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CHRONIC ETHANOL CONSUMPTION DEPRESSES HYPOTHALAMIC-PITUITARY-ADRENAL FUNCTION IN AGED RATS

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Summary

In separate experiments, nine (n=20) and fifteen (n=12) month old rats were treated with either 6% ethanol or 12% sucrose (to balance caloric intake) in the drinking water to examine the effect of chronic ethanol consumption on the hypothalamic-pituitary-adrenal axis of aged rats. Rats were maintained on these treatment regimens for thirty days and were killed by decapitation. Blood was collected and plasma concentrations of adrenocorticotropin (ACTH) and corticosterone were determined by radioimmunoassay. Adrenal glands were cleaned, quartered and used to test *in vitro* responsiveness to ACTH. Anterior pituitary glands from all 15 month old rats and one half of the nine month old rats were collected, frozen and extracted for measurement of tissue ACTH concentration. The remaining anterior pituitary glands from the nine month old rats were challenged with corticotropin releasing hormone (CRH) to test *in vitro* responsiveness. In nine month old rats, chronic ethanol consumption decreased plasma ACTH and corticosterone ($P<0.05$). Pituitary ACTH concentrations were unchanged in treated nine month old rats, but the amount of pituitary ACTH released in response to CRH was decreased ($P<0.05$) in rats consuming ethanol. *In vitro* responsiveness of the adrenal gland to ACTH in nine month old rats consuming ethanol was unchanged ($P>0.05$). Plasma ACTH and corticosterone concentrations were also decreased in 15 month old rats chronically consuming ethanol ($P<0.05$). No differences were noted in responsiveness of the adrenal gland or in the amount of pituitary ACTH due to ethanol consumption in 15 month old rats ($P>0.05$). The results of these experiments indicate that chronic ethanol consumption decreases hypothalamic-pituitary-adrenal function in aged rats.

Alcohol abuse and dependence are serious health problems that affect approximately 10% of the adult population in the United States and contribute to at least 3% of all deaths annually (1). A recent report indicated that a history of heavy alcohol consumption was a predictor of impaired physical, psychological and social health, as well as social functioning in elderly men (2). Studies examining the effect of chronic ethanol consumption in laboratory animals have largely been limited to mature animals but have not examined the effect of chronic ethanol consumption in the aged (9 to 15 month) rat. For example, the following studies have been conducted in rats of 3 to 6 months of age. Chronic exposure to ethanol has been shown to result in elevated concentrations of ACTH due to increased CRH secretion (3) and to increase sensitivity of the anterior pituitary gland to CRH (4,5). Other researchers have shown depressed hypothalamic-pituitary-adrenal (HPA) function in response to chronic ethanol consumption. Dave and coworkers (6) found that long term consumption of ethanol decreased pituitary CRH receptor number, pituitary adenyl cyclase activity and anterior pituitary proopiomelanocortin levels. Rivier et al. (7) recently reported that basal ACTH and corticosterone

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were elevated after chronic exposure to ethanol vapors, but that the magnitude of stress-induced ACTH secretion was decreased.

Aging is also known to activate the hypothalamic-pituitary-adrenal axis. Basal concentrations of ACTH and corticosterone increase with advancing age (8-12). Responsiveness to stress does not appear to be influenced by aging (12,13). However, aged rats are less sensitive to dexamethasone suppression of plasma corticosterone (13) and plasma corticosterone remains elevated for a longer time period following exposure to a stressor (12). These observations suggest that corticosterone is less capable of regulating the hypothalamic-pituitary-adrenal axis with increasing age, a response similar to what is caused by chronic ethanol consumption. The objective of this study was to examine the effect of chronic ethanol consumption on the hypothalamic-pituitary-adrenal axis of aged (9 and 15 month old) rats.

Methods

Two separate experiments were conducted using aged male Sprague-Dawley rats. Rats (9 and 15 months) were obtained from Charles River, Portage, MI and maintained on a controlled light:dark cycle (12 hours light:12 hours dark) with lights on at 0600 and lights off at 1800. Rats were permitted food (Purina Rodent Chow, Ralston Purina Co., St. Louis, MO) ad libitum. Chronic ethanol treatment was administered though the drinking water. Rats consuming ethanol drank tap water containing 6% ethanol (v/v). Control rats received tap water containing 12% sucrose (w/v) in order to balance caloric intake. Rats were weighed weekly and water consumption was monitored daily for thirty days. All rats (either 9 month or 15 month experiments) daily consumed amounts of fluid that were similar to the controls.

Experiment I (Nine Month Old Rats):

At the end of the treatment period rats (10 treated; 10 controls) were killed by decapitation at 0900. Blood was collected, processed to yield plasma, and stored at -70 C until plasma ACTH and corticosterone were determined by radioimmunoassay (RIA). One half of the anterior pituitary glands from treated and control rats (five pituitary glands per group) were collected, placed in medium 199 (M199; GIBCO Laboratories, Grand Island, NY), and incubated at 37 C in the presence of 100 μg CRH for 90 minutes to assess responsiveness *in vitro*. The remaining anterior pituitary glands were collected, frozen immediately in liquid nitrogen, and stored at -70 C until they were extracted with 0.1 N HCl as described by Procknor (14) for determination of ACTH content. Tissues were lyophilized and homogenized with a glass rod in 12 x 75 mm borosilicate glass test tubes. Two ml of 0.1 N HCl was added and tissues were vortexed for one minute. Samples were then centrifuged (2000 x g) at 4 C for 30 minutes. The supernatant was removed and transferred to a test tube and stored at -70 C until quantitated for ACTH by RIA. Adrenal glands from all rats were collected and placed in M199 and incubated at 37 C in the presence of 100 μg ACTH for 90 minutes to assess responsiveness *in vitro*.

Experiment II (15 Month Old Rats):

Experiment II was conducted as described for Experiment I, except twelve fifteen month old rats (6 treated; 6 controls) were utilized. Anterior pituitary glands were collected only for determination of ACTH content.

Corticosterone and ACTH in both the plasma and media were quantitated utilizing commercial kits (ICN Biochemicals Inc. Costa Mesa, CA; Diagnostic Products Company, Los Angeles CA, respectively). All assays were conducted as described by the manufacturers' protocols. Sensitivity of the corticosterone RIA was 8ng/ml plasma and the intra and interassay coefficients of variation were 7 and 8 percent, respectively. Sensitivity of the ACTH RIA was 10pg/ml plasma and the intra and interassay coefficients of variation were both 10 percent. Data were analyzed by Students t-test with the level of significance set at $P<0.05$.

Results

Nine Month Old Rats:

Body weights at the end of the thirty day treatment period were not changed by ethanol consumption ($P>0.05$). At the end of the study control and treated rats weighed 566 ± 7.0 and 550 ± 5.2 g, respectively. Plasma concentrations of ACTH in nine month old rats were decreased to 60.7% of controls in response to chronic ethanol consumption ($P<0.05$; Table 1). Plasma corticosterone concentrations were decreased to 51.0% of control corticosterone concentrations ($P<0.05$; Table 1). Pituitary concentration of ACTH was not affected by chronic ethanol consumption ($P>0.05$; Table 1).

Responsiveness of the anterior pituitary gland to CRH stimulation *in vitro* is shown in Table 2. In response to 100 μg CRH, the anterior pituitary glands of rats chronically consuming ethanol released less ACTH compared to the anterior pituitary glands from control rats ($P<0.05$). Anterior pituitary glands from treated rats released only 35.3% of the ACTH that the anterior pituitary glands from control rats released.

TABLE 1

Hormone	Control	Treated
Plasma ACTH (pg/ml)	31.2 ± 4.8	19.0 ± 1.7*
Plasma Corticosterone (ng/ml)	37.5 ± 7.0	19.2 ± 2.7*
Pituitary ACTH (ng/pituitary)	123.5 ± 26.8	97.7 ± 18.7

TABLE 1. Plasma hormone and pituitary ACTH concentrations in 9 month old rats following chronic ethanol consumption. Animals were treated as described in Methods. Hormone concentrations in plasma and the pituitary gland were determined by RIA. Asterisks denote differences due to chronic ethanol consumption ($P<0.05$).

Responsiveness of the adrenal gland to ACTH stimulation *in vitro* is also shown in Table 2. The amount of corticosterone released in response to 100 μg ACTH was not affected by chronic ethanol consumption.

TABLE 2

Hormone	Control	Treated
Pituitary ACTH secretion (ng/ml)	18.1 ± 2.4	6.4 ± 1.4*
Adrenal Corticosterone secretion (ng/ml)	1.40 ± 0.21	1.49 ± 0.33

TABLE 2. *In vitro* responsiveness of the anterior pituitary and adrenal glands of 9 month old rats chronically consuming ethanol. Tissues were removed and stimulated with either CRH (anterior pituitary glands) or ACTH (adrenal glands) as described in Methods. Hormone concentrations in the media were determined by RIA. Asterisks denote differences due to chronic ethanol consumption ($P<0.05$).

Fifteen Month Old Rats:

Body weights at the end of the thirty-day treatment period (controls 550g ± 13; treated 553g ± 12) were not changed by ethanol consumption (P>0.05).

As shown in Table 3, chronic ethanol consumption also decreased plasma ACTH and corticosterone concentrations in fifteen month old rats (P<0.05). Plasma ACTH concentrations in ethanol treated rats were 53.6% of control rat plasma ACTH concentrations. Plasma corticosterone was decreased to only 8.8% of control concentrations by chronic ethanol consumption. Anterior pituitary gland concentration of ACTH was unaffected (P>0.05) by chronic ethanol consumption (Table 3).

Adrenal responsiveness to ACTH stimulation *in vitro* for 15 month old rats was not altered by chronic ethanol consumption (P>0.05). Corticosterone release in response to 100 μg ACTH was similar for control and treated tissues. Adrenal glands from control and treated rats released 0.69 ± 0.08 and 0.58 ± 0.07 ng corticosterone, respectively.

TABLE 3

Hormone	Control	Treated
Plasma ACTH (pg/ml)	48.0 ± 8.3	25.7 ± 4.7*
Plasma Corticosterone (ng/ml)	147.6 ± 29.3	13.0 ± 2.6*
Pituitary ACTH (ng/pituitary)	352 ± 59	350 ± 24

TABLE 3. Plasma hormone and pituitary ACTH concentrations in 15 month old rats following chronic ethanol consumption. Animals were treated as described in Methods. Hormone concentrations in plasma and the pituitary gland were determined by RIA. Asterisks denote differences due to chronic ethanol consumption (P<0.05).

Discussion

Chronic ethanol consumption by aged rats decreases basal tone (as determined by the plasma concentrations of ACTH and corticosterone at 0900) of the hypothalamic-pituitary-adrenal axis. Such decreased basal tone is evident in the depression of plasma ACTH and corticosterone concentrations in nine and fifteen month old rats chronically consuming ethanol. In addition to decreased HPA tone, these data also suggest that the effects of chronic ethanol consumption on the fifteen month old rat are different than those in the nine month old rat. In both ages, there was decreased plasma ACTH and corticosterone concentrations, but in the older (fifteen month old rats) there was a greater depression of plasma corticosterone than was observed in the nine month old rats (compare Tables 1 and 3).

There were also effects on *in vitro* responsiveness of the anterior pituitary gland to trophic stimulation in the nine month old rats. Anterior pituitary glands of nine month old treated rats were less capable of releasing ACTH than were control tissues. However, adrenal responsiveness to ACTH stimulation was not changed by ethanol consumption. This suggests that there are differences in the effect of chronic alcohol consumption on these two target tissues.

The results of the present study demonstrate that aged rats may respond differently to chronic ethanol consumption than the younger rats routinely utilized in laboratory studies. In

younger rats (3 to 6 months of age) chronic ethanol consumption has been reported to result in both activation and depression of the HPA. For example, it has been reported that hypothalamic CRH secretion (3) and the ability of CRH to stimulate secretion of ACTH from the anterior pituitary gland (4,5) are increased following chronic ethanol exposure. It has also been reported that pituitary CRH receptor number, pituitary adenyl cyclase activity, and anterior pituitary proopiomelanocortin levels are decreased after chronic ethanol exposure (6), suggesting a depression in HPA function. The data obtained from the experiments described herein demonstrate that aged (9 and 15 month old) rats have decreased HPA function in response to chronic ethanol consumption.

It is possible that the effects observed in the present study are the result of the interaction between aging and chronic ethanol consumption on the HPA. It is well established that as rats age, changes occur within the HPA. Basal serum ACTH and corticosterone concentrations increase progressively throughout the lifetime of the rat (8-12). As shown in Tables 1 and 3, ACTH and corticosterone concentrations are greater in the control rats at 15 months of age than in the control rats at nine months of age, indicating that the rats used in these studies are responding normally to the effects of aging. It has been suggested that in aged rats corticosterone is less capable of regulating the HPA. Aged rats are less sensitive to dexamethasone suppression of circulating corticosterone (13), and corticosterone remains elevated for a longer period of time following a stressor in aged rats (12). In the present studies, ethanol consumption did not impair the ability of the adrenal gland to respond to stimulation *in vitro*, suggesting that decreased corticosterone concentrations observed in the rats consuming ethanol are not due to decreased adrenal responsiveness. However, chronic ethanol consumption did decrease pituitary responsiveness to CRH stimulation *in vitro*. These findings indicate that function of the anterior pituitary gland may be compromised in aged rats chronically consuming ethanol. Decreased anterior pituitary function may be responsible, in part, for the decreased plasma ACTH and corticosterone concentrations observed in both ages of rats.

Recently, Meites and coworkers (15) proposed that advancing age may make dysfunctions in the neuroendocrine system that interrupt normal regulation of body functions more prevalent. The HPA is known to regulate nutrient metabolism and immune function. Alterations in the HPA due to aging may interact with the effects of chronic ethanol consumption to further alter nutrient absorption, metabolism and immune function. Chronic alcohol consumption by humans is reported to alter carbohydrate, lipid and protein metabolism (16,17), nutrient absorption (16) and increase the susceptibility to infection (18-20). The resulting interaction between chronic ethanol consumption and aging could have potentially serious consequences. The data presented in the present study suggest that chronic ethanol consumption by aged rats decreases the function of the HPA. Further studies are warranted to examine the mechanism by which chronic ethanol consumption depresses HPA function in the aged rat. Such studies should examine the interaction between aging and ethanol consumption on decreased HPA function.

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