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Gender, Sex Steroids, Corticotropin-Releasing Factor, Nitric Oxide, and the HPA Response to Stress

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RIVIER, C. *Gender, sex steroids, corticotropin-releasing factor, nitric oxide, and the HPA response to stress.* PHARMACOL BIOCHEM BEHAV **64**(4) 739–751, 1999.—We used two stresses—exposure to mild electrofoot shocks (a neurogenic stress) and acute alcohol injection (a systemic stress)—to investigate the influence of gender and circulating sex steroids on ACTH and corticosterone released by adult rats. Both types of stresses significantly increased plasma levels of these hormones. Following exposure to shocks, intact females secreted significantly more ACTH than intact males, a difference that was abolished by ovariectomy. Gender differences in corticosterone responses were sometimes, but not always, present. In contrast, in this series of experiments males released more ACTH when acutely injected with alcohol, while there was no obvious effect of sex on corticosterone secretion. Corticotropin-releasing factor (CRF) antagonists were more effective at reducing ACTH compared to corticosterone levels. Finally, pituitary response to CRF, but much less so to vasopressin (VP), was larger in intact females compared to intact males. Blockade of endogenous nitric oxide formation slightly enhanced the effect of CRF in males, but not in females, and while it produced the expected enhancement of VP-induced ACTH release, this effect was more pronounced in females. Collectively, these results provide evidence for an influence of circulating sex steroids on pituitary and adrenal activity under some, but not all circumstances. © 1999 Elsevier Science Inc.

HPA axis Stress Gender Rat

THE fact that exposure to homeostatic threats ("stresses") stimulates the activity of the hypothalamic–pituitary–adrenal (HPA) axis represents one of the fundamental facts of neuroendocrinology. A primary component of this response is the synthesis of the hypothalamic peptides corticotropin-releasing factor (CRF) and vasopressin (VP) in the paraventricular nucleus (PVN) of the hypothalamus, the primary site from which these peptides are transported to the pituitary. CRF and VP, whose production in the PVN is under the control of a complex system of afferent neurons that include catecholamines and other neurotransmitters, essential amino acids and steroids [see, e.g., (3,43,62)], activate their specific receptors in the corticotrophs. The resulting increased ACTH secretion in the general circulation stimulates the adrenal cortex, and leads to the production of glucocorticosteroids.

There are many reports that female rodents release more ACTH and adrenal steroids than males in response to a variety of stresses [see, e.g., (9,11,15,29,30,44,47–49,63)]. As these stresses rely on the activation of several compartments of the HPA axis to induce ACTH release, each compartment can, at least in theory, be regulated in a sex-specific manner. The present consensus is that changes in CRF and possibly VP gene expression play an important role in this gender difference. However, controversy remains in this regard. Indeed, the human CRF gene is reported to contain estrogen-responsive elements in its promoter region, with estrogen exerting a stimulatory influence on its gene transcription (57), while in contrast, these steroids were found to decrease PVN CRF expression in the rat (10). Similarly, CRF content in the median eminence (which is thought to reflect PVN synthesis) has been found not altered (27), increased (10), or decreased (14) by estrogens. Nevertheless, the HPA axis of female rats often appears more active during proestrus than during other parts of the cycle [see, e.g., (4,59)], although this may not be true in all models (13). The fact that PVN CRF mRNA levels are elevated in the afternoon of proestrus (6) would, therefore, argue in favor of at least a partial stimulatory role of estrogens in these responses, even though other factors might sometimes mask this gender difference. For example, in the endotoxemia model in which no clear influence of the stage of the cycle was found (13), it is possible that the complex events leading to the activation of the HPA axis might involve elements that are both stimulated and inhibited by circulating sex steroids. Finally, little is known regarding the potential influence of gender on PVN afferents, with the exception of a sex difference in the number, the activity, and the distribution of type II corticosteroid receptors in the hippocampus (1,7). In the case of the pituitary, there is one article reporting that

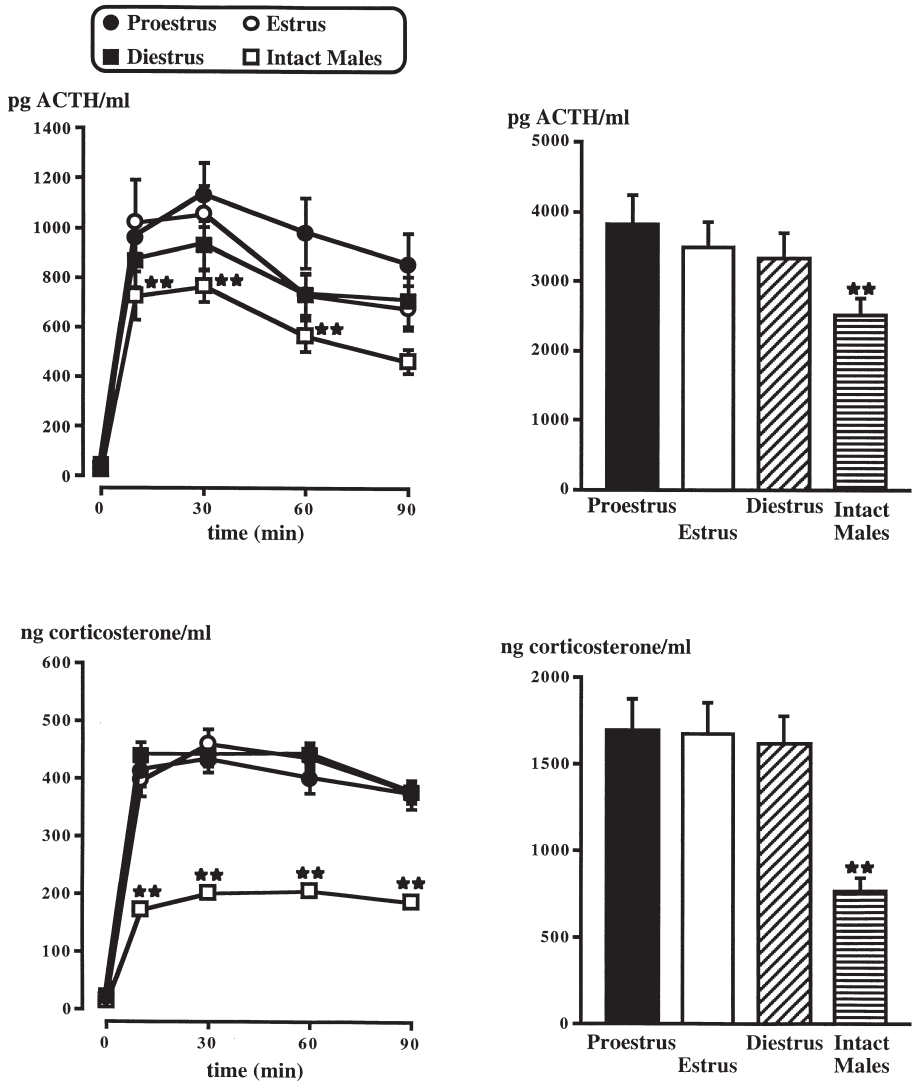

FIG. 1. (*Left panels*) Time-related increases in shock-induced plasma ACTH and corticosterone levels in intact male and female rats in diestrus, proestrus, or estrus. (*Right panels*) Cumulative ACTH levels measured at the 30-, 60-, 90-, and 120-min time points. Each point/bar represents the mean ± SEM of six to seven animals. Left panels: $^{**}p < 0.01$ vs. proestrus females for ACTH, all females for corticosterone. Right panels: $^{**}p < 0.01$ vs. all females.

females of at least some rat strains appear to release more ACTH in response to CRF and VP compared to males (47).

The present article illustrates results of experiments conducted in our laboratory that were aimed at investigating the influence of gender and circulating sex steroids on the rat ACTH and/or corticosterone response to two acute stresses, mild electrofoot shocks (a neurogenic stress), and alcohol (a systemic stress). In view of the gender-specific influence of sex steroids on CRF, we also asked whether removal of this peptide differentially altered the ACTH/corticosterone response to these stresses. This hypothesis was tested by administering newly developed CRF antagonists related to astressin (12,20,42). To examine potential male–female differences in pituitary activity, we also determined whether the intravenous (IV) injection of CRF and VP elicited similar ACTH secretory rates in both genders. Finally, in view of our finding that endogenous nitric oxide (NO) exerts a powerful inhibitory influence on VP-, but not CRF-induced ACTH release (37), we though it of interest to determine whether this influence was gender specific.

METHOD

Animals

Male and female Sprague–Dawley rats (55–65 days of age) were kept under standard light and feeding regimens. They were equipped with intravenous (IV) and/or intraperitoneal (IP) cannulae as previously described (33). Castration or ovariectomy (OVX) was carried out under halothane anesthesia 10 days prior to the assay, a time considered sufficient for the absence of circulating sex steroids to become influential (5,15). Synchronization of the female's estrous cycle was achieved as previously described (41). All procedures were approved by the Salk Institute Animal Care and Use Committee.

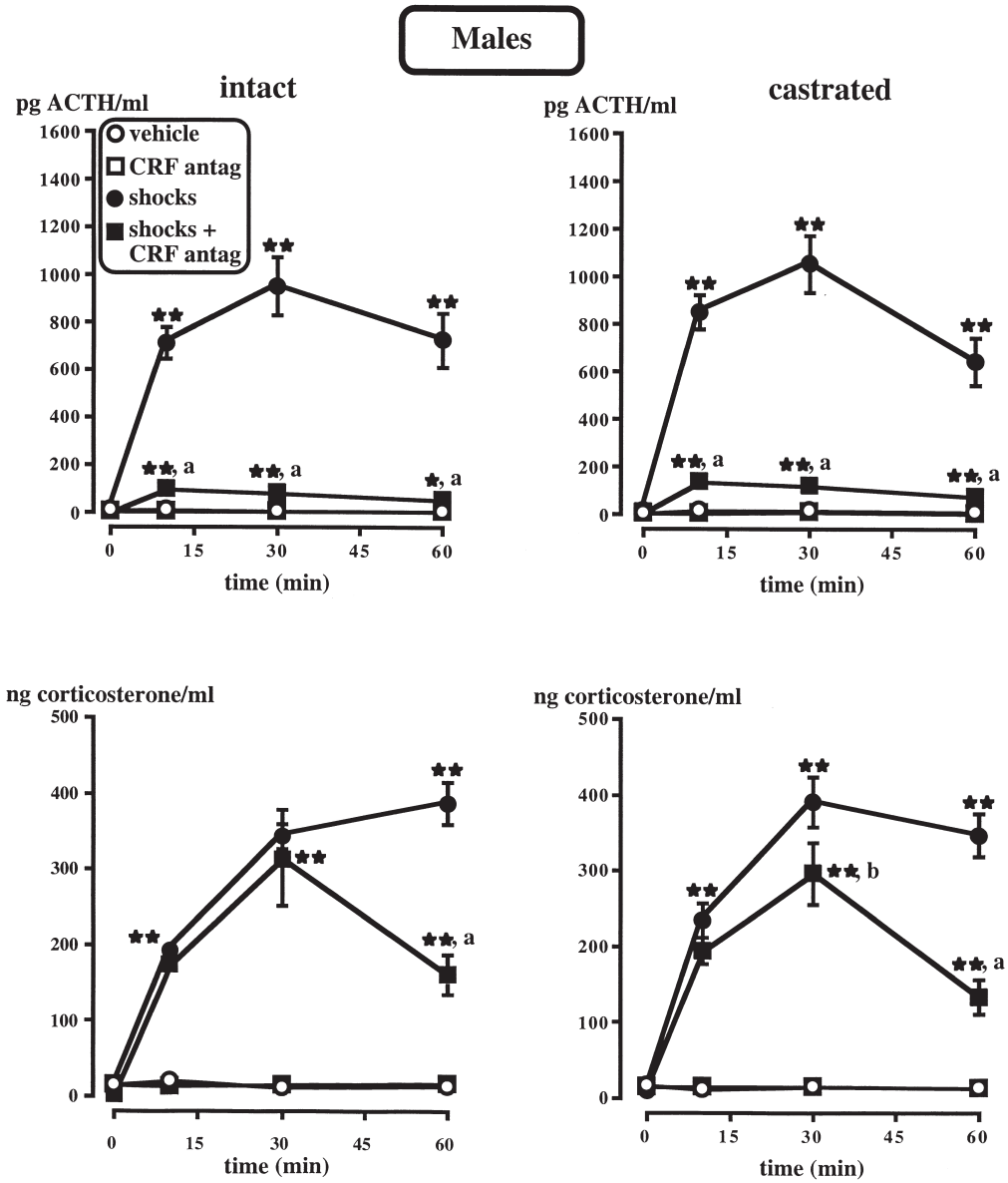

FIG. 2. Time-related increases in plasma ACTH and corticosterone levels in intact or castrated males exposed to mild electrofoot shocks (1 mA, 1-s duration, 2 shocks/min) in the presence or absence of a CRF antagonist (antag). Each point represents the mean ± SEM of five to seven animals. $^{**}p < 0.01$ vs. vehicle; a, $p < 0.01$ and b, $p < 0.05$ vs. shocks.

Reagents

CRF antagonists of the astressin family, as well as CRF and VP, were synthesized by solid phase methodology (21) and generously provided by Dr. Jean Rivier (The Salk Institute, La Jolla, CA). They were diluted in 0.04 M phosphate-buffered saline, pH 7.4, that contained 0.1% BSA and 0.01% ascorbic acid. The antagonists were administered IV 5–10 min prior to the experiment, at a dose (100–120 μg/kg) previously shown to be maximally effective over a 2–4 h period (42). The CRF antibodies, raised in sheep and generously provided by Dr. Wylie Vale and Ms. Joan Vaughan (54), were injected IV 5–10 min prior to the experiment, at a dose (0.6 ml/kg) shown to fully block CRF (52). Blockade of NO formation was achieved with the administration of the arginine derivative N_{ω}nitro-L-arginine-methylester (L-NAME) (37,53). L-NAME, purchased from Sigma Corp. (St. Louis, MO), was diluted in 0.04 M phosphate buffered-saline, and injected subcutaneously (SC) 3 h prior to the experiment, a dose that inhibits brain NOS activity by >85% (19,50).

Assays

All assays were conducted in awake, freely moving animals. The shocks were delivered to the paws of the animals as previously described (40). An average of two shocks/min, 1-s duration each at 1 mA, were delivered for 30 min. Alcohol,

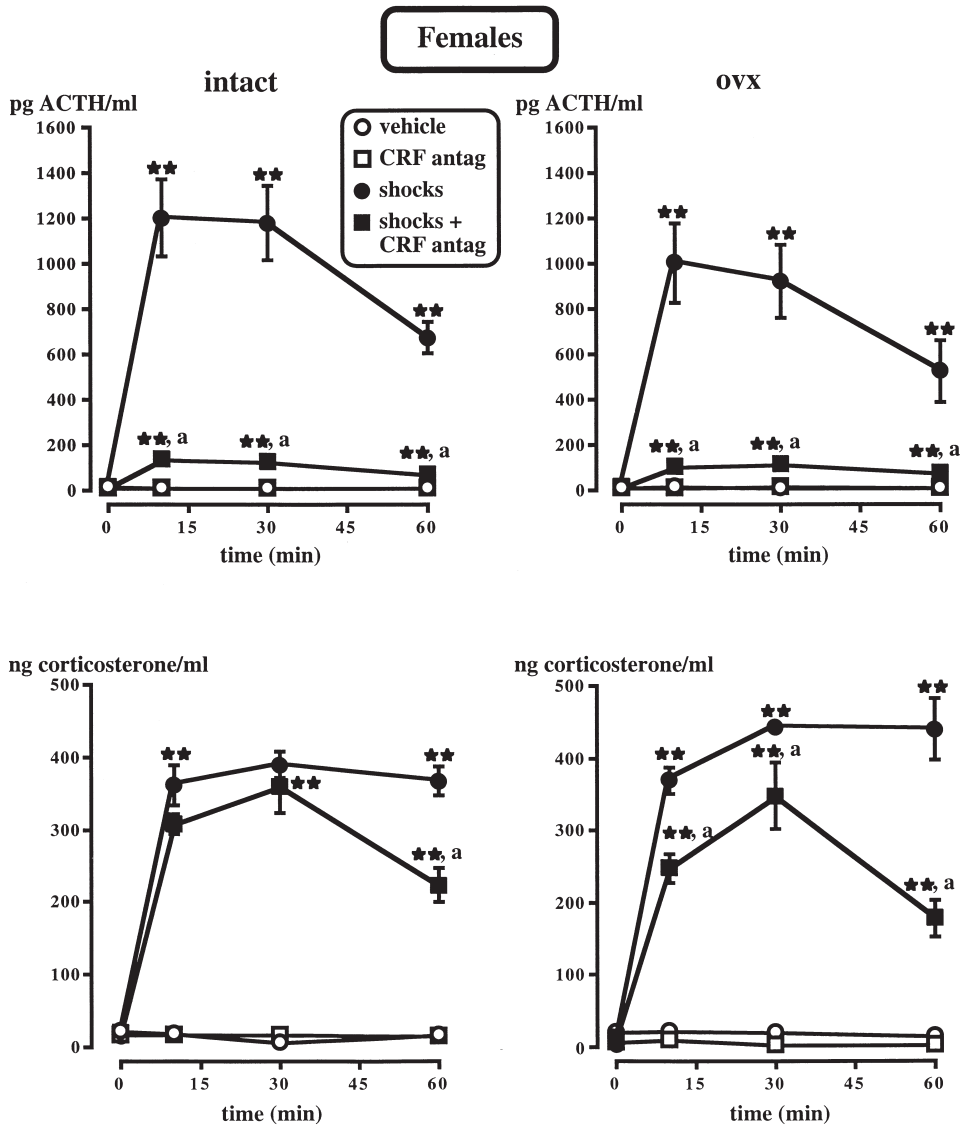

FIG. 3. Time-related increases in plasma ACTH and corticosterone levels in intact or OVX females exposed to mild electrofoot shocks in the presence or absence of a CRF antagonist (antag); **, $p < 0.01$ vs. vehicle; a, $p < 0.01$ vs. shocks. Legend as in Fig. 1.

diluted in saline at <20% v/v, was injected IP in a volume of 5 ml over a 3-min period as previously described (23,26,29). Reagents were delivered, and bloods collected remotely via the IV cannulae.

Statistical Analysis

Data were analyzed by two-way ANOVA. When necessary, post hoc analysis was accomplished using the least squares means test. To facilitate visualization of possible gender differences over the time course of ACTH and corticosterone release, we also presented data as a function of total hormone secreted over a specific period. Cumulative release of a hormone [areas under the curve (AUC)] was assessed by adding values for each time point. Baselines were not included in these calculations.

RESULTS

Effect of Shocks in Intact Males and Females Tested at Specific Stages of Their Cycle

Because HPA axis activity is reported by some investigators to vary during the cycle, we first determined whether diestrous, proestrous, or estrous females exhibited significantly different ACTH and/or corticosterone responses to mild electrofoot shocks. If this was not the case, it would allow us to carry out subsequent experiments in females at only one stage of the cycle. Although exposure to mild electrofoot shocks produced the expected rise in plasma ACTH and corticosterone levels, there were slight, but not statistically significant overall differences between the response of females tested during three stages of the cycle (Fig. 1). In contrast, the re-

TABLE 1

STATISTICAL ANALYSIS OF THE INFLUENCE OF GENDER AND CIRCULATING SEX STEROIDS ON THE ACTH ANDCORTICOSTERONE RESPONSE TO MILD ELECTROFOOT SHOCKS

Treatment	Vehicle	CRF Antagonist	Percent Decrease
ACTH			
Intact males	2386 ± 255	239 ± 28*	90
Castrated males	2544 ± 270	341 ± 36*	87
Intact females	3063 ± 328†	346 ± 25*	89
OVX females	2438 ± 170‡	471 ± 30*	81
Corticosterone			
Intact males	971 ± 88	650 ± 60*	33
Castrated males	923 ± 89	634 ± 58*	31
Intact females	1119 ± 98	891 ± 50*	20§
OVX females	1252 ± 100	774 ± 70*	38

Data (means ± SEM, n = 5–7) are presented as cumulative ACTH (pg/ml) or corticosterone (ng/ml) levels for 30 min after the onset of the shocks. These results are identical to those presented in Figs. 2 and 3, but are expressed numerically to facilitate comparison between groups.

*$p < 0.01$ vs. vehicle; †$p < 0.05$ vs. intact male; ‡$p < 0.05$ vs. intact females; §$p < 0.01$ vs. the three other groups.

TABLE 2

STATISTICAL ANALYSIS OF THE INFLUENCE OF GENDER AND CIRCULATING SEX STEROIDS ON THE ACTH AND CORTICOSTERONE RESPONSE TO ACUTE ALCOHOL INJECTION

Treatment	Vehicle	CRF Antagonist	Percent Decrease
ACTH			
Intact males	2427 ± 238	321 ± 28†	87
Castrated males	2120 ± 201	408 ± 36†	81
Intact females	1898 ± 200	178 ± 18†	90
OVX females	1792 ± 193	212 ± 19†	88
Corticosterone			
Intact males	1238 ± 120	954 ± 91*	23
Castrated males	1126 ± 110	883 ± 81*	22
Intact females	1490 ± 151	941 ± 96†	67‡
OVX females	1524 ± 151	870 ± 86†	43‡

Data (means ± SEM) are presented as cumulative ACTH (pg/ml) or corticosterone (ng/ml) levels for 120 min after the IP injection of alcohol (3.0 g/kg). These results are identical to those presented in Figs. 4 and 5, but are expressed numerically to facilitate comparison between groups.

*$p < 0.05$ and †$p < 0.01$ vs. vehicle; ‡$p < 0.01$ vs. intact or castrated males.

sponse of intact males was significantly ($p < 0.01$) smaller than that of females.

Effect of Circulating Sex Steroids on the ACTH and Corticosterone Response to Mild Electrofoot Shocks Model

The purpose of these experiments was to investigate the influence of circulating sex steroids on the ACTH and corticosterone response to mild electrofoot shocks, and on the ability of a CRF antagonist to decrease these responses. In view of the results of Fig. 1, experiments carried out in intact females used either animals at random stages of the cycle, or females in diestrus. Results were statistically comparable, and Fig. 3 illustrates data obtained during random stages of the cycle.

ACTH. Shocks induced the expected rise in plasma ACTH levels (Figs. 2 and 3, Table 1). ANOVA for both repeated-measures and cumulative ACTH release indicated a significant interaction between treatment and gender, $F(1, 44) = 1.655$, $p < 0.05$, and $F(1, 62) = 10.549$, $p < 0.05$, respectively, with intact females exhibiting a larger ACTH response than intact males. There was no significant difference between intact and castrated males, but OVX females released significantly ($p < 0.05$) less ACTH than intact females. Gonadectomy also abolished abolished the difference between males and female, $F(1, 44) = 0.336$, $p > 0.05$. The CRF antagonist significantly ($p < 0.01$) reduced ACTH levels in all animals, with no obvious difference between groups, $F(1, 44) = 0.428$, $p > 0.05$.

Corticosterone. Overall, the corticosterone response to shocks was comparable in intact and gonadectomized animals of both sexes (Figs. 2 and 3, Table 1). ANOVA for both repeated measures indicated no significant interaction between treatment and gender, $F(1, 37) = 0.064$, $p > 0.05$, and no effect of gonadectomy, $F(1, 37) = 0.433$, $p > 0.05$. Comparable results were obtained for the AUC. The CRF antagonist significantly ($p < 0.01$) reduced corticosterone levels in intact, $F(1, 37) = 18.992$, $p < 0.01$, and gonadectomized, $F(1, 37) = 42.911$, $p < 0.01$, animals, but this effect was significantly less marked in intact females ($p < 0.01$) compared to the other three groups. In addition, the time course of the corticosterone response to shocks was differentially altered by the antagonist: in OVX females, it was already significantly ($p < 0.01$) reduced at the 10-min time point, while the earliest decrease was noted at the 30-min point in the other groups.

Effect of Circulating Sex Steroids on the ACTH and Corticosterone Response to Acute Alcohol Injection

The purpose of these experiments was to investigate the influence of circulating sex steroids on the ACTH and corti-

TABLE 3

STATISTICAL ANALYSIS OF THE INFLUENCE OF GENDER AND NO ON THE ACTH RESPONSE TO THE IV INJECTION OF CRF OR VP

Treatment	Vehicle	L-Name	Percent of Increase
Males			
0.05 μg CRF/kg	99 ± 15	146 ± 18	47
0.1 μg CRF/kg	412 ± 49	521 ± 58	26
Females			
0.05 μg CRF/kg	161 ± 21†	177 ± 21	10
0.1 μg CRF/kg	947 ± 101‡	976 ± 104‡	3
Males			
0.1 μg VP/kg	68 ± 8	186 ± 2*	174
0.5 μg VP/kg	266 ± 31	811 ± 90*	205
Females			
0.1 μg VP/kg	96 ± 12†	380 ± 43*	296
0.5 μg VP/kg	356 ± 40	1247 ± 130†	250

Data (means ± SEM, n = 4–5) are expressed as cumulative ACTH levels (pg/ml) for 30 min after CRF/VP injection. These results are identical to those presented in Fig. 7 and 8, but are expressed numerically to facilitate comparison between groups.

*$p < 0.01$ vs. vehicle. †$p < 0.05$ and ‡$p < 0.01$ vs. males.

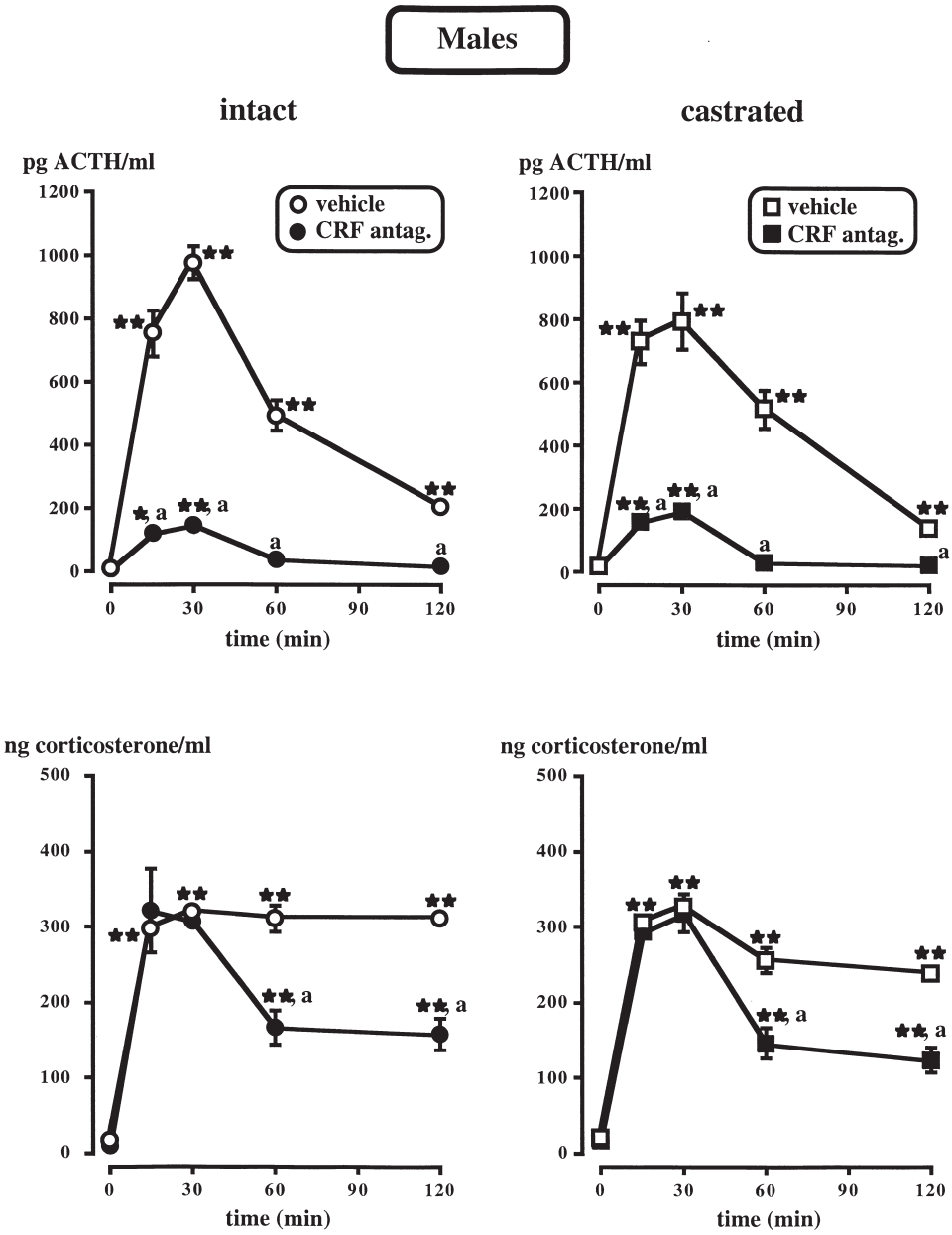

FIG. 4. Time-related increases in plasma ACTH and corticosterone levels in intact or castrated males injected with alcohol (3.0 g/kg, IP) in the presence or absence of a CRF antagonist (antag). $^{**}p < 0.01$ vs. animals not injected with alcohol (which, for reasons of clarity, are not illustrated in the figure); a, $p < 0.01$ vs. vehicle. Each point represents the mean ± SEM of five to seven animals.

costerone response to acute alcohol injection, and on the ability of a CRF antagonist to decrease these responses. Females were used during diestrus.

ACTH. Alcohol induced the expected (32,34) rise in plasma ACTH and corticosterone levels (Figs. 4 and 5). ANOVA for repeated-measures over the time course of the ACTH response indicated a significant effect of gender, $F(1, 60) = 0.633$, $p < 0.05$, with the largest response observed in intact males. There was an effect of male circulating sex steroids, $F(1, 60) = 5.184$, $p < 0.05$, with castration significantly ($p < 0.05$) reducing the ACTH response. Cumulative ACTH release also indicated a significant effect of gender, $F(1, 62) = 6.343$, $p < 0.05$, with values being largest in intact males. However, the effect of circulating sex steroids was lost, $F(1, 62) = 0.185$, $p < 0.05$. The CRF antagonist significantly ($p < 0.01$) reduced these responses in all groups of animals with regard to the time course of ACTH release, $F(1, 60) = 240.912$, $p < 0.01$, and AUC, $F(1, 62) = 201.763$, $p < 0.01$, with a significant interaction between treatment and gender for both measures, $F(1, 60) = 1.789$, $p < 0.05$, and $F(1, 62) = 1.248$, $p < 0.05$, respectively, such that this effect was significantly ($p < 0.05$) smaller in castrated, compared to intact males, and also signif-

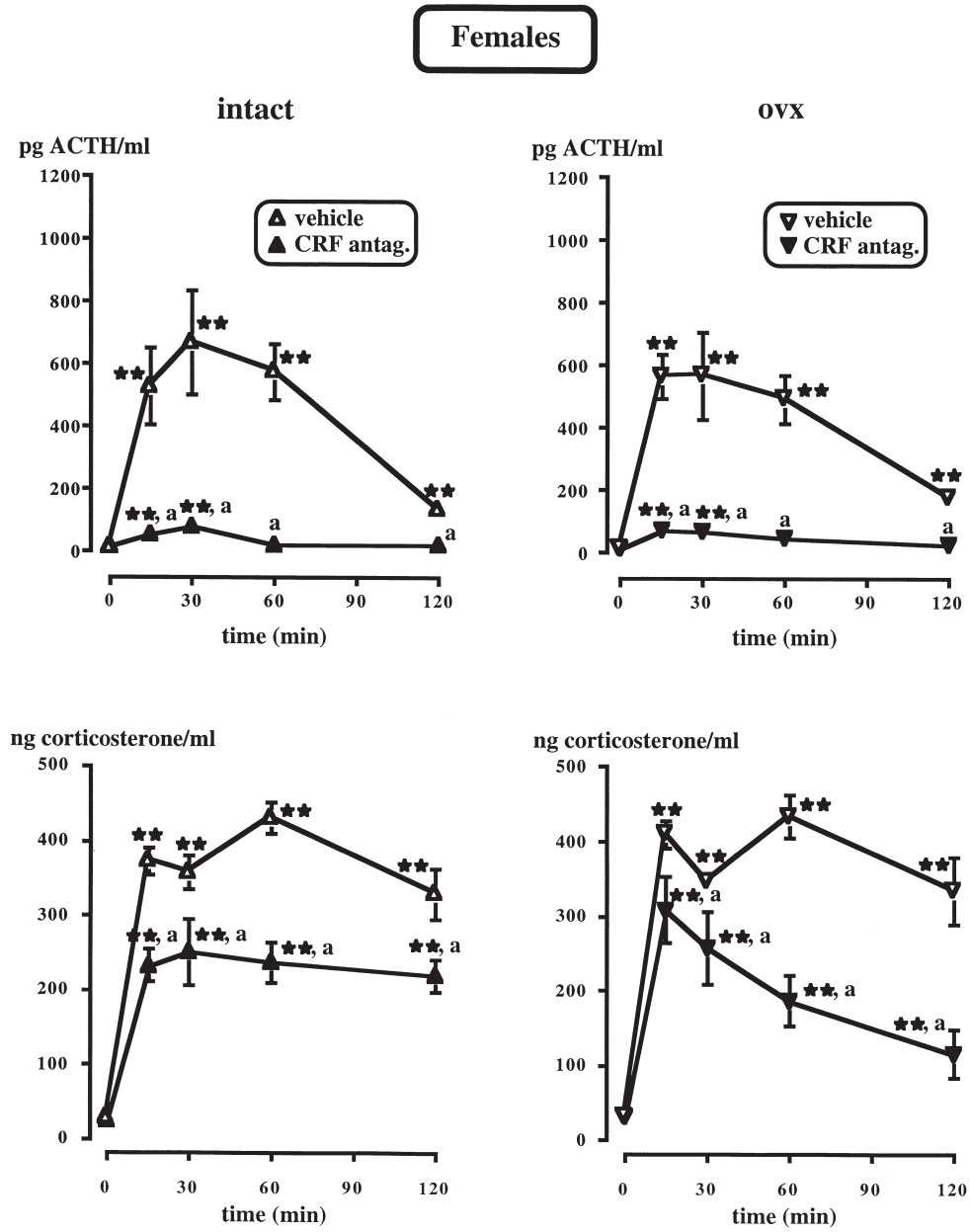

FIG. 5. Time-related increases in plasma ACTH and corticosterone levels in intact or OVX females injected with alcohol (3.0 g/kg, IP) in the presence or absence of a CRF antagonist (antag). $^{**}p < 0.01$ vs. animals not injected with alcohol (which, for reasons of clarity, are not illustrated in the figure); a, $p < 0.01$ vs. vehicle. Each point represents the mean ± SEM of five to seven animals.

icantly ($p < 0.01$) larger in intact females, compared to castrated males (Table 2).

Corticosterone. Alcohol induced statistically comparable increases in plasma corticosterone levels in all groups of animals (Figs. 4 and 5, Table 2). ANOVA for repeated measures over the time course of the corticosterone response indicated an effect of gender, $F(1, 50) = 22.332$, $p < 0.01$, with the largest response observed in females. There was no effect of circulating sex steroids, $F(1, 50) = 0.777$, $p > 0.05$. Cumulative corticosterone release also indicated a significant effect of gender, $F(1, 62) = 10.549$, $p < 0.01$, but not of circulating sex steroids, $F(1, 62) = 1.008$, $p > 0.05$. The CRF antagonist significantly ($p < 0.01$) reduced these responses in all groups of animals with regard to the time course, $F(1, 50) = 93.676$, $p < 0.01$, and cumulative values, $F(1, 62) = 73.902$, $p < 0.01$, with a significant interaction between treatment and gender for both measures, $F(1, 60) = 13.582$, $p < 0.01$, and $F(1, 62) = 13.630$, $p < 0.01$, respectively, such as the CRF antagonist significantly reduced the corticosterone response to alcohol at all time points studied in females (Fig. 5), but only at the 60- and

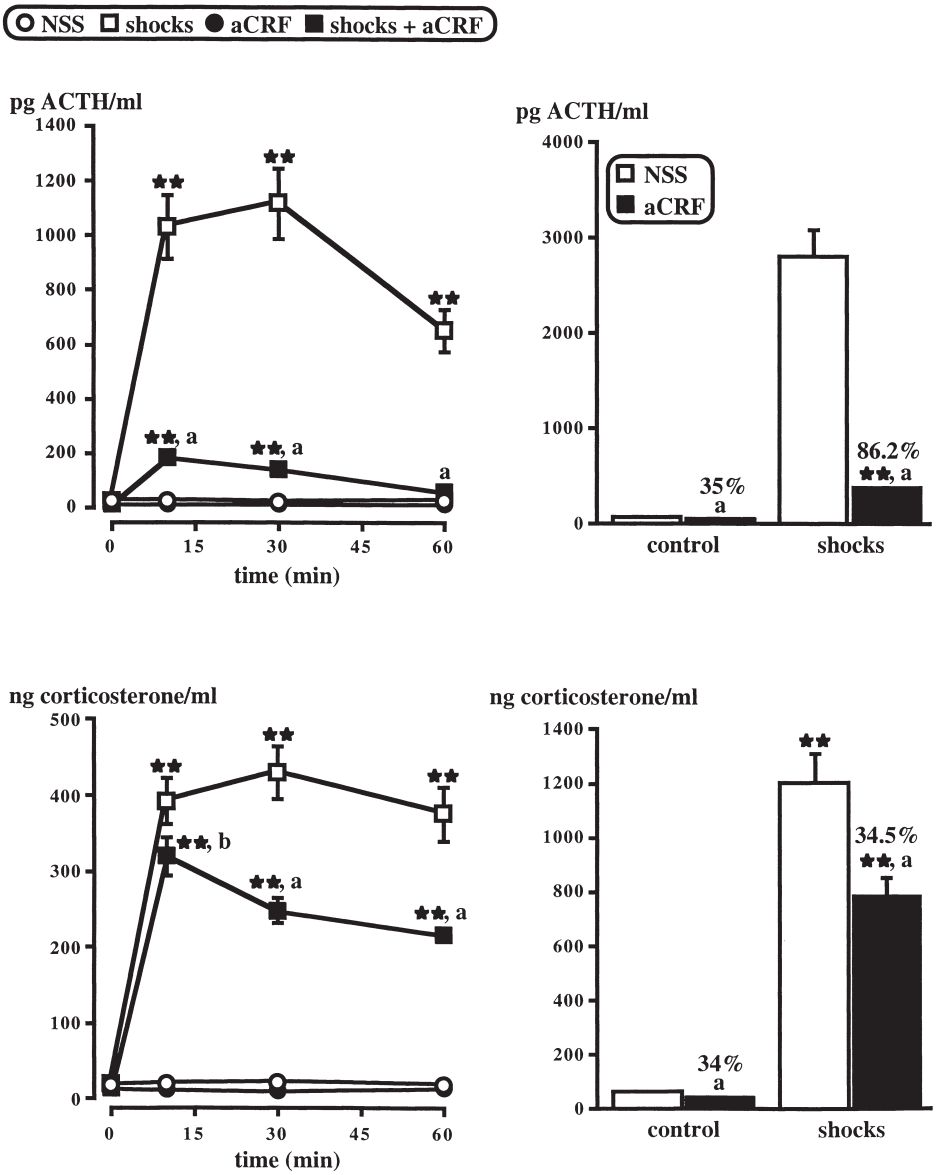

FIG. 6. Effect of prior treatment with normal sheep serum (NSS) or CRF antibodies (aCRF) on the ACTH and corticosterone response of control intact males, or animals exposed to mild electrofoot shocks. (*Left panels*) Time-related increases in plasma ACTH and corticosterone levels. (*Right panels*) Cumulative ACTH or corticosterone levels measured at the 30-, 60-, 90-, and 120-min time points. Each point/bar represent the mean ± SEM of six to seven animals. **, $p < 0.01$ vs. NSS. a, $p < 0.01$ and b, $p < 0.05$ vs. NSS. Numbers above the aCRF columns indicate the percentage decrease induced by removal of endogenous CRF.

120-min time points in males (Fig. 4). As a result, this decrease was significantly ($p < 0.01$) larger in OVX females, compared to male (Table 2).

Effect of CRF Antibodies on Shock-Induced ACTH and Corticosterone Release in Intact Male Rats

As the CRF antagonists we used are new and have not yet been extensively studied by other investigators, we thought it useful to demonstrate that their ability to decrease ACTH and corticosterone levels was comparable to that of specific and potent CRF antibodies. The expected rise in plasma ACTH and corticosterone responses to shocks was significantly ($p < 0.01$) decreased by removal of endogenous CRF, but as in the case of the antagonists, the decrease in corticosterone release was significantly less marked than that observed for ACTH (Fig. 6).

ACTH Response to CRF or VP as a Function of Gender

These experiments were designed to determine whether the stimulatory effect of CRF or VP, and the influence of NO on

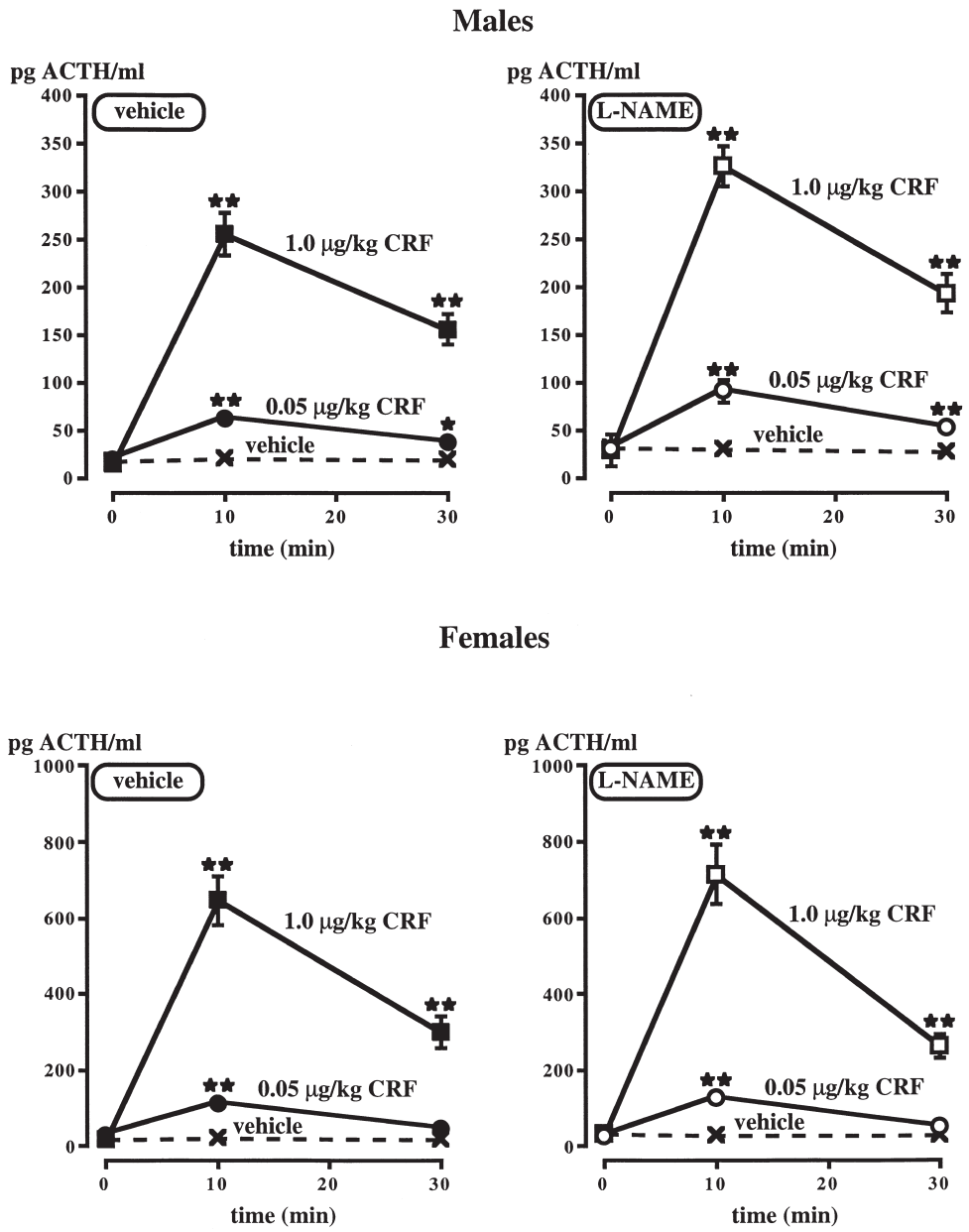

FIG. 7. Effect of blockade of NO formation with L-NAME (30 mg/kg) on the ACTH response of intact males and females to the IV injection of CRF. Each point represents the mean ± SEM of five animals. *$p <$ 0.05 and **$p < 0.01$ vs. vehicle.

these responses, was influenced by gender. Intact male or diestrous female rats showed that expected rise in plasma ACTH levels in response to the IV injection of ovine CRF (Fig. 7). In this experiment, there was a significant interaction between treatment and gender, $F(1, 43) = 11.470$, $p < 0.01$, with males showing a smaller response than females (Table 3). Pretreatment with L-NAME (30 mg/kg, SC; 3 h) produced a small and insignificant augmentation of the ACTH response [no interaction between treatment and L-NAME, $F(1, 43) = 1.047$, $p > 0.05$].

In the rat, the IV injection of VP induces ACTH release through a mechanism that depends on endogenous CRF (35,38), and, contrary to its modest effect on isolated pituitary cells (55), results in significant elevations in plasma ACTH levels (Fig. 8). Comparison between responses of intact males and diestrous females indicated a small, but insignificant difference. The augmenting influence of L-NAME, which was significant ($p < 0.01$) and expected (37), more marked than that exerted over the ACTH response to CRF, was larger in females (Table 3) [interaction between L-NAME and gender, $F(1, 49) = 10.932$, $p < 0.01$].

DISCUSSION

The influence exerted on the rodent HPA axis by sex steroids is well established. However, the mechanisms responsible for this phenomenon remain to be fully explained. One

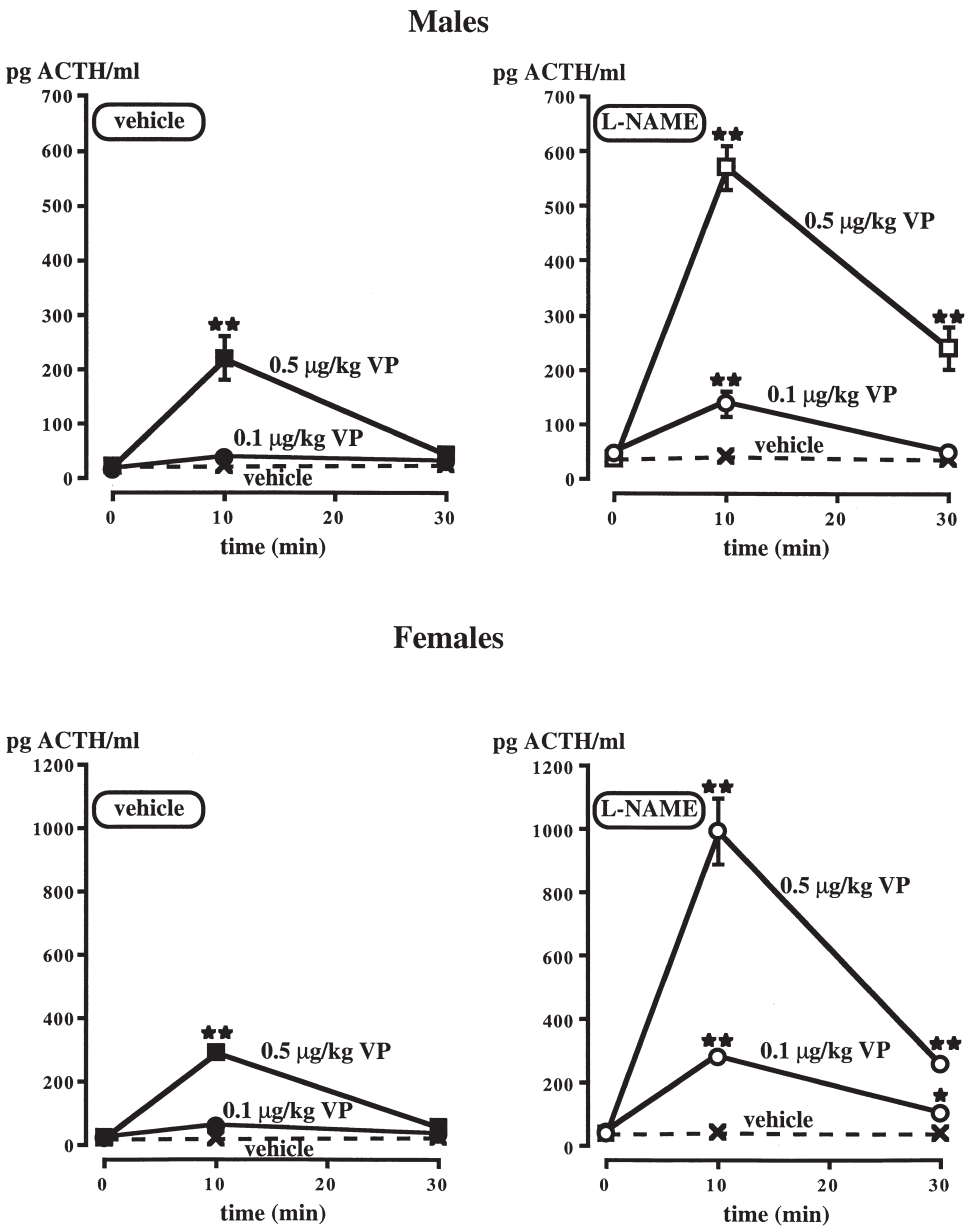

FIG. 8. Effect of blockade of NO formation with L-NAME (30 mg/kg) on the ACTH response of intact males and females to the IV injection of VP. Each point represents the mean ± SEM of five animals. $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. vehicle.

complicating factor is the fact that different stresses activated PVN CRF and VP neurons through different circuitries, each of which could respond to sex steroids in a specific way. Sex differences in HPA axis activity could, therefore, originate both at the level of neural pathways in the brain, and within peripheral organs (15). The release of peptides from the median eminence, their ability to activate pituitary receptors, as well as the characteristics of these receptors, represent potential targets for sex steroids, but whether this is indeed the case has not been extensively studied. It, therefore, should come as no surprise that controversy exists in the published literature regarding gender- and/or cycle-dependent changes in the HPA axis response to various stresses. The data presented in this article are, therefore, in no means intended to provide information that could or should be generalized to all paradigms of homeostatic threats. They can, however, provide the basis for future studies aimed at uncovering novel mechanisms.

Overall, data obtained in rats exposed to shocks or alcohol support the concept that the activity of the HPA axis is gender specific, but they do not provide strong evidence for a role of sex steroid. As discussed in more detail below, this might be due to several factors, including the intensity of the stressors and/or the duration of removal of circulating sex steroids before the experiments were performed. The general view is that testosterone inhibits, and estrogen enhances, HPA axis function [see, e.g., (15,16,49,60)], and that circulating sex ste-

roids exert their effects through an activational effect on neuronal circuits that include the hippocampus and the medial preoptic nucleus. In females, the localization of estrogen receptors in the PVN (45), although still controversial for the type beta (2,22), suggests that these steroids may directly influence CRF perikarya. Both electroshocks and alcohol activate the HPA axis by mechanisms that include stimulation of PVN CRF neurons (17,24,32), but the set of neurotransmitters (catecholamines, prostaglandins, opiates, etc.) that convey to the PVN the occurrence of each particular stress, is probably different. It was, therefore, not totally unexpected to find that sex steroids exerted a somewhat dissimilar influence in these two models. In animals submitted to shocks, the largest ACTH response was observed in intact females, compared to males, and ovariectomy removed the difference with the three other groups. Gender-dependent differences in corticosterone responses were less apparent, and did not reach statistical significance with regard to the AUC, even though the timing of the response was slightly quicker in OVX females.

Following alcohol administration, on the other hand, the largest ACTH response was observed in intact males. These results are at variance with our previous report that after the injection of smaller doses of alcohol, intact females released significantly more ACTH than intact males, and that removal of circulating sex steroids abolished the gender difference in terms of ACTH secretion (29). It is, therefore, possible that treatment with a relatively large dose of alcohol may have masked differences previously observed with smaller concentrations. Another intriguing aspect of the experiments conducted with alcohol is that in many other models, castration enhances and ovariectomy reduces the ACTH response to stress, an effect attributed to respective inhibitory and stimulatory influence of testosterone and estrogen, respectively, on PVN activity (see above). In the present experiments however, castrated males exhibited a slightly (although not statistically significant) smaller ACTH response to alcohol than intact animals of the same gender.

We were interested in determining whether at least part of the influence of sex steroids above reflected gender specificity in pituitary activity. We show here that, as previously reported (47), pituitary responsiveness to CRF, but not VP was largest in females. This was an unexpected finding in view of the very modest effect of gonadectomy in the ACTH response of females in our models. It is, therefore, possible that other components of the HPA axis, particular at the level of the PVN and/or its afferents, respond to sex steroids in a manner different from the pituitary. Studies conducted in gonadectomized animals injected with CRF may help resolve this issue. It should be noted, however, that another possibility is that even though we conducted our experiments at a time considered sufficient by several investigators to remove the activational influence of sex steroids (5,15), some endocrine responses may take longer to develop (5). The other salient point of our studies focused on the pituitary response to its trophic signals was that the inhibitory influence exerted by NO on the ACTH response to VP, but not CRF, may also be influenced by sex steroids. This suggests that at least under certain circumstances, the enhanced HPA axis response to stress may reflect sites of action of steroids both within and outside the PVN. We had previously shown that the interaction between NO and interleukin-1β in releasing ACTH appeared comparable in adult males and females (51). Inasmuch as the ACTH response to this cytokine is primarily dependent on CRF (31), our two sets of results are not conflicting. However, this illustrates the difficulty encountered is assessing the influence of sex steroids in paradigms that put multiple areas of the HPA axis into play.

Until very recently, most experiments aimed at investigating the influence of endogenous CRF relied on pretreatment of the animals with specific antibodies to immunoneutralize this peptide [see, e.g., (34,36)]. This was due in part to the fact that CRF antagonists with sufficient potency and duration of action were not available. The recent development in our laboratory of analogs that significantly reduced ACTH levels for several hours (12,20,42,52) now permits their use in in vivo assays. Comparison between results obtained with CRF antibodies or potent antagonists indicated that neither was capable of totally abolishing the ACTH response to shocks or alcohol, a phenomenon that we attribute to the contribution of VP in these models. Indeed, we have shown that removal of both CRF and VP is necessary for ACTH levels to return to values close to those of controls (32,34,39), indicating the probable release of both peptides during these stresses, as well as their known interaction at the level of the pituitary (35,38). With regard to corticosterone, experiments conducted with CRF antibodies or antagonists have consistently shown a large residual degree of hormone release after this peptide has been immunoneutralized, or its receptors have been blocked. This is probably due to the fact that because the rat adrenal is exquisitely sensitive to ACTH (18), even a small degree of increased ACTH secretion is capable of inducing significant corticosteroid production.

Modest gender differences were observed following CRF antagonist treatment. In the shock paradigm, blockade of CRF receptors was somewhat less effective in reducing the corticosterone response in intact females compared to the other groups, while it produced a more rapid decrease in corticosterone levels in OVX females. However, opposite results were observed in the alcohol model, in which the antagonist was most effective in intact females. As these data are, to our knowledge, the first to address potential differences in the effect of CRF antagonists as a function of stress paradigms and gender, this rather surprising observation still lacks an explanation. It is hoped that the new availability of potent and long-lasting antagonists will allow us and others to test hypotheses that would shed light on this phenomenon.

In conclusion, we have shown that although gender differences in ACTH and corticosterone responses can be demonstrated in some stress models, this may not represent a universal phenomenon. At least to our knowledge, few experiments have compared the effect of two different stresses in the same strain of rats and in the same laboratory (as done in the present work). In addition, the absence of systematic studies aimed at comparing ACTH and corticosterone responses to several stresses in intact males and females at specific stages of their cycles, renders wide-ranging conclusions difficult. It also remains possible, as mentioned above, that stresses of different kinds (e.g., systemic or neurogenic), or even the same stress given at different intensities (e.g., alcohol or cytokines administered at different doses), activate different parts of the HPA axis, which may be differentially influenced by sex steroids. Finally, there are many circumstances that can influence the adult rat HPA axis, but that elude the investigator. For example, it is well known that a variety of even mild stresses or differences in housing conditions during pre/neonatal life alter the response of the mature animal's CRF, ACTH, and corticosterone secretion [see, e.g., (8,25,28,46,56,58,61,64)]. As it is difficult to assess the impact that these early conditions may have in the animals we study, it

should come as no surprise that, at least in the field of gender differences, some controversy remains regarding the precise nature of the influence exerted by sex steroids. Many more studies will be necessary before these issues are convincingly settled.

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