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In the Rat, Endogenous Nitric Oxide Modulates the Response of the Hypothalamic-Pituitary-Adrenal Axis to Interleukin-1β, Vasopressin, and Oxytocin

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Nitric oxide (NO) synthase (NOS), the enzyme responsible for NO formation, is found in hypothalamic neurons containing oxytocin (OT), vasopressin (VP), and to a lesser extent corticotropin-releasing factor (CRF). Because NO is reported to modulate endocrine activity, we have investigated the hypothesis that endogenous NO participates in ACTH released by various secretagogues in the rat.

In the adult male rat, the intravenous injection of interleukin-1β (IL-1β; 0.2–0.3 μg/kg), VP (0.3–0.9 μg/kg), and OT (30 μg/kg) significantly increased plasma ACTH and corticosterone levels. Pretreatment with the L-form, but not the D-form, of N_ω-nitro-L-arginine-methylester (L-NAME; a specific inhibitor of NOS) markedly augmented the effects of these secretagogues whether it was injected acutely or over a 4 d period. Blockade of NOS activity also caused significant ($P < 0.01$) extensions of the duration of action of IL-1β, VP, and OT. In contrast, L-NAME did not significantly alter the stimulatory action of peripherally injected CRF, or centrally administered IL-1β. Administration of L-arginine, but not D-arginine (100 mg/kg), used as a substrate for basal NO synthesis and which did not by itself alter the activity of the hypothalamic-pituitary-adrenal (HPA) axis, blunted IL-1–induced ACTH secretion, and reversed the interaction between L-NAME and IL-1β. The stimulatory action of endotoxin, a lipopolysaccharide that releases endogenous cytokines, was also augmented by inhibition of NO formation.

The observation that blockade of NO formation specifically augmented the hormonal effect of signals released peripherally during immune stimulation suggests that endogenous NO may restrain the stimulation of the HPA axis during conditions of increased cytokine production.

[Key words: nitric oxide, ACTH, corticosterone, interleukin-1β, vasopressin, oxytocin]

Nitric oxide (NO) is rapidly emerging as a major and ubiquitous modulator of a variety of physiological parameters (Moncada et al., 1991; Snyder and Bredt, 1992). Though peripheral NO was initially believed to be primarily involved in the regulation of cardiovascular functions (Rees et al., 1989; Whittle et al., 1989), it is now also considered essential in participating in defense mechanisms responsible for destroying invading pathogens (Moncada et al., 1991). Recently, the observation that NO synthase (NOS) is present in perikarya of the hypothalamus [specifically in nuclei closely associated with the regulation of pituitary activity (Sagar and Ferreiro, 1987; Arevalo et al., 1992; Schmidt et al., 1992; Vincent and Kimura, 1992)], as well as in the median eminence and the pituitary itself (Sagar and Ferreiro, 1987; Bredt et al., 1990), has suggested that NO might play a physiological role in regulating neuroendocrine functions. This concept has been further supported by the detection of NOS staining in hypothalamic cells containing oxytocin (OT), vasopressin (VP), and to a lesser degree corticotropin-releasing factor (CRF) (Torres et al., 1993). Because of the functional link between interleukins and NO (Moncada et al., 1991; Lancaster, 1992; Snyder and Bredt, 1992), and because peripheral administration of interleukin-1 (IL-1) increases delivery of CRF, VP, and OT to the pituitary (Sapolsky et al., 1987; Naito et al., 1991), we used these ACTH secretagogues to test the hypothesis that endogenous NO participates in the activation of the hypothalamic-pituitary-adrenal (HPA) axis.

Materials and Methods

Animals. For evaluation of *neuroendocrine functions*, adult male Sprague–Dawley rats (220–240 gm) were kept under standard light and feeding regimens. For central administration of the treatments, intracerebroventricular cannulas were inserted into one lateral ventricle 10 d prior to the experiment (Rivier and Vale, 1989). All rats underwent aseptic insertion of a right jugular venous catheter cannula 24–48 hr prior to the experiment (Rivier et al., 1989). Treatments were delivered via the intravenous (0.2 ml) or intracerebroventricular (5 μl, infused at the rate of 1 μl/10 sec) cannulas, and blood samples (0.3–0.5 ml) were obtained through the intravenous catheter. Correct placement of the intracerebroventricular cannulas was assessed by histological evaluation of brain slices. Rats with incorrect placement were not included in the statistical analysis of the results. For peripheral administration of the treatments, intravenous cannulas were inserted as mentioned above. All experiments were performed in undisturbed animals as previously described (Rivier et al., 1989; Rivier and Vale, 1991), and each experiment was repeated twice.

For evaluation of *cardiovascular functions*, adult rats were implanted with an indwelling femoral arterial catheter for blood pressure monitoring, and a femoral venous catheter for drug administration. Forty-eight hours later, awake rats were tested after a 2 hr rest period. Mean arterial blood pressure (MAP) and heart rate (HR) were monitored continuously with the use of a Beckman type 4-327-0 transducer (Beckman Industries, Anaheim, CA) and a SensorMedics Dynograph R611 (SensorMedics Corp., Anaheim CA). After stabilization of MAP and HR, the vehicle or NAME (defined below) was injected intravenously, followed 5 min later by the vehicle or IL-1β.

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Table 1. Measurement of ACTH levels in serial dilutions of plasma from ADX rats

Dilution	Plasma (μl/tube)	Initial ACTH (pg/ml)	Final ACTH (pg/ml)
1:1	100	1205	1205
1:2	50	582	1164
1:4	25	276	1104
1:8	12.5	130	1040
1:16	6.25	74.4	1184
1:32	3.125	38.5	1232
1:64	1.56	18.6	1190
1:128	0.78	9.0	1152

All procedures were approved by the Salk Institute and UCSD Institute Animal Care and Use Committees.

Treatments. Recombinant human IL-1β was a generous gift of Dr. Gillis, Immunex, Seattle, WA. Vasopressin (VP), oxytocin (OT), and CRF were synthesized by solid-phase methodology and provided by Dr. Jean Rivier, The Salk Institute, La Jolla, CA (Kornreich et al., 1992). The treatments were prepared in phosphate-buffered saline that contained 0.1% BSA and 0.01% ascorbic acid. The arginine derivatives N_ω-nitro-L-arginine-methylester (L-NAME), N_ω-nitro-D-arginine-methylester (D-NAME), and L- or D-arginine (L-, D-Arg) were purchased from Sigma Chemical Corp. (St. Louis, MO), and dissolved in saline followed by adjustment of the pH as necessary. Endotoxin (lipopolysaccharide, LPS; #3755) was purchased from Sigma.

CRF gene expression. The rats were deeply anesthetized and perfused with saline followed by 4% paraformaldehyde in 0.1 M borate buffer. Frozen sections (30 μm) were cut on a sliding microtome, stored in antifreeze solution, mounted onto gelatin/poly-L-lysine–coated slides, and air dried. The hybridization protocol has been previously described (Lee and Rivier, 1992). Briefly, brain sections were dried under vacuum overnight, and digested by proteinase K (10 μg/ml, 37°C, 30 min), acetylated, and dehydrated. After being vacuum dried, 90 μl of hybridization mixture (10^7 cpm/ml) was applied to each side and sealed under coverslips. Hybridizations were performed at 60°C overnight. The coverslips were removed, and the slides were rinsed in 4× SSC (1× SSC: 15 mM trisodium citrate buffer, pH 7.0, 0.15 M NaCl) at room temperature. The slides were then digested by RNase A (20 μg/ml) for 30 min, washed in two 5 min changes of 2× SSC, 10 min in 1× SSC, and 10 min in 0.5× SSC at room temperature, and 30 min in 0.1× SSC at 55°C. All sections were exposed to x-ray film for 1–2 d, dipped in NTB2 nuclear emulsion, (Kodak; diluted 1:1 with H_2O), exposed for 7 d, and developed. Subsequently, all sections were counterstained with thionin.

Radioimmunoassays. Plasma ACTH levels were measured in duplicates, using a two-site IRMA assay (Allegro kit from Nichols Institute, San Juan Capistrano, CA). The characteristics of this assay have been previously described (Rivest and Rivier, 1991), and its validation for the measure of rat ACTH is presented in Results. Corticosterone values were measured by radioimmunoassay with an antiserum generously provided by Dr. G. Niswender, Colorado State University, Fort Collins, CO, and the methodology has been published by our laboratory (Lee and Rivier, 1992).

Statistical analysis. All results are expressed as mean ± SEM. They were analyzed by repeated-measured analysis of variance (ANOVA). When the interactions between factors were significant ($P < 0.05$), post hoc comparisons were made separately at each time point on the curve by means of the Dunn-Sidak procedure (Kirk, 1982) or Fisher's protected least significant test.

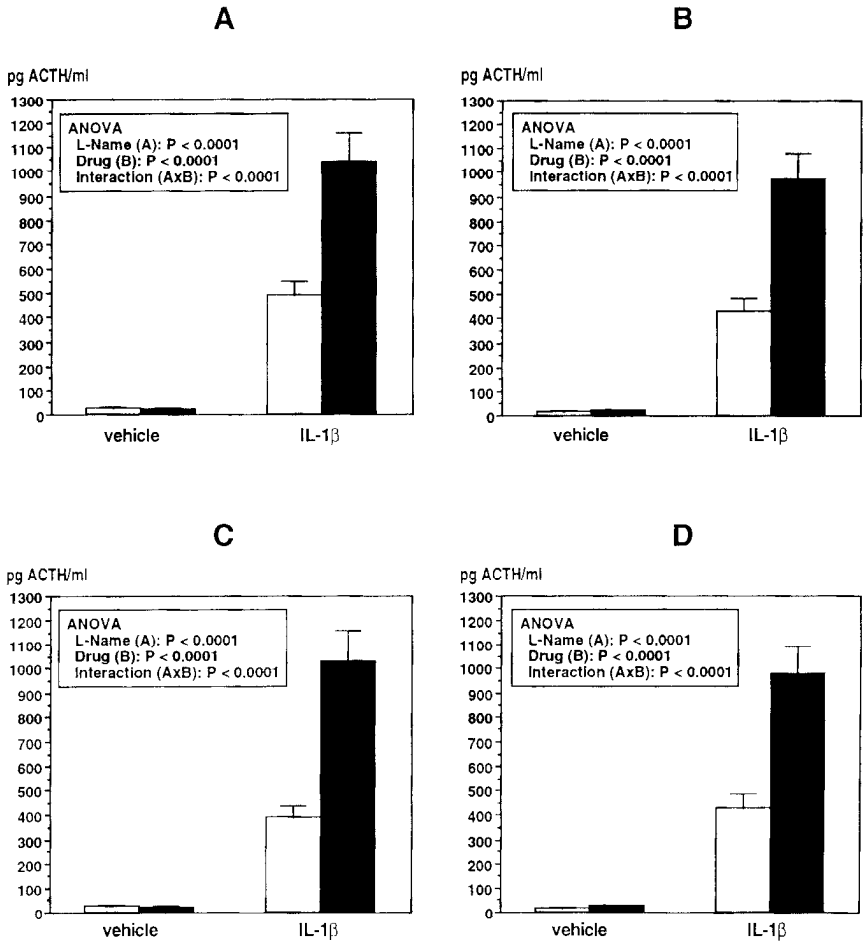

Figure 1. Effect of the administration of the vehicle or L-NAME [30 mg/kg, i.v., at −2 min (*A*) or at −3 hr (*B*); or s.c. daily for 24 hr (*C*) or for 4 d (*D*)] on ACTH secretion induced by the intravenous injection of the vehicle or 0.4 μg of IL-1β/kg. Blood samples were obtained 30 min after injection of the vehicle or IL-1β. Each bar represents the mean ± SEM ($N = 5$). *Open bars*, rats not injected with L-NAME; *solid bars*, rats injected with L-NAME. Statistical differences were assessed using a 2 × 2 ANOVA. The factors were L-NAME, which comprised two levels (vehicle or L-NAME), and drug, which also comprised two levels (vehicle or IL-1β). Post hoc comparisons were made with the Dunn-Sidak procedure.

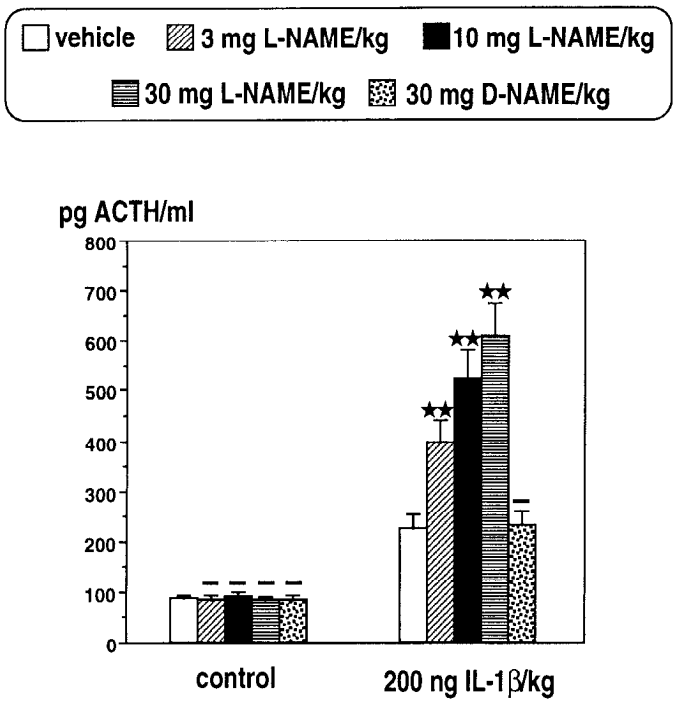

Figure 2. Effect of the intravenous injection of L- or D-NAME 2 min prior to IL-1β (200 ng/kg, i.v.). Control rats were administered the appropriate vehicle. Each bar represents the mean ± SEM of five animals. –, $P > 0.05$; **, $P < 0.01$ from vehicle.

Results

Validation of the two-site IRMA assay for rat plasma

Plasma from adrenalectomized (ADX) rats was diluted with equine hypophysectomized plasma (provided by Nichols Institute). The displacement curve obtained with increasing volumes of ADX plasma was parallel to that obtained with human ACTH standard, and the calculated final concentrations were statistically comparable across all dilutions (Table 1).

Effects of various regimens of peripheral L-NAME administration on basal and IL-1β–induced activation of the HPA axis

NAME, which inhibits NO formation (Rees et al., 1990) as well as brain NOS activity (Knowles et al., 1990), crosses the blood–brain barrier and has been used to investigate the role played by NO in CNS-mediated events (Bluthe et al., 1992; Lerner-Natoli et al., 1992). Because of reports that long-term administration of NOS inhibitors to rodents, caused a greater inhibition of the enzyme's activity than short-term treatments (Dwyer et al., 1991), we first investigated interactions between various regimens and doses of L-NAME and IL-1β in order to determine the best protocol. Plasma ACTH levels of rats injected with the vehicle or 30 mg/kg L-NAME twice daily (subcutaneously) for 4 d, or intravenously 2, 30, or 180 min prior to the experiment, remained at basal values (<30 pg ACTH/ml; Fig. 1). No measurable changes in CRF gene expression in the hypothalamus were observed under any treatment regimen (data not shown). The intravenous administration of 0.4 μg IL-1β/kg significantly increased plasma ACTH levels measured 30 min later, and this effect was markedly augmented under all regimens of L-NAME pretreatment.

In a next experiment, we investigated the importance of the dose of L-NAME, as well as the specificity of the L-isomer. As illustrated in Figure 2, 30 mg/kg L-NAME produced the largest augmenting effect in rats injected with IL-1β intravenously. No further augmentation was observed at larger doses (results not shown). In contrast, D-NAME was without effect. In view of these results, 30 mg/kg L-NAME was injected intravenously 2 min before the treatments in all subsequent experiments.

Table 2. Effect of NAME (30 mg/kg, i.v., at −2 min) on corticosterone released by secretagogues

Treatment	− NAME	+ NAME
Vehicle	65 ± 10	70 ± 9
10 μg OT/kg	103 ± 15	298 ± 38**
0.3 μg VP/kg	282 ± 38	529 ± 58**
0.02 μg IL-1β/kg	281 ± 36	527 + 61**
3 μg CRF/kg	421 ± 31	409 ± 35

Results are expressed as ng corticosterone/ml ± SEM. Blood was obtained 30 min after treatment.

** $p < 0.01$ from treatment without NAME.

Effect of L-NAME on the time course of IL-1β–induced ACTH secretion

Neither the vehicle nor L-NAME (30 mg/kg, i.v., at −2 min) measurably altered ACTH release (Fig. 3*A*). Both 0.02 and 0.1 μg of IL-1β/kg, injected intravenously, significantly ($P < 0.01$) increased plasma ACTH levels at the 15 and 30 min time points; only the higher dose of the cytokine maintained elevated ($P < 0.01$) ACTH values at the 60 min time point. L-NAME significantly ($P < 0.01$) augmented the effect of both 0.02 and 0.1 μg of IL-1β/kg, and extended the duration of the ACTH release curve. Calculation of the total amount of ACTH released over the 60 min period (Fig. 3*B*) indicated a two- to threefold increase in integrated areas when IL-1β was injected to NAME-pretreated rats, compared to vehicle-injected animals. Measurement of plasma corticosterone levels also indicated significant ($P < 0.05$–0.01) differences between rats treated with NAME or the vehicle prior to IL-1β administration (results not shown). In subsequent experiments, we observed similar effects of lower doses of NAME (5–20 mg/kg; results not shown).

Effect of NAME on the time course of ACTH secretion stimulated by secretagogues (injected intravenously)

The intravenous injection of the vehicle or L-NAME (30 mg/kg, i.v., at −2 min) did not alter ACTH secretion (Fig. 4). Intravenous administration of VP (0.9 μg/kg) or OT (30 μg/kg) significantly increased plasma ACTH levels. In rats injected with VP or OT, prior treatment with L-NAME significantly ($P < 0.01$) augmented peak ACTH secretion at the 10 min point, and extended the duration of action of the peptides. Similarly, corticosterone secretion was significantly ($P < 0.01$) increased in rats injected with VP, OT, and IL-1β 2 min after NAME, while there was no measurable differences following CRF administration (Table 2).

Effect of L-NAME on the ACTH dose–response curve to IL-1β, VP, OT, or CRF (injected intravenously)

As previously reported (Rivier and Vale, 1983, 1985a; Rivier et al., 1989), IL-1β, CRF, VP, and OT caused dose-related increases in plasma ACTH levels (Fig. 5). In the absence of L-NAME, the minimal effective doses for IL-1β, VP, and OT in this particular assay were 0.1, 0.3, and 30 μg/kg, respectively.

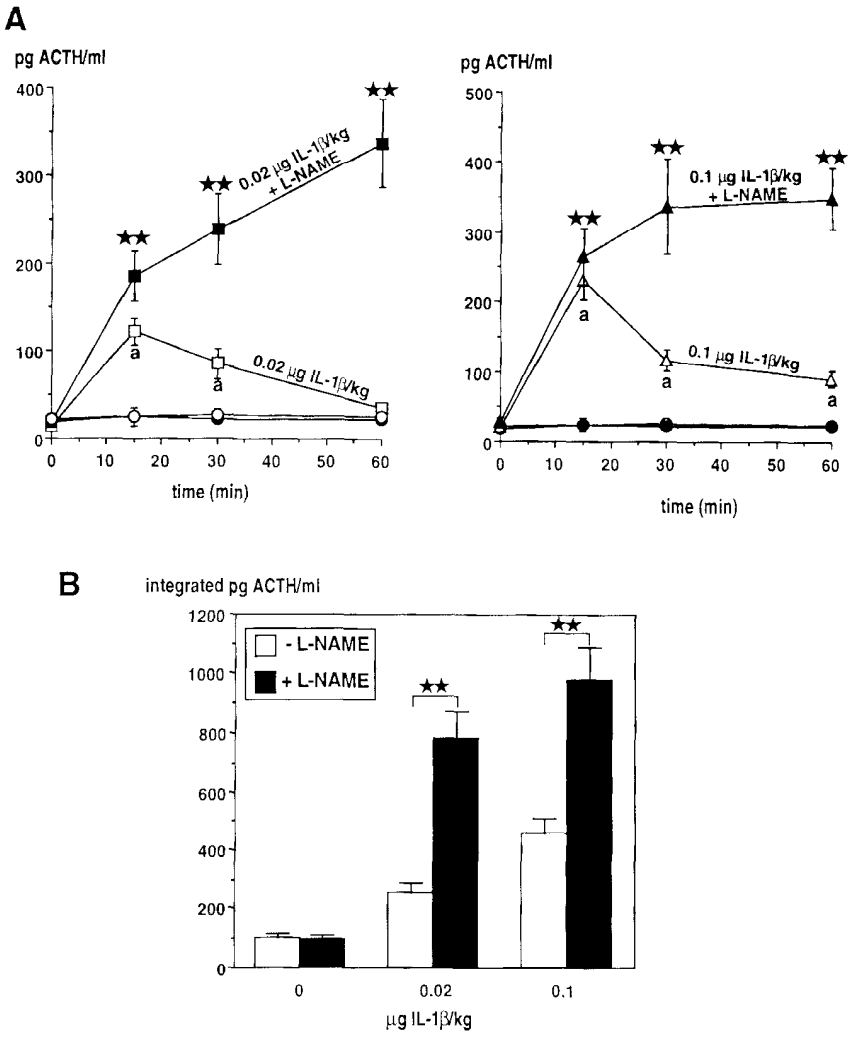

Figure 3. *A*, Time course of ACTH secretion in rats pretreated with L-NAME (30 mg/kg, i.v., at −2 min) or its vehicle, and then injected intravenously with 0.02 or 0.1 µg of IL-β/kg. *Open symbols*, rats without L-NAME; *solid symbols*, rats with L-NAME. Each point represents the mean ± SEM ($N = 5$). *a*, $P < 0.01$ from vehicle or L-NAME; **, $P < 0.01$ from treatment without L-NAME. *B*, Integrated ACTH release over a 60 min period. *Open bars*, rats without L-NAME; *solid bars*, rats with L-NAME. **, $P < 0.01$.

L-NAME caused a significant ($P < 0.01$) shift in the dose–response curves, with a decrease of the minimal effective doses to <0.03, 0.1, and 10 µg/kg, respectively. In contrast, the stimulatory effect of CRF was not statistically altered ($P > 0.05$) by L-NAME.

Interaction between L-NAME and L-Arg on IL-1β–induced ACTH secretion

Neither the intravenous injection of NAME (30 mg/kg) or L-Arg (100 mg/kg) measurably altered ACTH levels in control rats

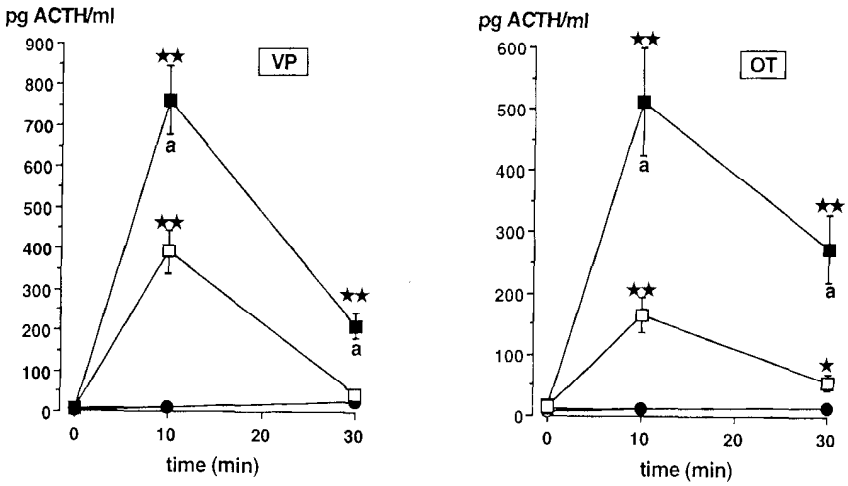

Figure 4. Time course of ACTH secretion in rats pretreated with L-NAME (30 mg/kg, i.v., at −2 min) or its vehicle, and then injected intravenously with VP (0.9 µg/kg) or OT (30 µg/kg). *Open symbols*, rats without L-NAME; *solid symbols*, rats with L-NAME. Results are expressed as mean ± SEM ($N = 5$). *, $P < 0.05$ from vehicle or L-NAME only; **, $P < 0.01$ from vehicle or L-NAME only; *a*, $P < 0.01$ from treatment without L-NAME.

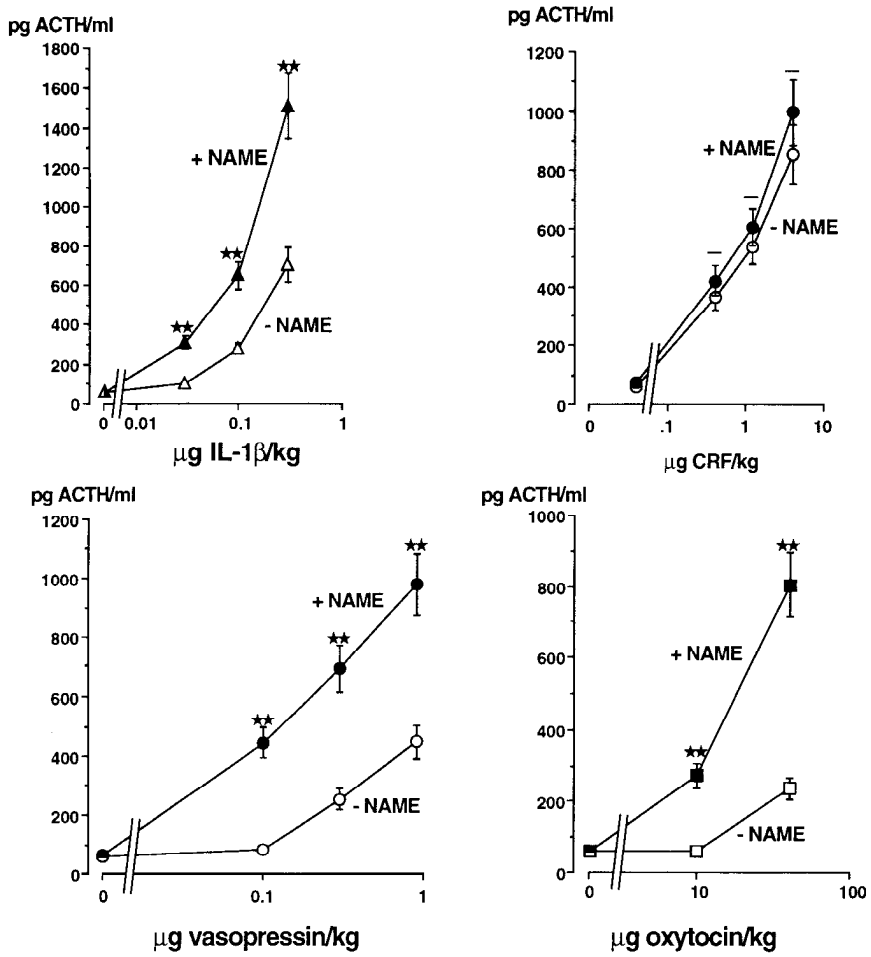

Figure 5. ACTH dose–response curves to increasing concentrations of intravenously injected IL-1β, CRF, VP, OT, or CRF in the absence or presence of L-NAME (30 mg/kg, i.v., at −2 min). Each point, which shows integrated plasma ACTH levels (calculated without subtraction of baseline) measured over a 30 min period, represents the mean + SEM ($N = 5$). *Open symbols*, rats without L-NAME; *solid symbols*, rats with L-NAME. –, $P > 0.05$; **, $P < 0.01$ from treatments without L-NAME.

(Fig. 6). Administration of L-Arg inhibited IL-1–induced ACTH release (intravenous injection), and counteracted the effect of NAME.

Effect of L-NAME (injected intravenously) on LPS-induced ACTH and corticosterone secretion

LPS caused dose-related increases in plasma ACTH and corticosterone levels (Fig. 7). Removal of endogenous NO significantly augmented this effect at all the doses at which LPS was tested. Indeed plasma ACTH levels, which were not significantly altered over control values in rats injected with the lowest dose of LPS (0.25 μg/kg), showed a twofold increase in the presence of L-NAME. Corticosterone release by rats injected with L-NAME and 0.25–4 μg, but not 20 μg, of LPS/kg was also significantly ($P < 0.05$–0.01) increased over those of animals injected without NAME.

Effect of L-NAME (injected intravenously) on ACTH secretion stimulated by IL-1β (injected intracerebroventricularly)

The intracerebroventricular injection of the vehicle did not measurably alter ACTH secretion (Fig. 8). Central administration of 10 ng of IL-1β significantly ($P < 0.01$) increased plasma ACTH levels, and this effect was not altered ($P > 0.05$) by L-NAME. Comparable results were obtained with 1 ng of IL-1β, a dose of cytokine that by itself only produced a small increase in plasma ACTH levels (results not shown).

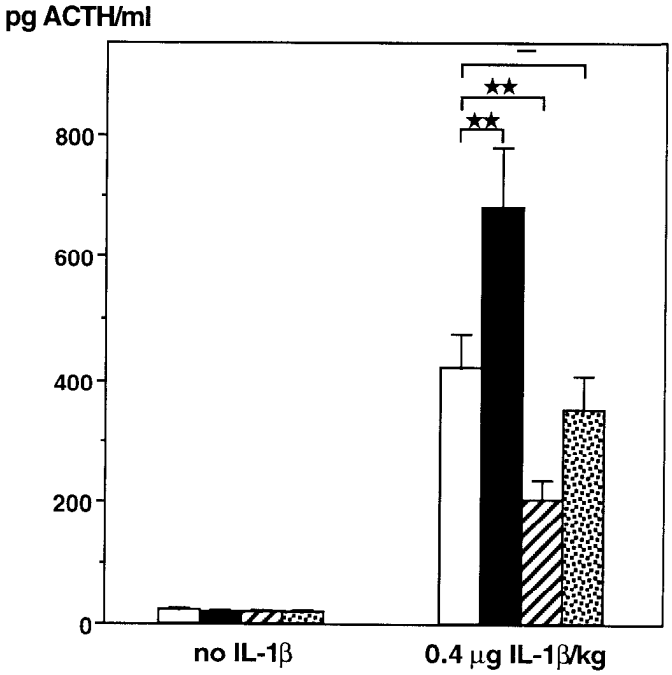

Figure 6. Interaction between L-NAME (30 mg/kg, i.v.) and L-Arg (100 mg/kg, i.v.) in rats injected with the vehicle or 0.4 μg of IL-1β/kg (i.v.). Blood samples were obtained 30 min after IL-1 injection. Each bar represents the mean ± SEM ($N = 5$). –, $P > 0.05$; **, $P < 0.01$ from IL-1β alone. *Open bars*, vehicle; *solid bars*, L-NAME; *hatched bars*, L-Arg; *stippled bars*, L-NAME + L-Arg.

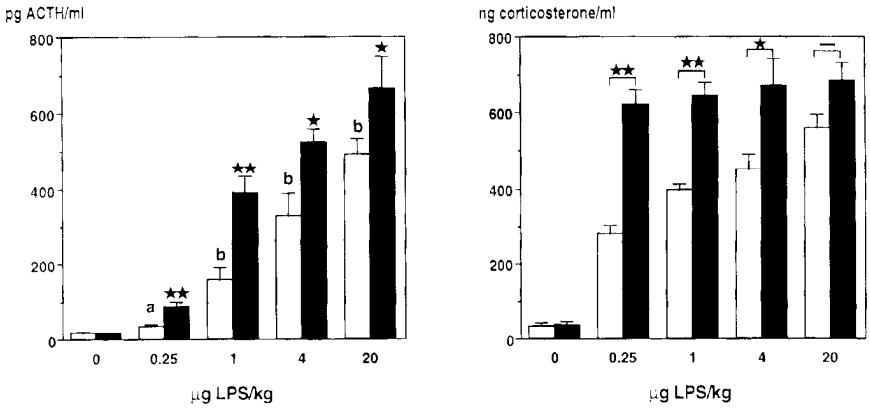

Figure 7. Interaction between L-NAME (30 mg/kg, i.v., at −2 min) and LPS on ACTH and corticosterone secretion. Blood samples were obtained 30 min after injection. *Open bars*, rats without L-NAME; *solid bars*, rats with L-NAME. Each bar represents the mean ± SEM ($N = 5$). –, $P > 0.05$; *, $P < 0.05$ from LPS alone; **, $P < 0.01$ from LPS alone; *a*, $P > 0.05$ from vehicle; *b*, $P < 0.01$ from vehicle.

Interaction between L-NAME (injected intravenously) and IL-1β on cardiovascular function

The intravenous injection of IL-β (0.4 μg/kg) caused a significant ($P < 0.05$) increase in HR and MAP (Fig. 9). L-NAME (30 mg/kg, i.v.) induced the previously reported (Whittle et al., 1989; Rees et al., 1990) decrease in HR and increase in MAP, regardless of whether it was administered by itself or with IL-1β. These results indicate a dissociation between the cardiovascular and neuroendocrine consequences of the administration of L-NAME (which decreased HR, increased MAP, and did not alter ACTH levels) or L-NAME + IL-1β (which decreased HR, increased MAP, and caused the release of ACTH).

Discussion

In the present work, we show that the peripheral administration of an arginine derivative that interferes with the activity of NOS significantly augmented the stimulatory action of IL-1β, VP, and OT on ACTH secretion. Conversely, injection of L- (but not D-) arginine (the non-rate-limiting substrate for NOS) blunted the effect of IL-1β. This compound also appeared to reverse the effect of L-NAME [a finding reported by other investigators (Whittle et al., 1989; Rees et al., 1990)], though of course one might argue that this apparent reversal could merely represent the net effect of the inhibitory action of L-Arg and the augmenting effect of L- (but not D-) NAME. In agreement with previous reports (Bluthe et al., 1992; Rettori et al., 1992; Costa et al., 1993), we did not observe any effect of L-NAME or L-Arg under basal circumstances; this suggests that NOS is not activated in unstimulated rats. In contrast, increases in circulating levels of IL-1β, VP, and/or OT appear to trigger NO synthesis, which then alters the activity of the HPA axis. As circulating levels of VP and OT are increased by the peripheral administration of cytokines (Naito et al., 1991), our findings suggest that endogenous NO may exert a restraining influence on the activity of the HPA axis during exposure to inflammatory or infectious agents. These results bring forth the questions of (1) what are the mechanisms that mediate the effect of NAME on the HPA axis, and (2) how do we explain that the effect of CRF, a peptide that is also released by IL-1 (Sapolsky et al., 1987), does not appear to be altered by removal of endogenous NO?

Mechanisms that might mediate the effect of endogenous NO include changes in hypothalamic activity, altered release of peptides and/or neurotransmitters in the median eminence, and changes in pituitary responsiveness to secretagogues. The brain structures that are important for the control of ACTH secretion, and that represent potential targets for NAME, are the hypothalamus, the infundibulum/median eminence, and the pituitary (Swanson et al., 1983). In the brain, the hypothalamus (and in particular the paraventricular nucleus, PVN) contains neurons that express peptides essential for the regulation of pituitary activity, such as CRF, VP, and OT (Swanson, 1987). The presence of NOS in hypothalamic neurons (Sagar and Ferriero, 1987; Arevalo et al., 1992; Vincent and Kimura, 1992), the observation that NO inhibits KCl-induced CRF and VP secretion by hypothalamic explants (Costa et al., 1993; Grossman et al., 1993), and the recent report that NO causes changes in hypothalamic portal blood flow (Ceccatelli et al., 1992), all suggest that this gas exerts significant effects at the level of the CNS. Nevertheless, the inability of NAME to augment ACTH response to the intracerebroventricular injection of IL-1β [two stimuli that stimulate the HPA axis primarily through the activation of CRF-secreting cells in the hypothalamus (Rivest et al., 1992)], and the absence of any changes in hypothalamic CRF biosynthesis (measured by *in situ* hybridization) of rats treated with NAME (S. Lee and C. Rivier, unpublished observations), argue against the hypothesis that in our model, NO acts primarily at the level of the PVN and/or the transport of CRF to the pituitary.

The acute release of ACTH caused by the intravenous injection of IL-1 is believed to result primarily from an effect of the cytokine at the level of the median eminence (Matta et al., 1990; Rivest et al., 1992; Ericsson and Sawchenko, 1993). Having observed that arginine derivatives blocked prostaglandin (PG) secretion by explanted hypothalamic fragments exposed to norepinephrine, Rettori et al. (1992) proposed that NO formation induced by this catecholamine might diffuse to peptide terminals and alter the release of PGs. Though these findings support the concept that an important site of action of NO is the median eminence, the functional relationship between increased NO production in rats injected with IL-1β and changes in the levels of neurotransmitters known to influence the activity of the HPA axis remains to be determined. Further studies investigating the secretory profile of catecholamines, PGs, and/or peptides from terminals present in the infundibulum should allow us to determine whether this hypothesis is valid.

At the level of the pituitary, the ability of VP and OT to stimulate ACTH secretion is greatly enhanced by endogenous CRF (Rivier and Vale, 1985a,b). This observation accounts for

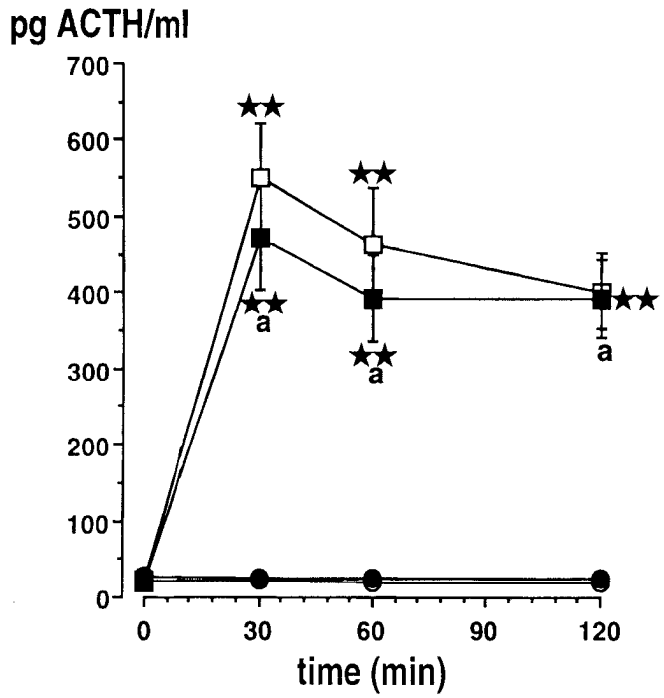

Figure 8. Effect of L-NAME (30 mg/kg, i.v., at −2 min) on ACTH secretion induced by the intracerebroventricular administration of the vehicle or IL-1β (10 ng). ○, Vehicle; ●, L-NAME; □, IL-1β; ■, L-NAME + IL-1β. Each point represents the mean ± SEM (N = 5–6). **, P < 0.01 from vehicle or L-NAME; *a*, P > 0.05 from IL-1β alone.

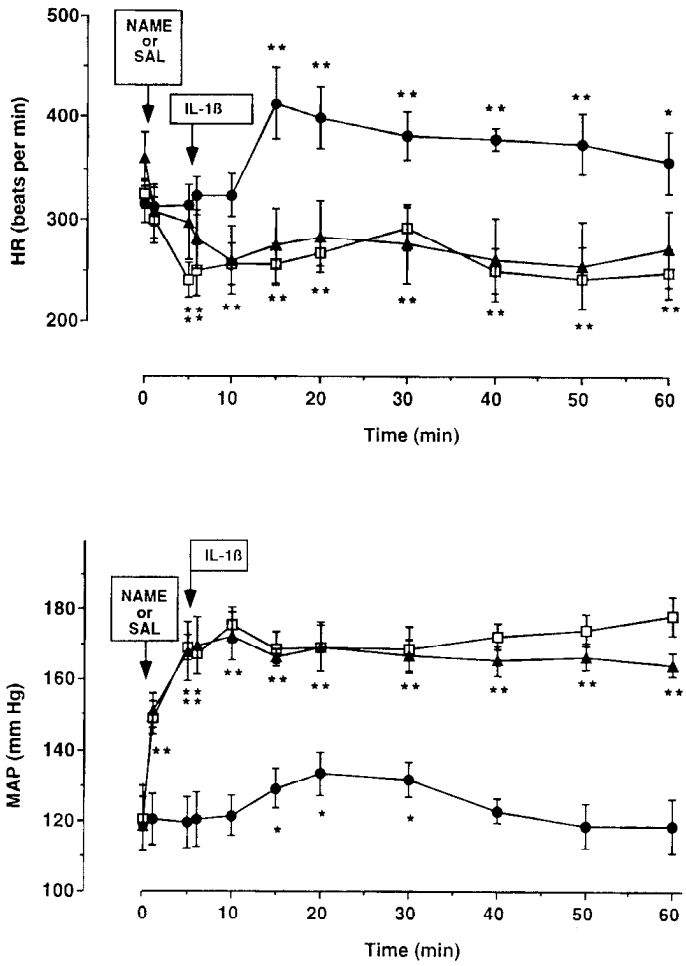

Figure 9. Effect of L-NAME (30 mg/kg, i.v., at −2 min) and/or IL-1β (0.4 μg/kg) on mean arterial blood pressure (*MAP*) or heart rate (*HR*) in awake rats. *SAL*, saline. ●, saline + 0.4 μg IL-1β/kg; □, L-NAME + IL-1β; ▲, L-NAME alone. *, P < 0.05 from preinjection values; **, P < 0.01 from preinjection values. Each point represents the mean ± SEM.

the significant discrepancy between the weak ACTH-releasing activity of VP and OT in cultured pituitary cells preparations (Vale et al., 1983), and their potent stimulatory influence in the intact rat (Rivier and Vale, 1985a). As illustrated in Figure 4, the doses of OT necessary to increase ACTH levels were significantly larger than that of VP, a finding already reported by our laboratory (Rivier and Vale, 1985a). Interestingly, plasma OT levels reached following injection of IL-1β are reported to be markedly higher than those measured for VP, and to be sustained for longer periods of time (Naito et al., 1991). Direct comparisons between the circulating concentrations of a peptide under physiological circumstances and the minimal effective doses of this peptide, are often difficult to establish. It is nevertheless tempting to speculate that the higher rate of release of OT compared to VP bears some relationship to the weaker potency of OT in releasing ACTH. At present, the role of this peptide in the male remains largely speculative. If however OT is involved in the interaction between immune signals and activation of the HPA axis, these findings might provide a novel perspective into the function of this peptide. The inability of NAME to increase the stimulatory action of CRF argues against changes in the corticotrophs' responsiveness to this peptide itself as playing a major role. It is possible, on the other hand, that NAME alters the response of the pituitary to secretagogues other than CRF. The shifts in the dose–response curves to IL-1, VP, and OT that we observed in rats injected with NAME indeed suggest that these changes may be caused through potentiation of endogenous CRF by VP or OT at the level of the pituitary (Rivier and Vale, 1983, 1985a). Thus, NAME might induce, for example, changes in second messengers (such as protein kinase, adenylate cyclase) or in calcium-dependent pathways important for signal transduction, which modulate the interactions between CRF and other ACTH secretagogues (Aguilera et al., 1986). This would explain how NAME can augment the effect of VP or OT [whether injected exogenously, or released by IL-1 administered intravenously (Naito et al., 1991)]. Such a hypothesis, while seductive, fails nevertheless to account for the inability of NAME to alter the stimulatory action of IL-1 infused directly into the brain, unless one assumes that the concentrations of VP and OT released by centrally injected IL-1 are negligible compared to those released following intravenous administration of the cytokine.

A striking feature of our studies is the specificity of the site at which NO appears to act, of the secretagogues whose effects are altered by removal of endogenous NO, and of the endocrine functions that are sensitive to these effects. Indeed, the ability of L-Arg to alter ACTH released by IL-1β, but not electroshocks, supports our hypothesis that CRF terminals, not cell bodies, are primarily targeted by NO. It is also particularly important to note that the influence of endogenous NO appears to be restricted to activation of the HPA axis by specific secretagogues associated with increased cytokine levels (such as OT and VP), but not by CRF. This may ensure that ACTH release during conditions other than exposure to inflammatory and/or infec-

tious processes, and which primarily relies on CRF (but not VP and/or OT) secretion, is not impaired by increases in NO formation. Finally, we have consistently failed to observe any changes in LH or PRL secretion, in paradigms designed to determine whether endogenous NO played a role in modulating the release of these hormones (results not shown).

In conclusion, we show here that removal of endogenous NO augments the ability of IL-1β, VP, and OT to release ACTH in the intact rat, though the mechanisms responsible for this effect presently remain elusive. The ability of NAME also to increase the stimulatory effect of endotoxin on ACTH secretion suggests that our findings can be extended to circumstances of increased endogenous cytokine production. Overproduction of ACTH and corticosteroids is detrimental to both the homeostasis of the organism and the normal function of the immune system. Thus, we propose that one of the roles of endogenous NO might be to restrain activation of the HPA axis during conditions of increased production of cytokines and/or neuropeptides such as occurs during immune stimulation (Kasting, 1986; Dinarello et al., 1992). While earlier work had suggested that there might be a very close relationship between immune activation and the release of corticosteroids (Besedovsky and Sorkin, 1975), changes in the activity of the HPA axis are now believed to occur only when the immune responses reach a certain threshold (Besedovsky and Sorkin, 1992). Indeed, we have recently reported that while all rats injected with keyhole limpet hemocyanin coupled to phosphocholine showed an increased release of antigen-specific IgM 5–7 d after immunization, only those animals that had received a very high dose of the antigen exhibited an increase in plasma corticosterone levels that coincided with the appearance of gamma globulins (Stenzel-Poore et al., 1993). As activated macrophages produce both NO (Moncada et al., 1991) [which inhibits CRF release (Costa et al., 1993)], and CRF [which stimulates ACTH secretion (Sapolsky et al., 1987; Rivier et al., 1989)], it is tempting to propose that the net effect of these inhibitory and stimulatory influences depends, as suggested above, on the intensity of the immune response. Thus, under conditions of moderate immune activation, the ability of cytokines to release CRF would be counteracted by the inhibitory effect of NO, and the inappropriate and putatively detrimental release of hormones from the HPA axis would be prevented. Following exposure to more intense immune stimuli, on the other hand, the amount of CRF secreted would be sufficient to increase circulating levels of proopiomelanocortin (POMC) peptides and corticosteroid, and thus prevent the possibly lethal overproduction of interleukins.

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