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Pituitary–Adrenal Axis Responses to Acute Amphetamine in the Rat

N. R. SWERDLOW,*[1] G. F. KOOB,† M. CADOR,‡ M. LORANG† AND R. L. HAUGER*

**Department of Psychiatry, UCSD School of Medicine, La Jolla, CA 92093-0804*
†Department of Neuropharmacology, Scripps Research Institute, La Jolla, CA 92037
‡Inserm, U 161, 2 Rue Camille Saint Saens, 33077 Bordeaux, France

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SWERDLOW, N. R., G. F. KOOB, M. CADOR, M. LORANG AND R. L. HAUGER. *Pituitary-adrenal axis responses to acute amphetamine in the rat.* PHARMACOL BIOCHEM BEHAV **45**(3) 629–637, 1993. – After acute administration of amphetamine (AMPH), a characteristic behavioral response occurs in the rat, involving increased locomotion and stereotyped licking, grooming, and biting. AMPH administration also activates several neuroendocrine systems, including the pituitary-adrenal axis. Because recent evidence has supported a role for glucocorticoids in modulating the behavioral response to AMPH, the purpose of the present study was to examine the relationship between behavioral and hypothalamic-pituitary-adrenal (HPA) responses to AMPH and determine the physiological substrates responsible for the AMPH-induced release of adrenal steroids. AMPH administration produced the often-reported "inverted-U" shaped behavioral response. Specifically, locomotion was increased by low doses (0.5–1.0 mg/kg, SC) significantly more so than by the highest dose (5.0 mg/kg, SC), which instead elicited intense focused stereotyped movements. Plasma levels of adrenocorticotropic hormone (ACTH) and corticosterone were increased by AMPH in a monotonic dose-response function, with highest levels measured in rats exhibiting the most intense stereotyped behaviors. Plasma ACTH levels then declined 10–30 min after AMPH administration, while AMPH-induced locomotion and stereotyped behavior persisted well beyond this period. In a parallel study, AMPH failed to elevate plasma levels of vasopressin, an important ACTH secretagogue, and AMPH reduced levels of corticotropin-releasing factor (CRF) immunoreactivity in the median eminence, providing indirect evidence of CRF release from this region. AMPH-stimulated ACTH and corticosterone release were prevented by immunoneutralization of CRF. These results show that the "nonlinear" behavioral response to AMPH is accompanied by activation of adrenocorticoids mediated by AMPH-stimulated CRF release from the median eminence and suggest that the stereotyped movements do not represent an active coping response to the stress-enhancing actions of amphetamine.

Adrenocorticotropic hormone Amphetamine Behavior Corticosterone Locomotion
Stereotyped behaviors Stress

THE behavioral response to amphetamine (AMPH) in the rat is highly dose dependent. Thus, low doses of AMPH stimulate behaviors that can be elicited by appetitive stimuli, and include locomotor activation, sniffing, and rearing (7,10). Higher doses of AMPH stimulate stereotyped, repetitive behaviors, including gnawing, licking, and "head-down" sniffing with movement restricted to one point in space (7,10), which can be elicited only by stressful stimuli (1). AMPH also stimulates several autonomic and endocrine changes that typically occur as part of the physiological response to stress. For example, AMPH increases heart rate, blood pressure (23), and the startle reflex (8) and stimulates the release of pituitary-adrenal hormones such as growth hormone and corticosterone (19,28).

The behavioral response to AMPH is believed to modify—and to be regulated by—the hypothalamic-pituitary-adrenal (HPA) axis. Adrenal responsivity is a known determinant of the behavioral response to AMPH. For example, adrenalectomy decreases the behavioral responses to chronic AMPH treatment, while adrenal hyperresponsiveness after acute stress enhances the development of behavioral sensitization to psychostimulants (28). It has also been proposed that the behavioral response to high doses of AMPH provides a "coping function" that limits the stressful effects of AMPH-induced activation (16). AMPH-induced stereotyped behaviors are potentiated by prior exposure to stressors such as food deprivation, noncontingent foot-shock, tail-pinch (1), novelty (14,33), and isolation rearing (34), and neurochemical lesions of the striatum that prevent the expression of AMPH-stereotyped behaviors prolong the neuroendocrine stress response to AMPH (16). Consequently, the behavioral response to AMPH may have an important role in modulating the neuroendocrine effects of this drug and, in converse, HPA function may have an important role in modulating the behavioral effects of AMPH.

While the neurochemical substrates of AMPH-induced behaviors are well known (7), relatively less is known about

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

the physiological substrates responsible for AMPH-induced activation of the HPA axis. The present studies were undertaken to assess the relationship between the neuroendocrine and behavioral effects of AMPH in rats and determine the physiological substrates of the pituitary-adrenal response to AMPH.

GENERAL METHOD

Adult, male Sprague-Dawley rats (250–350 g; Charles River, Boston, MA) were housed in groups of three with a normal 12 L : 12 D cycle and free access to food and water. Rats were allowed to acclimate for at least 2 weeks after arrival in the animal facility, followed by daily handling for several days prior to testing; testing occurred during the light phase, between 0800 and 1200 h.

BEHAVIORAL MEASURES

Locomotor activity after AMPH injections was measured using wire mesh photocell cages (22 × 35 × 15 cm) fitted with two parallel infrared beams 1 cm above the floor perpendicular to the long axis of the cage. Fifteen identical cages were monitored simultaneously; interruption of any photocell beam was registered by a computer and summed over 10-min intervals. During measurements of locomotor activity, ratings of stereotyped behaviors were made every 10 min by an experimenter who was blind to drug dose.

PLASMA COLLECTION

Trunk blood samples were collected by decapitation within 30 s following removal of the animal from its cage with precautions taken to minimize handling stress. Approximately 5 ml blood were collected in plastic conical centrifuge tubes containing 200 μl of a solution of 50 mg/ml EDTA and 500 kallikrein inhibitor units (KIUs) of aprotonin (Sigma Chemical Co., St. Louis, MO). Experiments involving trunk blood collection are described in the text and indicated in the figure legend.

In other experiments, a Silastic catheter was surgically implanted under halothane anesthesia in the right atrium via the jugular vein. The distal end of the catheter was passed under the skin, brought to the exterior between the scapulae, and filled with heparin (500 mU/ml in 0.9% NaCl). After surgery, rats were housed individually for 1 day to allow recovery from surgery. The next day, rats were transported in their home cages to a laboratory. For time course studies, blood samples (0.5–1.0 ml) were withdrawn at baseline and at multiple time points following AMPH administration (+10 to +120 min post-AMPH). Within 5 s of collection, the blood samples were placed in a polypropylene microfuge tube containing 1 mg/ml EDTA and 100 KIU/ml aprotonin. The total volume of blood withdrawn via the IV catheter was <1% of the body weight. Each blood sample was replaced with an equal volume of isotonic saline to compensate for blood loss during serial collection. All blood samples from trunk or catheters were collected on ice and plasma was prepared within 30–60 min by centrifugation at 4°C. Plasma samples were stored at −70°C until time of assay.

HORMONE ASSAYS

For plasma ACTH measurements, samples were extracted onto C18 Sep-Pak cartridges (Waters Associates, Inc., Milford, MA) and eluted with 60% acetonitrile in triethylamine formate buffer, pH 3.2. ACTH immunoreactivity was measured in lyophilized eluates by a double-antibody radioimmunoassay (RIA) using anticorticotropin serum immunoglobulin (Ig)G–ACTH-1 (IgG Corp., Nashville, TN), which is directed at the ACTH-(5-18) sequence, as previously described (29). The ACTH assay sensitivity was 2–4 pg/ml and the inter- and intraassay coefficients of variation (CV) are 7.8 and 4.8%, respectively. Plasma corticosterone concentrations were measured using a commercially available RIA kit that employs an antibody produced against corticosterone 21-hemisuccinate: bovine serum albumin (BSA) (ICN Biomedicals Inc., Costa Mesa, CA) (17). Plasma arginine-vasopressin levels were measured in extracts of plasma using a double-antibody nonequilibrium RIA method. Plasma extraction was accomplished using Amberlite CG-50 (Sigma) cation exchange resin (13). Plasma samples were acidified to a pH of 4.5 with 50% glacial acetic acid, and after centrifuging for 10 min the supernatant was decanted into tubes containing 100 mg Amberlite resin. After mixing for 30 min on a Labquake rotary mixer (Labindustries, Inc., Berkeley, CA), the resin suspension was centrifuged and the supernatant discarded. The resin pellet was then washed with 2.0 ml distilled water (pH 4.5) followed by a second wash with 50% ethanol/50% distilled water, and each supernatant was discarded. Elution of AVP from Amberlite was performed using a mixture of 75% ethanol and 25% water (pH 1.5) and the sample fraction was then lyophilized. The AVP RIA was performed at 4°C in RIA phosphate buffer (0.05 M sodium, phosphate, 0.15 M sodium chloride, 0.25% BSA, 0.1% sodium azide, 0.025 M eidetic acid, pH 7.4). The AVP antiserum was developed by Dr. Laurel Fisher and the ^{125}I-AVP tracer was obtained from Dupont-NEN (Wilmington, DE).

In some rats, regional brain levels of corticotropin-releasing factor (CRF) immunoreactivity were measured 60 min after AMPH administration. Following decapitation, the median eminence (ME), locus coeruleus (LC), amygdala, nucleus of the tractus solitarius (NTS), and neurointermediate pituitary were removed by free-hand dissection under a dissecting microscope and for each structure 500 μl (100 μl for the ME) was placed immediately into vials containing 100 μl 1 N HCl. The tissue was rapidly frozen on dry ice and stored at −70°C. Prior to the CRF assays, the tissue was rapidly thawed, homogenized by ultrasonic disruption, and centrifuged (10,000 rpm at 4°C for 15 min) as previously described (13). The acid extract was lyophilized in 10 × 75 polystyrene tubes and then reconstituted in 150 μl buffer (SPEA with 0.1% BSA, 0.05% Triton X-100, pH 7.4). Protein content was measured by the Pierce BCA assay. The CRF concentration in individual tissues was measured in duplicate using a modification of the RIA protocol described by Vale et al. (38). In brief, the reconstituted tissue or plasma samples were incubated at 4°C for 24 h with 100 μl CRF antiserum (rC70 raised in rabbits against rat/human CRF) at a final dilution of 1 : 666,666 (approximately 30% binding). At the end of this incubation, the tubes were incubated with 50 μl ^{125}I-Tyr°-rat/human CRF (15,000 cpm, SA-2200 Ci/mmol; Dupont-NEN) at 4°C for an additional 24 h. Afterward, sheep antirabbit gamma globulin (second antibody) was added with 10% polyethylene glycol to precipitate the bound CRF. The tubes were then centrifuged at 2,300 × g for 15 min at 4°C. After the supernatants were decanted, the pellets were counted in a gamma counter for 1 min to measure bound reactivity.

STATISTICS

For data analysis, the dependent variables included photocell counts, stereotyped behavior ratings, tissue CRF content,

and corticosterone, ACTH, and vasopressin levels. Photocell counts were analyzed using a two-way analysis of variance (ANOVA), with repeated measures on AMPH dose and time. Stereotyped behavior ratings were analyzed using the nonparametric Information Statistic test (20). Plasma hormone levels were analyzed using a two-way ANOVA with repeated measure on AMPH dose. Regional tissue CRF levels were compared by Student's t-test. Alpha was 0.05.

EXPERIMENT 1

Previous reports described the behavioral (7,10) and neuroendocrine (19,26) effects of AMPH administration in rats. The present study was designed to demonstrate the sensitivity of our paradigm to these effects and study the temporal relationship between AMPH-stimulated locomotor activity, stereotyped behaviors, and neuroendocrine activation.

METHOD

One day prior to testing, rats were placed individually for 180 min in the activity monitors described above. On the day of testing, rats were returned to the cages for 90 min. Each rat was then randomly assigned to receive one of four doses of AMPH sulfate (0.0, 0.5, 1.0, or 5.0 mg/kg, SC, in saline 1 cc/kg). These doses of AMPH were selected because they have been reported to stimulate a range of behaviors, including locomotor activation and focused stereotyped behaviors (1,7,10). Following drug treatment, rats were returned immediately to their cages. Their activity was monitored for 60 min. At 10-min intervals, rats were observed by an investigator who was not visible to the rats and was blind to their treatment (N.R.S.). Rats were observed for 20 s each, and the appearance of any of the following behaviors during that period was noted: still (no movement), sniffing, locomotion, rearing (both front paws elevated), grooming, licking, and head-down behavior in a restricted spot (10).

Sixty minutes after AMPH treatment, rats were removed from their cages and decapitated, and trunk blood was collected for measurement of plasma ACTH and corticosterone. This time point was chosen because previous studies reported that this is the peak point of intensity of the AMPH-induced stereotyped behaviors (7,10).

To assess the time course of the neuroendocrine response to AMPH, circulating levels of plasma ACTH and corticosterone were measured in a separate group of rats equipped with indwelling jugular vein catheters, as described above. Samples were taken prior to injection with 0, 0.5, 1.0, or 5.0 mg/kg AMPH SC and at times 10, 30, and 60 min after AMPH injection. Difference scores were calculated for each rat by subtracting the baseline (preinjection) hormone level from the level at each of the three points postinjection; in this manner, each rat served as its own "baseline" control. This experimental design reduced the variance caused by differences in individual baseline levels and by the "nonspecific" stressful effects of SC injections.

RESULTS

The locomotor response to AMPH is represented in Fig. 1. Two-way ANOVA with repeated measures on AMPH dose and time revealed a significant effect of AMPH dose, $F(3, 47) = 23.16$, $p < 0.0001$, a significant effect of time, $F(5, 235) = 17.99$, $p < 0.0001$, and a significant dose $\times$ time interaction, $F(5, 235) = 11.84$, $p < 0.0001$. Posthoc ANOVAs revealed a significant effect of 0.5, 1.0, and 5.0 mg/kg AMPH ($F = 18.76$, 53.56, and 4.72, respectively), while locomotor activity was lower in the 5-mg/kg dose group than it was in the 0.5-mg/kg dose group, $F(1, 23) = 10.28$, $p < 0.05$, or in the 1.0-mg/kg dose group, $F(1, 24) = 39.85$, $p < 0.0001$. Thus, as previously reported (7,10), locomotor activity in response to AMPH follows an "inverted-U" shaped dose responsivity, with maximal locomotor activity stimulated by submaximal doses of AMPH.

The behaviors exhibited 10, 30, and 60 min post-AMPH are represented in Table 1. Analysis by the Information Statistic (20) revealed that compared to vehicle-treated rats (0 mg/kg) exploratory behaviors (sniffing, locomotion, and rearing) were increased in rats treated with 0.5, 1.0, or 5.0 mg/kg AMPH in the first 10 min post-AMPH administration. By 20 min post-AMPH, there was the onset of repetitive, restricted stereotyped movements of the head and mouth in rats treated with the highest dose (5.0 mg/kg) of AMPH, with an associated decline in locomotor activity in these rats (data not shown). By 30 min post-AMPH, behaviors in rats treated with the highest dose of AMPH were restricted entirely to focused stereotypies, while rats treated with lower doses of AMPH (0.5 and 1.0 mg/kg) exhibited continuous locomotion, sniffing, and rearing, the latter of which remained pronounced only in the 1.0-mg/kg group by the end of the 60-min session. Thus, as previously reported, rats treated with low doses of AMPH exhibited activated behaviors, including locomotor activation, and ambulatory sniffing and rearing, while in rats treated with the highest dose of AMPH these ambulatory behaviors were rapidly replaced by repetitive stereotyped movements including licking, biting, and head-down sniffing in a restricted space.

Plasma levels of ACTH in AMPH-treated rats are represented in Table 2 and Fig. 2A. Samples from trunk blood taken 60 min after AMPH injection revealed no significant effect of AMPH on ACTH levels ($F < 1$) (Table 2). Consistent with this finding, plasma ACTH levels in samples taken via jugular vein catheters increased to a maximum at 30 min post-AMPH, followed by a decline at 60 min post-AMPH, where ACTH levels were significantly less than the +30-min levels. ANOVA with repeated measures over time on the ACTH response to SC AMPH challenge (difference from pre-AMPH baseline levels) revealed a significant effect of AMPH, $F(3, 15) = 4.67$, $p < 0.02$, a significant effect of time, $F(2, 30) = 13.20$, $p < 0.0001$, and a significant AMPH $\times$ time interaction, $F(6, 30) = 5.74$, $p < 0.0005$. Posthoc analysis revealed that the ACTH response to AMPH was maximal 30 min post-AMPH, $F(3, 16) = 8.55$, $p < 0.001$; at this point, the increase in plasma ACTH was the largest with the highest dose of AMPH [0 mg/kg vs. 5.0 mg/kg, $t(8) = 3.23$, $p < 0.012$], and this ACTH response was significantly diminished by 60 min post-AMPH [5-mg/kg dose, 30 min vs. 60 min, $t(4) = 4.56$, $p < 0.01$].

Plasma levels of corticosterone in AMPH-treated rats are represented in Table 2 and Fig. 2B. AMPH stimulated a significant dose-dependent increase in plasma corticosterone (Table 2). ANOVA revealed a significant effect of AMPH, $F(3, 47) = 10.14$, $p < 0.001$; posthoc analysis revealed that statistically significant increases in corticosterone were produced by the 1.0- and 5.0-mg/kg doses of AMPH, $t(8) = 3.74$ and 4.68, respectively, $p < 0.001$. Consistent with this effect, plasma corticosterone levels in samples taken via jugular vein catheters were significantly increased at 10, 30, and 60 min post-AMPH. ANOVA with repeated measures over time revealed a significant effect of AMPH, $F(3, 16) = 5.43$, $p < 0.01$, a significant effect of time, $F(2, 32) = 5.82$, $p < 0.01$,

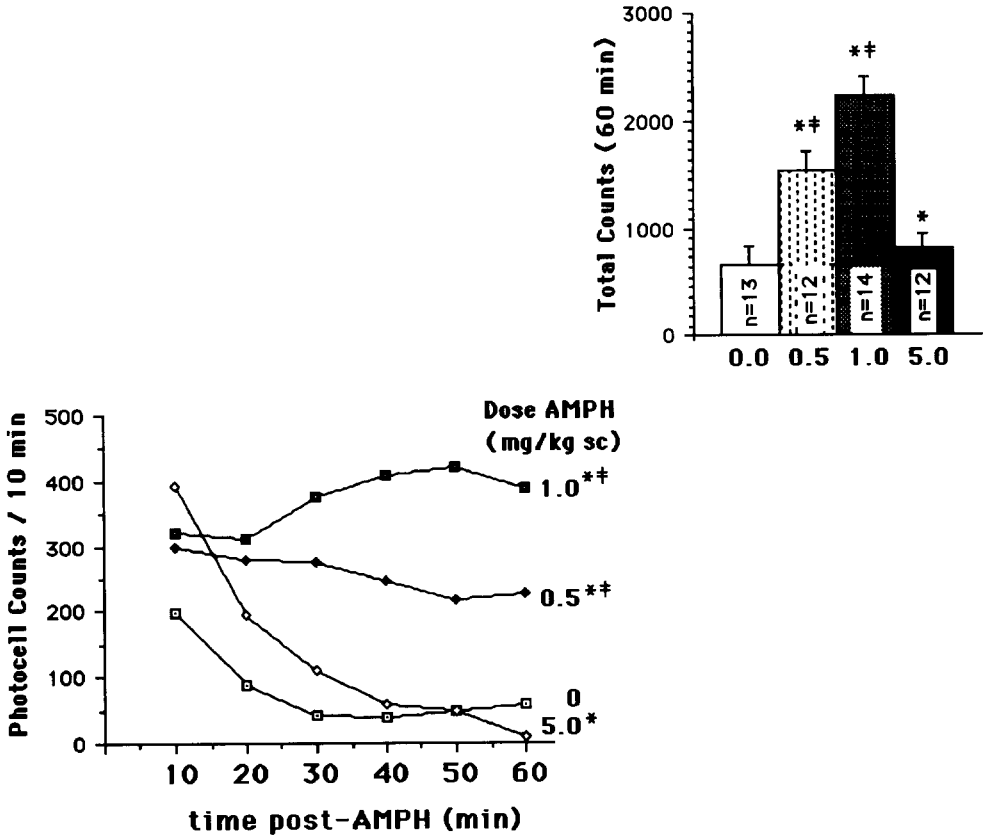


FIG. 1. Photocell activity (mean ± SEM) in rats treated with amphetamine (AMPH) (0, 0.5, 1.0, or 5.0 mg/kg, SC). *Significantly different from 0 mg/kg by posthoc analysis of variance (ANOVA) after significant overall effect of dose by ANOVA and significant dose × time interaction. †Significantly greater than 5.0-mg/kg dose by posthoc ANOVA.

and no significant AMPH × time interaction, $F(6, 32) = 1.44$, n.s. Posthoc ANOVAs revealed that the plasma corticosterone response in the 5.0-mg/kg group was significantly greater than the response in the 0-mg/kg group, $F(1, 8) = 10.50$, $p < 0.02$. Inspection of the data revealed that compared to pre-AMPH baseline levels corticosterone was elevated in all groups 10 min after SC injection. Corticosterone levels returned to baseline values by 60 min postinjection in rats treated with 0 mg/kg AMPH, plasma levels peaked at 30 min for rats treated with 0.5 or 1.0 mg/kg AMPH and began to decline by 60 min in these rats, while levels remained elevated over the 60-min post-AMPH period in rats treated with 5.0 mg/kg AMPH (Fig. 2B).

It is important to note that informal observation of post-AMPH behaviors exhibited by catheterized rats revealed behavioral activation consistent with patterns noted using blinded and objective measures in noncatheterized rats. Consequently, the presence of an indwelling jugular cannula and the withdrawal of serial blood samples did not appear to alter the behavioral responses to AMPH.

These results demonstrate the behavioral and neuroendocrine activation produced by acute treatment with AMPH. AMPH stimulates a dose- and time-dependent behavioral activation, characterized by the early stimulation of locomotor activity, which at higher doses evolves into intense and spatially restricted stereotyped behaviors. These AMPH-stimulated behaviors are paralleled by dose- and time-dependent changes in plasma levels of ACTH and corticosterone. Plasma ACTH levels are significantly increased shortly after AMPH administration, with the highest ACTH responses occurring at the 5.0-mg/kg dose during the emergence of high-intensity stereotyped behaviors. However, plasma ACTH levels return to baseline by 60 min post-AMPH despite the continued expression of AMPH-stimulated locomotion and stereotyped behaviors. Interestingly, rats treated with lower doses of AMPH exhibit a smaller and earlier peak in ACTH levels that begins to decline by 30 min post-AMPH despite the fact that these rats never exhibit stereotyped behaviors. AMPH also stimulates a dose-dependent increase in plasma corticosterone levels, and this elevation is sustained over the time course of the AMPH-induced behavioral changes, particularly in rats treated with 5.0 mg/kg AMPH. Thus, while the emergence of stereotyped behaviors in rats treated with 5.0 mg/kg AMPH is temporally contiguous with a reduction in plasma ACTH, the reduction in ACTH is noted even earlier in rats treated with lower doses of AMPH in the absence of any stereotyped behaviors. Further, plasma corticosterone levels remain elevated long after the emergence of stereotyped behaviors. Sixty minutes post-AMPH, low corticosterone levels are found in rats that are "still," moderately elevated corticosterone levels are found in rats that exhibit locomotion and rearing, and highest corticosterone levels are found in rats that exhibit restricted high-intensity stereotyped behaviors. Thus, while the appearance of AMPH-stimulated stereotyped behaviors may

TABLE 1

NUMBER OF RATS SHOWING SPECIFIC BEHAVIORS FOLLOWING AMPH

Dose AMPH (mg/kg)	Minutes Post-AMPH	Still	Sniff	Locomote	Rear	Groom	Lick	Gnaw	Head Down
0	10	7/13	4/13	3/13	0/13	0/13	0/13	0/13	0/13
0.5	10	1/12*	10/12*	7/12	5/12	0/12	0/12	0/12	0/12
1.0	10	0/14*	14/14*	14/14*	7/14*	0/14	0/14	0/14	0/14
5.0	10	0/12*	12/12*	12/12*	9/12*	1/12	0/12	0/12	3/12*
0	30	10/13	3/13	2/13	0/13	0/13	0/13	0/13	0/13
0.5	30	1/12*	10/12*	7/12*	5/12*	0/12	0/12	0/12	0/12
1.0	30	0/14*	14/14*	12/14*	10/14*	0/14	0/14	0/14	0/14
5.0	30	0/12*	12/12*	0/12*	3/12*	0/12	0/12	8/12	12/12*
0	60	11/13	1/13	1/13	0/13	0/13	0/13	0/13	0/13
0.5	60	2/12*	10/12*	7/12*	0/12	0/13	0/12	0/12	0/12
1.0	60	0/14*	14/14*	14/14*	9/14*	0/13	0/14	0/14	0/14
5.0	60	0/12*	10/12*	0/12	1/12	0/13	9/12*	11/12*	12/12*

10 min, statistically significant increase compared to 0 mg/kg: 0.5 mg/kg, sniff ($2\hat{I}$ = 7.43), still ($2\hat{I}$ = 6.52); 1.0 mg/kg, sniff ($2\hat{I}$ = 18.32), locomote ($2\hat{I}$ = 21.55), rear ($2\hat{I}$ = 11.50), still ($2\hat{I}$ = 12.93); 5.0 mg/kg, sniff ($2\hat{I}$ = 16.62), locomote ($2\hat{I}$ = 19.61), rear ($2\hat{I}$ = 19.17), still ($2\hat{I}$ = 11.7), head down ($2\hat{I}$ = 4.85). 30 min, statistically significant increase compared to 0 mg/kg: 0.5 mg/kg, sniff ($2\hat{I}$ = 9.76), locomote ($2\hat{I}$ = 5.21), rear ($2\hat{I}$ = 8.72), still ($2\hat{I}$ = 13.37); 1.0 mg/kg, sniff ($2\hat{I}$ = 21.55), locomote ($2\hat{I}$ = 14.75), rear ($2\hat{I}$ = 22.84), still ($2\hat{I}$ = 21.55), 50 mg/kg, sniff ($2\hat{I}$ = 19.61), gnaw ($2\hat{I}$ = 16.07), head down ($2\hat{I}$ = 34.62), still ($2\hat{I}$ = 19.61), rear ($2\hat{I}$ = 4.85). 60 min, statistically significant increase compared to 0 mg/kg: 0.5 mg/kg, sniff ($2\hat{I}$ = 16.43), locomote ($2\hat{I}$ = 7.99), still ($2\hat{I}$ = 12.64); 1.0 mg/kg; sniff ($2\hat{I}$ = 30.05), locomote ($2\hat{I}$ = 30.05), rear ($2\hat{I}$ = 16.12), still ($2\hat{I}$ = 25.33); 5.0 mg/kg, sniff ($2\hat{I}$ = 16.43), lick ($2\hat{I}$ = 19.17), still ($2\hat{I}$ = 23.13), gnaw ($2\hat{I}$ = 27.41), head down ($2\hat{I}$ = 34.62).

reflect a behavioral "coping" strategy aimed at reducing the stressful effects of AMPH, the emergence of these behaviors does not appear to specifically modify the pituitary-adrenal response to AMPH, and the restoration of baseline ACTH levels actually occurs most rapidly in rats that exhibit pronounced locomotor activation but do not exhibit stereotyped behaviors.

While ACTH appeared to return to baseline levels by the close of the 60-min observation period, corticosterone levels remained elevated at that time. This leaves open the possibility that later measurements might have detected a reduction in plasma corticosterone that was related to the coping properties of stereotyped behaviors. This possibility is made less likely by the observation that a substantial reduction in plasma corticosterone was noted between 30 and 60 min post-AMPH in rats treated with lower doses of AMPH (1.0 mg/kg), which stimulated locomotor activation, but a decrease in corticosterone was not seen in rats treated with higher doses of AMPH (5.0 mg/kg), which stimulated intense stereotyped behaviors (Fig. 2B).

EXPERIMENT 2

While the behavioral and neuroendocrine responses to acute AMPH injection are documented in Experiment 1, these findings do not provide any information about the physiological substrates by which AMPH stimulates the neuroendocrine "stress" response. Two known ACTH secretagogues are vasopressin and CRF (31). In the present study, we assessed the effects of acute AMPH administration on plasma levels of vasopressin and on levels of CRF in the median eminence and other brain regions. CRF levels in the median eminence may be an indirect measure of CRF turnover, and decreased median eminence CRF may correlate with increased release of CRF into the portal circulation (13).

METHOD

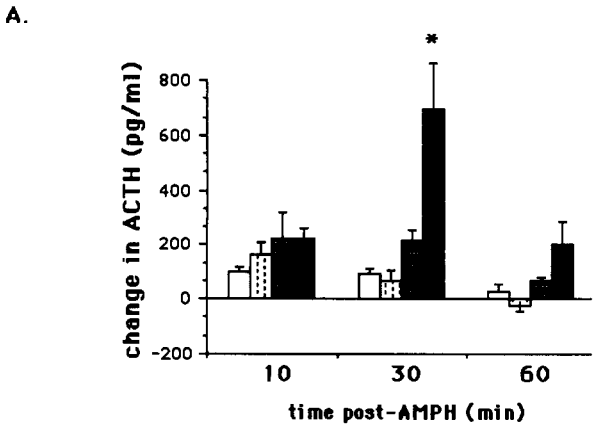
Twenty rats were equipped with indwelling jugular catheters as described above. One week later, plasma samples were obtained from some rats as previously described 10–15 min prior to and 15, 30, 60, and 120 min after injection of AMPH

TABLE 2

ACTH AND CORTICOSTERONE LEVELS 60 min POST-AMPH

	Dose AMPH (mg/kg, SC)			
	0	0.5	1.0	5.0
Plasma ACTH (ng/ml) ± SEM	70.4 ± 10.8	89.7 ± 17.7	77.9 ± 12.6	67.7 ± 13.9
Plasma CORT (pg/ml) ± SEM	91.8 ± 24.5	159.6 ± 28.3	214.5 ± 22.0*	320.2 ± 43.4*

*Significantly different from 0-mg/kg value, $p < 0.001$ by t-test after significant main effect of dose by ANOVA.



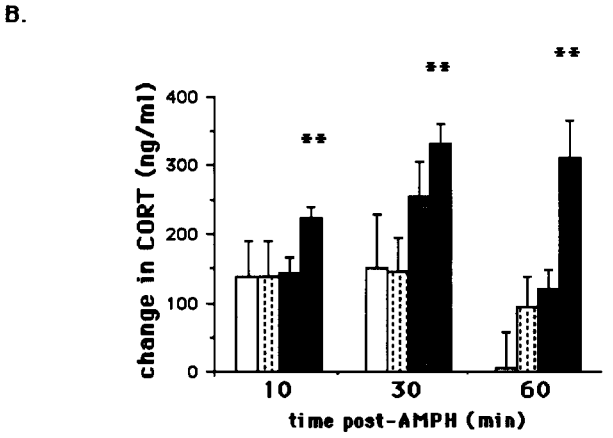


FIG. 2. Effects of amphetamine (AMPH) on plasma adrenocorticotropic hormone (ACTH) and corticosterone (CORT). (A) ACTH response (mean change from pre-AMPH baseline ± SEM) from IV samples in catheterized rats collected 10, 30, or 60 min post-AMPH. *Significantly greater than 0-mg/kg dose by unpaired *t*-test after significant main effect of dose and dose × time interaction by analysis of variance (ANOVA), and a significant effect of dose at 30-min time point by independent ANOVA. (B) CORT response (mean change from pre-AMPH baseline ± SEM) from IV samples in catheterized rats collected 10, 30, or 60 min post-AMPH. **Significantly greater than 0-mg/kg dose by independent ANOVA after significant main effect of dose by ANOVA and no significant dose × time interaction.

(0 or 5.0 mg/kg, IV, $n = 4$ each dose) and assayed for vasopressin as described above. IV injections of AMPH were used in this study to avoid the nonspecific stressful effects of SC injections noted in Experiment 1. Some rats ($n = 12$) were sacrificed 60 min post-AMPH injection and brain regions were obtained for analysis of CRF levels, as described above.

RESULTS

While behavioral measures were not quantified in these rats, nonblind observation revealed a clear pattern of AMPH-induced behavioral activation consistent with patterns noted in rats treated with 5.0 mg/kg AMPH SC using more formal measures in Experiment 1.

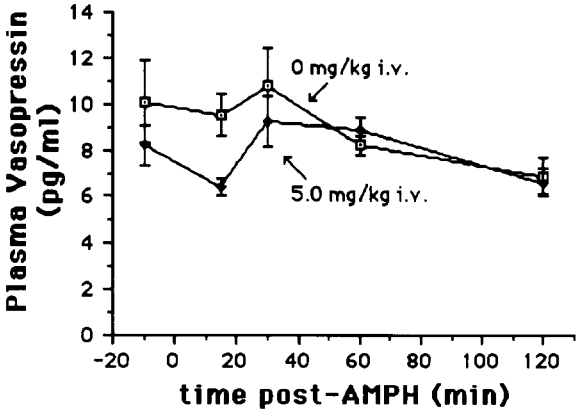


FIG. 3. Effects of IV amphetamine (AMPH) (0 or 5.0 mg/kg) on plasma levels of vasopressin (mean ± SEM). There is no significant effect of AMPH on vasopressin levels.

Findings from neuroendocrine measures in these rats are presented in Fig. 3. Basal levels of vasopressin did not differ significantly between groups, $t(6) < 1$, n.s. In contrast to our findings with ACTH and corticosterone (Experiment 1), AMPH did not significantly increase plasma levels of vasopressin (Fig. 3), $F(1, 7) = 1.75$, n.s. There was a significant effect of time, $F(3, 18) = 3.67$, $p < 0.04$, but no significant dose × time interaction, $F(3, 18) = 1.34$, n.s. Thus, AMPH-stimulated ACTH release is not accompanied by a significant increase in plasma levels of vasopressin, an important ACTH secretagogue.

Regional brain levels of CRF are presented in Table 3. As previously reported (13), CRF content is highest in the median eminence. These levels are significantly reduced 60 min post-AMPH injection ($t = 3.8$, $p < 0.05$). This decrease is equivalent to over 32% of total median eminence CRF content and suggests that AMPH stimulates CRF release from this structure. In contrast, no post-AMPH reduction in CRF is noted in the amygdala, locus coeruleus, NTS, or neurointermediate pituitary ($p > 0.10$ all comparisons).

These results provide preliminary evidence that AMPH-stimulated increases in plasma levels of ACTH and corticosterone are accompanied by the release of CRF from the median eminence but are not accompanied by increases in plasma levels of vasopressin. This observation adds to our understanding of the physiological substrates of AMPH-induced neuroendocrine activation because many physiological manip-

TABLE 3

EFFECT OF AMPH (0–5.0 mg/kg, IV) ON TISSUE CRH IMMUNOREACTIVITY (pg/mg PROTEIN) [MEAN (SEM)]

Region	0 mg/kg	5.0 mg/kg
Median eminence	26,960 (2,277)	18,200 (2,642)*
Amygdala	141 (18)	120 (12)
Locus coeruleus	504 (52)	406 (31)
Nucleus tractus solitarius	111 (14)	104 (16)
Neurointermediate pituitary	704 (70)	697 (132)

*$p < 0.05$, 0 mg/kg vs. 5.0 mg/kg.

ulations that potentiate plasma ACTH levels—such as restraint or hemorrhage (13,18,27,31)—cause the release of both vasopressin and CRF. We should use caution in interpreting the failure to detect changes in plasma vasopressin, which might reflect the insensitivity of plasma levels to changes in pituitary vasopressin release. The AMPH-induced changes in CRF activity appear to be anatomically localized to the median eminence and are not noted in other brain regions that have been implicated in the behavioral and neuroendocrine effects of AMPH and CRF (6,22,36,39).

EXPERIMENT 3

These findings suggest that AMPH stimulates CRF release and that this stimulation may be responsible for the AMPH-induced elevation of plasma ACTH and corticosterone. More direct evidence is required, however, before one can conclude that AMPH-stimulated CRF release is necessary for the observed changes in ACTH and corticosterone. Support for such a causal link might be provided by evidence that disruption of CRF activity prevents AMPH-induced increases in plasma ACTH and corticosterone. Previous work demonstrated that ACTH and corticosterone secretion are not stimulated by either acute stress or an IV injection of cocaine if rats are pretreated with a CRF antiserum (30). In the present experiment, we assessed the effects of CRF immunoneutralization on the neuroendocrine response to acute AMPH administration.

METHOD

Twenty-four hours after recovery from surgery, IV-cannulated rats were divided into two groups (n = 15–16 per group). The IV cannula line was flushed with 0.5 ml heparinized 0.9% saline. Approximately 1 h later, a baseline sample (1 ml) was withdrawn. Ten minutes later, one group received IV injection of 0.25 ml CRF antiserum while the other rats were administered 0.25 ml normal rabbit serum (control group). The CRF antiserum was kindly provided by Dr. Wylie Vale (Salk Institute, La Jolla, CA), while the normal rabbit serum was purchased from Peninsula Laboratories (Belmont, CA). One minute after the above pretreatment, each group was injected IV with 5.0 mg/kg AMPH. Previous work has shown that basal pituitary-adrenal hormone levels are not altered by IV injection of CRF antiserum or normal rabbit serum. In addition, our data demonstrated a large three- to fourfold stimulation of pituitary-adrenal hormone release after AMPH without any concomitant changes in plasma ACTH or corticosterone following IV saline injection. Consequently, the effects of CRF antiserum or normal rabbit antiserum pretreatment on AMPH responses were only examined. Following IV AMPH injection, additional blood samples (1.0 ml) were collected at +10, +30 min, and +60 min post-AMPH. An equal volume of isotonic saline was injected into individual animals following each blood sample to replace circulatory volume. Assays for ACTH (n = 31 rats) and corticosterone (n = 16 rats) were completed as described above.

RESULTS

Findings from neuroendocrine measures in these rats are presented in Fig. 4. Rats treated with control serum (Fig. 4) exhibited the expected AMPH-induced increases in plasma ACTH and corticosterone, as noted in Experiments 1 and 2. This AMPH-induced neuroendocrine activation was prevented by CRF immunoneutralization. Basal levels of ACTH did not differ significantly between normal serum and antiserum groups, $t(29) = 1.24$, n.s. Analysis of ACTH levels using ANOVA with repeated measures over time revealed a significant effect of antisera, $F(1, 29) = 32.88$, $p < 0.001$, a significant effect of time, $F(2, 58) = 45.55$, $p < 0.001$, and a significant antisera × time interaction, $F(2, 58) = 16.62$, $p < 0.0001$. Posthoc planned comparisons were performed using a one-way ANOVA to determine individual time point differences. Serum and antiserum groups were significantly different at +10 min, $F(1, 29) = 22.89$, $p < 0.0001$, +30 min, $F(1, 29) = 29.23$, $p < 0.0001$, and +60 min, $F(1, 29) = 20.59$, $p < 0.0001$. Plasma ACTH in the CRF antiserum group was significantly lower at +60 min than it was at baseline (paired t-test, $p < 0.001$), whereas plasma ACTH in the normal rabbit serum group returned to baseline by this time point.

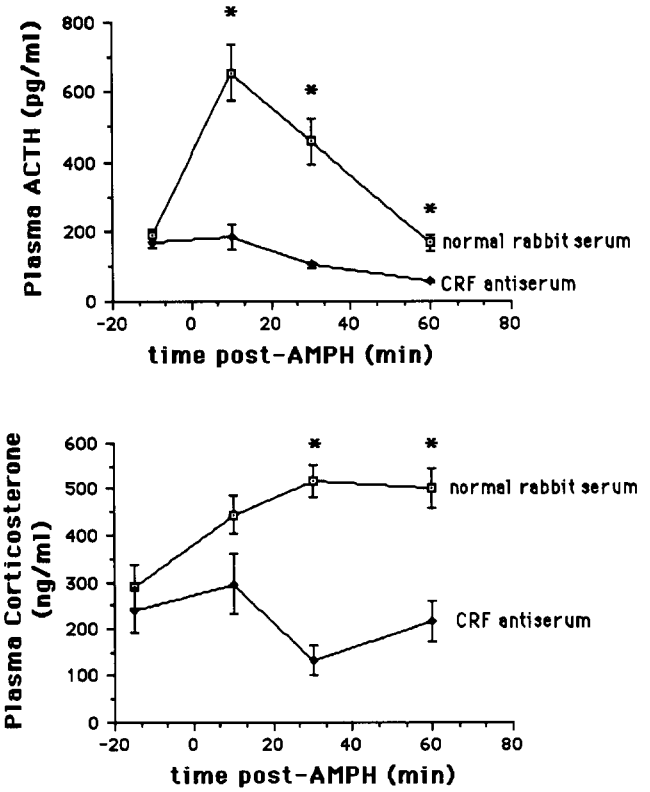


FIG. 4. Effects of corticotropin-releasing factor (CRF) immunoneutralization on the plasma adrenocorticotropic hormone (ACTH) (mean ± SEM) (top) and corticosterone (mean ± SEM) (bottom) responses to amphetamine (AMPH). Immunoneutralization significantly decreases AMPH-induced plasma ACTH and corticosterone stimulation. *Significantly greater than CRF antiserum group by independent analysis of variance (ANOVA) after significant main effect of antisera by ANOVA.

Basal levels of corticosterone did not differ between normal serum and antiserum groups, $t(14) < 1$, n.s. Analysis of corticosterone levels using ANOVA with repeated measures over time revealed a significant effect of antisera, $F(1, 14) = 4.34$, $p < 0.0001$, no significant effect of time, $F(2, 28) < 1$, n.s., and a significant antisera × time interaction, $F(2, 28) = 4.31$, $p < 0.025$). Posthoc planned comparisons were performed using a one-way ANOVA to determine individual time point differences. Antiserum groups were significantly differ-

ent at +30 min, $F(1, 14) = 61.34$, $p < 0.0001$, and +60 min, $F(1, 14) = 19.55$, $p < 0.0006$, but this difference only approached significance at +10 min, $F(1, 14) = 3.40$, $p < 0.09$.

GENERAL DISCUSSION

Numerous studies have documented the monoaminergic regulation of stress-induced neuroendocrine (2,3,9,11,15,19, 28,40) and behavioral (1,16,25,33,34) responses, as well as the robust changes in regional monoaminergic activity that occur in response to acute (40) and chronic (35) stressors. AMPH is an indirect monoamine agonist that stimulates behavioral and neuroendocrine changes that are similar to those produced by acute stressors, and several investigators proposed that AMPH-stimulated stereotyped behaviors serve to reduce the stressful properties of higher doses of AMPH (16). Similar hypotheses have been directed toward stereotyped gnawing or eating elicited by tail-pinch (1).

In the present experiments, behavioral and neuroendocrine changes in rats were examined after acute treatment with a dose range of AMPH. AMPH-stimulated behavioral changes followed the often-reported "inverted-U" shaped dose–response curve—low doses causing locomotor activation and high doses causing restricted, stereotyped behaviors—while the AMPH-stimulated changes in plasma ACTH and corticosterone levels followed simple monotonic ascending dose–response properties.Thus, if stereotyped behaviors serve to reduce the "stressful" properties of higher doses of AMPH this "stress" reduction was not reflected by a unique reduction in the ACTH or corticosterone responses to AMPH. The peak ACTH response to AMPH coincided temporally with the onset of stereotyped behaviors, so one might argue that the subsequent reduction in ACTH reflected the "stress-reducing" effects of stereotyped behaviors; such an interpretation is weakened by the observation that the increase in ACTH levels stimulated by low and middle doses of AMPH (0.5–1.0 mg/kg) peaks and declines sooner than those stimulated by higher doses of AMPH, despite the fact that these low and middle doses of AMPH elicit robust locomotor activity but no stereotyped behaviors. In other words, the intensity and latency of the ACTH "peak" response to AMPH is a function of AMPH dose rather than the appearance of any particular behavior.

The physiological substrates of AMPH-stimulated neuroendocrine changes are consistent with existing mechanisms that mediate the neuroendocrine response to other acute stressors (13,31). Thus, the results suggest that AMPH stimulates endogenous release of CRF from the hypothalamus but does not increase the plasma levels of vasopressin, another important ACTH secretagogue. A causal relationship between this CRF release and AMPH-induced increases in plasma ACTH and corticosterone is suggested by our finding that this AMPH-stimulated neuroendocrine response is prevented by immunologic disruption of the effects of hypothalamic CRF activity on the pituitary. CRF regulation of AMPH-induced neuroendicrine activation may be germaine to evidence linking CRF to the behavioral manifestations of stress, anxiety, and affective states in preclinical and clinical models (4,5,12).

Recent studies indicated that cocaine, another psychostimulant and indirect monoamine agonist, can stimulate ACTH release via D_1 and D_2 dopaminergic and 5-hydroxytryptamine$_2$ (5-HT_2) serotonergic mechanisms (32,37). Further, serotonin in particular has been shown to modulate ACTH secretion (11) and AMPH-induced corticosterone release (19) and to be a potent stimulus of hypothalamic CRF release (32,37). Because AMPH can increase both dopamine and serotonin release in the CNS, these two transmitters may also have a role in the activation of the HPA axis by AMPH. This is not to suggest that the monoamine-enhancing effects of AMPH provide the unitary mechanism for AMPH-induced HPA activation. Indeed, interactions between monoaminergic and endocrine systems are modulated by complex feedback and feedforward mechanisms (24) that may be behavior dependent and may involve both adrenal and extraadrenal sources of corticosterone release (21).

The present study focuses on the relationship between the behavioral and neuroendocrine responses to AMPH and on the physiological substrates that mediate the AMPH-induced stimulation of ACTH and corticosterone release. A temporal dissociation between the behavioral and neuroendocrine responses to AMPH has been shown, and because of this observation a causal link between AMPH-stimulated behaviors, CRF release, and increased plasma levels of adrenocorticoids is questioned. While the present study does not address whether AMPH-stimulated CRF release has a critical role in AMPH-induced behavioral activation, this issue is a focus of ongoing investigations.

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