC groups (DOPAC/DA) and all other groups except old male vehicle HC group (MHPG/NE). **Significantly ($p < 0.01$) greater than all other groups.

OF-stressed rats showed an increased NE turnover (MHPG/NE ratio) that was blocked by *d*-FEN treatment (Fig. 5).

Dopamine. Significant stress effects, $F(1, 65) = 10.8$-70.1, $p < 0.002$, were observed on all the parameters of DA metabolism measured: DA, DOPAC, and HVA levels, as well as DOPAC/DA and HVA/DA ratios. No significant age, drug, or interaction effects were observed. The marginal means (ng/g) for HC and OF rats were: HC-DA = 88, DOPAC = 104, HVA = 102; OF-DA = 75, DOPAC = 147, HVA = 115. Posthoc analysis confirmed that the stressed groups differed significantly from HC rats. Thus, exposure (20 min) to the OF led to a significant increase in DA turnover (DOPAC/DA ratio) in both young and old rats, which was not affected by *d*-FEN treatment or age (Fig. 5).

DISCUSSION

In the present study, we have shown that significant changes in the hormonal response to novelty stress occur in the aging male rat. These changes include an increased response in ACTH and CORT secretion and a decreased response in PRL release. In addition to these age-related

changes in anterior pituitary responses to novelty stress, we also observed that subchronic treatment with the 5-HT releaser and reuptake inhibitor *d*-FEN will prevent the age-related changes in the ACTH and CORT responses to stress.

In contrast, by examining the neurochemical response of a nonrelated, nonneuroendocrine tissue, the medial frontal cortex, to stress, we were able to determine that age-related changes and the effects of *d*-FEN are specific to neuroendocrine function. There were no age-related changes in the MFC DA or 5-HT response to novelty. Although there were age-related changes in MFC NE turnover, these were not altered by *d*-FEN treatment.

Our data concerning age-related changes in the CORT response to stress are consistent with previous reports showing elevated and prolonged secretion of CORT following immobilization (27), exposure to a novel environment (3), ether (19), and a conditioned emotional response paradigm (19). These elevated levels of CORT in aged male rats are believed to be responsible for hippocampal cell death (15–17,29) as well as other pathologic conditions (17).

Decreases in hippocampal CORT receptors coincident with elevations in CORT secretion suggest a deficit in the negative feedback regulation of the HPA axis in aging male rats (27). Other studies have shown changes in adrenal sensitivity to ACTH in senescent male rats, suggesting a peripheral mechanism responsible for the age-related changes in CORT secretion (32). The fact that we detected no age-related changes in basal CORT secretion but age-related increases in basal ACTH also suggests changes in adrenal sensitivity to ACTH can occur with age. However, our observation that basal and stimulated ACTH titers are elevated in the senescent male rat shows that age-related changes in the regulation of HPA function are probably of central origin.

It is well established that *d*-FEN is a potent releaser of 5-HT as well as an inhibitor of 5-HT reuptake (12). Because 5-HT is involved in the release of ACTH (5) and PRL (1), we subjected animals to subchronic low-dose *d*-FEN therapy to determine if age-related changes in ACTH could be related to changes in 5-HT activity. Although acute treatment with *d*-FEN will directly stimulate CORT and PRL secretion, *d*-FEN does not stimulate CORT or PRL secretion when given in a subchronic regimen (30). In the present study, subchronic *d*-FEN treatment reduced the poststress levels of ACTH and CORT in old male rats only, suggesting that changes in 5-HT availability might be a central factor underlying the age-associated hormonal changes. The fact that *d*-FEN treatment completely abolished the ACTH response to novelty of old rats, whereas it decreased but did not abolish the CORT response, is most probably a consequence of our experimental design. The increases in CORT most probably reflect earlier changes in ACTH that were not demonstrated in these studies using a single timepoint following the stressor. Indeed, our ongoing studies (unpublished) have shown maximal increases in ACTH occur 10 min following introduction to the novel environment whereas CORT levels are maximal after 20 min.

On the other hand, we did not observe any age-related differences or any *d*-FEN-induced alterations in MFC 5-HT metabolism. These observations suggest that the normalization of ACTH and CORT responses to stress in old male rats treated with *d*-FEN are not related to changes in MFC 5-HT turnover. However, this does not eliminate drug-induced alterations 5-HT turnover or receptor densities in other brain areas, such as the hypothalamus. Whether *d*-FEN acts by normalizing the negative feedback mechanism or the hypothalamic stimulation of ACTH remains to be determined.

In addition to age-associated changes in ACTH and CORT, significant differences in the pattern of PRL secretion were detected in old male rats introduced to the open-field arena. Prolactin increases were observed following novelty stress in young animals. Old animals showed increases in basal PRL secretion consistent with previous reports (9); however, increases in PRL were not present following the novelty stress. An age-associated decrease in the PRL response to restraint stress has previously been reported in female rats (10). The fact that the PRL response to novelty stress was absent in aged males is similar to data recently reported by Briski and Sylvester (4), who found that PRL bioactivity following novelty stress was lower in aged male rats. Because dopamine has been shown to be a powerful inhibitor and 5-HT a stimulator of PRL release (1), it is possible that age-associated changes in the dopaminergic or serotonergic systems could underlie this phenomenon. However, chronic *d*-FEN treatment had no effect on PRL secretion in young or old animals, possibly ruling out changes in the serotonergic system. It is also possible that age-related increases in CORT could be inhibiting the PRL response because previous studies have shown that chronic exposure to dexamethasone will inhibit the PRL response to restraint stress (18).

The presence of *d*-FEN effects on ACTH and CORT secretion in old animals and not in young animals can perhaps be explained in part by *d*-norFEN levels in brain tissue. *d*-norFEN levels were significantly greater in old males as compared to young males when given equivalent dosages, suggesting that *d*-FEN metabolism is reduced in old animals such that higher levels of the active metabolite can accumulate. However, observable changes in ACTH and CORT secretion were detected in old animals given the 0.2-mg/kg dose and not in young animals given the 0.6-mg/kg dose, even though concentrations of frontal cortex *d*-norFEN levels were similar. Consequently, these data suggest that chronic *d*-FEN treatment is only effective in modulating HPA function following age-associated perturbations of the system.

We have also shown a decrease in exploratory behavior in old male rats when introduced to an open-field arena. This age-related decrease in exploratory behavior is consistent with the elevated CORT and ACTH response, suggesting an increased stress response in old male rats. However, even though we detected significant effects of *d*-FEN therapy on age-related changes in hormone secretion there were no effects of *d*-FEN treatment on open-field behavior. These data show that *d*-FEN treatment is not acting to alter motor responses nor is it acting to alter the way aged animals perceive novelty.

Consistent with previous studies (19,22,26) using a variety of stressors, we have shown that the MFC DOPAC/DA ratio and the MFC MHPG/NE ratio, but not the MFC 5-HIAA/5-HT ratio, are increased following novelty stress. Most probably, these neurochemical changes in the MFC are not directly related to the changes in stress hormone secretion that we observed. Rather, changes in MFC neurochemistry represent a nonneuroendocrine measure of stress responsiveness. Similar to our findings of age-related changes in hormone secretion, age-related increases in the MHPG/NE but not the DOPAC/DA ratios were noted. *d*-FEN did not alter DA turnover in the MFC, consistent with its effects on behavioral parameters in these same animals. Interestingly, *d*-FEN treatment did significantly decrease MFC NE turnover in both young and old rats, which suggests a functional interaction between the serotonergic and noradrenergic systems that innervate the MFC. The nature of this interaction remains to be elucidated.

In a previous study, we demonstrated that *d*-FEN given in

a similar paradigm caused significant increases in several in vitro immune parameters of young but not old F344 males (7). Because acute *d*-FEN treatment will increase secretion of immunomodulatory hormones such as ACTH, CORT, and PRL (2,22), we hypothesized that the observed changes could be a consequence of an endocrine–immune interaction. Based upon the present results showing no effect of *d*-FEN on hormone secretion in young males, it is improbable that *d*-FEN-induced changes in immune function are a consequence of alterations in hormone secretion.

In summary, the data presented here show that the hormonal response following introduction to a novel environment is altered with age in a pattern unique to the hormone examined. Chronic treatment with *d*-FEN can ameliorate some of the age-associated changes occurring in the HPA axis but not for PRL. These data implicate 5-HT as playing a modulatory role in the age-associated changes in HPA function in the male rat.

ACKNOWLEDGEMENT

This research was supported in part by a grant from the Institut de Recherches Internationales Servier (France), and a preliminary report was presented at the 20th Annual Meeting of the Society for Neuroscience, St. Louis, MO, 1990.

REFERENCES

1. Ben-Jonathon, N.; Arbogast, L. A.; Hyde, J. F. Neuroendocrine regulation of prolactin release. Prog. Neurobiol. 33:399–447; 1989.
2. Bernton, E. W.; Bryant, H. U.; Holaday, J. W. Prolactin and immune function. In: Ader, R. et al., eds. Psychoneuroimmunology. New York: Academic Press; 1991;403–428.
3. Brett, L. P.; Chong, G. S.; Coyle, S.; Levine, S. The pituitary adrenal response to novel stimulation and ether stress in young adult and aged rats. Neurobiol. Aging 4:133–138; 1983.
4. Briski, K. P.; Sylvester, P. W. Effects of naltrexone on stress-induced bioactive prolactin release in aging male rats. Neurobiol. Aging 12:145–149; 1991.
5. Calogero, A. E.; Bagdy, G.; Szemeredi, K.; Tartaglia, M. E.; Gold, P.; Chrousos, G. P. Mechanisms of serotonin receptor agonist induced activation of the hypothalamic–pituitary–adrenal axis in the rat. Endocrinology 126:1888–1894; 1990.
6. Carnes, M.; Lent, S.; Hazel, D. Plasma adrenocorticotropic hormone in the rat demonstrates three different rhythms within 24 h. Neuroendocrinology 50:17–25; 1989.
7. Clancy J., Jr.; Petrovic, L. M.; Gordon, B. H.; Handa, R. J.; Campbell, D. B.; Lorens, S. A. Effects of subchronic *d*-fenfluramine on splenic immune functions in young and old male and female Fischer 344 rats. Int. J. Immunopharmacol. 13:1203–1212; 1991.
8. DeKosky, S. T.; Scheff, S. W.; Cotman, C. W. Elevated corticosterone levels: Possible causes of reduces axon sprouting in aged animals. Neuroendocrinology 38:33–38; 1984.
9. Demarest, K. T.; Moore, K. E.; Riegle, G. D. Adenohypophyseal dopamine content and prolactin secretion in the aged male and female rats. Endocrinology 116:1316–1323; 1985.
10. Demarest, K. T.; Moore, K. E.; Riegle, G. D. Restraint stress decreases the neurosecretory activity of tuberoinfundibular dopaminergic neurons in young but not in aged female rats. Neuroendocrinology 45:333–337; 1987.
11. Fuller, R. W. Serotonergic stimulation of pituitary-adrenocortical function in rats. Neuroendocrinology 32:118–127; 1981.
12. Garattini, S.; Bizzi, A.; Caccia, S.; Mennini, T.; Samanin, R. Progress in assessing the role of serotonin in the control of feed intake. Clin. Neuropharmacol. 11:S8–S32; 1988.
13. Godefroy, F.; Bassant, M. H.; Weil-Fugazza, J.; Lamour, Y. Age-related changes in dopaminergic and serotonergic indices in the rat forebrain. Neurobiol. Aging 10:187–190; 1989.
14. Kaler, L. W.; Neaves, W. B. The androgen status of aging male rats. Endocrinology 108:712–719; 1981.
15. Landfield, P.; Baskin, R.; Pitler, T. Brain aging correlates: Retardation by hormonal–pharmacological treatments. Science 214: 581–584; 1981.
16. Landfield, P.; Waymire, J.; Lynch, G. Hippocampal aging and adrenocorticoids: Quantitative correlations. Science 202:1098–1102; 1978.
17. Landfield, P. W.; Sundberg, D. K.; Smith, M. S.; Eldridge, J. C.; Morris, M. Mammalian aging: Theoretical implications of changes in brain and endocrine systems during mid and late life. Peptides 1(suppl. 1):185–196; 1980.
18. Lopez-Calderon, A.; Ariznavarreta, C.; Calderaon, M. D.; Tresguerres, J. A. F.; Gonzalez-Quijano, M. I. Role of the adrenal cortex in chronic stress-induced inhibition of prolactin secretion in male rats. J. Endocrinol. 120:269–273; 1989.
19. Lorens, S. A.; Hata, N.; Handa, R. J.; Van de Kar, L. D.; Guschwan, M.; Goral, J.; Lee, J. M.; Hamilton, M. E.; Bethea, C. L.; Clancy, J., Jr. Neurochemical, endocrine and immunological responses to stress in young and old Fischer 344 male rats. Neurobiol. Aging 11:139–150; 1990.
20. Lorens, S. A.; Van de Kar, L. D. Differential effects of serotonin (5-HT_{1A} and 5-HT_2) agonists and antagonists on renin and corticosterone secretion. Neuroendocrinology 45:305–310; 1987.
21. Munck, A.; Guyre, P. M. Glucocorticoids and immune function. In: Ader, R. et al., eds. Psychoneuroimmunology. New York: Academic Press; 1991;447–474.
22. Paris, J. M.; Lorens, S. A.; Van de Kar, L. D.; Urban, J. H.; Richarson-Morton, K. D.; Bethea, C. L. A comparison of acute stress paradigms: Hormonal responses and hypothalamic serotonin. Physiol. Behav. 39:33–43; 1987.
23. Petrovic, L. M.; Lorens, S. A.; George, M.; Cabrera, T.; Gordon, B. H.; Handa, R. J.; Campbell, D. B.; Clancy, J., Jr. Subchronic *d*-fenfluramine treatment enhances the immunological competence of old female Fischer 344 rats. In: Fozard, J. R.; Saxena, P. R., eds. Serotonin: Molecular biology, receptors and functional effects. Basel, Switzerland: Birkhauser Verlag; 1991: 389–397.
24. Reigle, G. D.; Meites, J. Effects of aging on LH and prolactin after LHRH, L-dopa, methyl-dopa and stress in male rat. Proc. Soc. Exp. Biol. Med. 151:507–511; 1979.
25. Richards, R. P.; Gordon, B. H.; Ings, R. M. J.; Campbell, D. B.; King, L. J. The measurement of *d*-fenfluramine and its metabolite, *d*-norfenfluramine in plasma and urine with an application of the method to pharmacokinetic studies. Xenobiotica 19: 547–553; 1989.
26. Roth, R. H.; Wolf, M. E.; Deutch, A. Y. Neurochemistry of midbrain dopamine systems. In: Meltzer, H. Y., ed. Psychopharmacology: The third generation of progress. New York: Raven Press; 1987:81–94.
27. Sapolsky, R. M.; Krey, L. C.; McEwen, B. S. Glucocorticoid sensitive hippocampal neurons are involved in terminating the adrenocortical stress response. Proc. Natl. Acad. Sci. USA 81: 6174–6177; 1984.
28. Sapolsky, R. M.; Krey, L. C.; McEwen, B. S. Corticosterone receptors decline in a site-specific manner in the aged-rat brain. Endocrinology 114:287–292; 1984.
29. Sapolsky, R. M.; Krey, L. C.; McEwen, B. S. Prolonged glucocorticoid exposure reduces hippocampal neuron number: Implications for aging. J. Neurosci. 5:1222–1227; 1985.
30. Serri, O.; Rasio, E. Temporal changes in prolactin and corticosterone response during chronic treatment with *d*-fenfluramine. Horm. Res. 31:180–183; 1989.
31. Simpkins, J. W.; Mueller, G. P.; Huang, H. H.; Meites, J. Evidence for depressed catecholamine and enhanced serotonin me-

tabolism in aging male rats: Possible relation to gonadotropin secretion. Endocrinology 100:1672–1678; 1977.
32. Sonntag, W. E.; Goliszek, A. G.; Brodish, A.; Eldridge, J. C. Diminished diurnal secretion of adrenocorticotropin (ACTH), but not corticosterone, in old male rats: Possible relation to increased adrenal sensitivity to ACTH in vivo. Endocrinology 120: 2308–2315; 1987.
33. Steiner, R. A.; Bremner, W. J.; Clifton, D. K.; Dorsa, D. M. Reduced pulsatile luteinizing hormone and testosterone secretion with aging in the male rat. Biol. Reprod. 31:251–258; 1984.
34. Tang, F.; Phillips, J. G. Some age-related changes in pituitary–adrenal function in the male laboratory rat. J. Gerontol. 33:377–382; 1978.
35. Timiras, P. S.; Hudson, D. B.; Segall, P. E. Lifetime brain serotonin: Regional effects of age and precursor availability. Neurobiol. Aging 5:235–242; 1984.
36. Van de Kar, L. D.; Karteszi, M.; Bethea, C. L.; Ganong, W. F. Serotonergic stimulation of prolactin and corticosterone secretion is mediated by different pathways from the mediobasal hypothalamus. Neuroendocrinology 41:380–384; 1985.
37. Wexler, B. C.; McMurtry, J. P. Cushingoid pathophysiology of old massively obese, spontaneously hypertensive rats (SHR). J. Gerontol. 38:148–154; 1983.
38. Winer, B. J. Statistical principles in experimental design. New York: McGraw-Hill; 1971.