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Effects of β-phenylethylamine on the hypothalamo–pituitary–adrenal axis in the male rat

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Abstract

β-Phenylethylamine (PEA) is a trace neuroactive amine implicated in the regulation of the hypothalamic–pituitary–adrenal (HPA) response to stress. To test this hypothesis, effects of subchronic levels of PEA (50 mg/kg/day treatment for 10 days) on the corticotroph function were studied. PEA treatment induces: (i) a significant increase of corticotrophin releasing hormone (CRH) immunoreactivity in the median eminence (ME), as measured by semi-quantitative immunofluorescence labeling techniques, (ii) a significant increase in CRH mRNA levels in paraventricular nuclei, as detected by in situ hybridization, and (iii) an increase in plasma adreno-corticotrophin hormone (ACTH) and corticosterone levels in responses to stress. PEA treatment has no effect on the number of binding sites and on the dissociation constant of the glucocorticoid receptors in any structure studied. Results of the dexamethasone suppression test were similar in PEA- and saline-treated rats. Taken together, these results suggest that PEA treatment stimulated the HPA axis activity levels directly via the CRH hypothalamic neurons, without altering the negative feed back control exerted by the glucocorticoids.

Keywords: β-Phenylethylamine; CRH; ACTH; Corticosterone; Glucocorticoid receptors; Median eminence; Paraventricular nucleus of the hypothalamus; Stress; Rat

1. Introduction

β-Phenylethylamine (PEA) is an endogenous amine that has been identified in the brain of several mammalian species including man. PEA is considered to be an endogenous amphetamine, and does, in fact, have their basic structure. Like amphetamines, it could mimic a sympathetic mimetic role identical to that of catecholamines (with respect to their effects and not their mechanisms), as the actions of amphetamines depend on the dose and the locus of treatments. PEA is found at lower levels than other monoamines in the brain except in the limbic system, which contains large amounts [3,39,40]. Unlike other catecholamines, PEA is not stored in vesicles: reserpine, which abolishes vesicular storage of catecholamines, increases the storage of PEA [16], and electrical stimulation of neurons does not cause PEA release [10]. Its release appears to be dependent on the concentration of PEA, showing that PEA is not a typical neurotransmitter. High affinity saturable PEA binding sites have, however, been demonstrated in the rat brain using [^{3}H]-PEA, and are different to the standard monoaminergic receptors [14]. Treatment with inhibitors of the enzyme monoamine oxidase-B (IMAO-B) abolish this binding [21].

Numerous studies have evidenced the role of PEA in the regulation of the central nervous system and in particular its neuromodulating effects on catecholaminergic activity in the mammalian brain. PEA increases the release of catecholamines and blocks catecholamine uptake [25]. Changes in PEA metabolism and their physiological consequences have been extensively studied, and relationships have been demonstrated between the plasma concentration of PEA and various affective disorders [29] and stresses [12,26]. Transgenic mice carrying a deletion of the gene encoding MAO-B have higher levels of PEA than wild-type mice. When the MAO-B deficient mice were exposed to stress that was

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inescapable (forced swim test), they showed a significantly increased mobility [12].

In view of these findings, it is likely that PEA is involved in the regulation of behavioral and hormonal responses to stress. To investigate this possibility, we studied the effects of chronic treatment with PEA on the hypothalamo-hypophyso-corticoadrenal axis. Various components of this axis were studied, under control and stress conditions. As PEA is structurally similar to amphetamines, and amphetamines affect the expression of glucocorticoid receptors in the hippocampus [35], we also studied the effects of chronic PEA treatment on these receptors.

2. Materials and methods

2.1. Animals

Ninety male Sprague–Dawley rats (250–350 g) were obtained from CE Depré (Saint Doulchard, France). For the duration of each experiment, they were housed in individual standard clear plastic housing chambers ($265 \times 205 \times 140$ mm) in light- and temperature-controlled rooms, photoperiod 12 h light/12 h dark (12L/12D, light on at 06:00) with food (pellets, UAR Paris, France) and water *ad libitum*.

The experimental protocols conformed to French laws concerning laboratory animals and were approved by the management committee of the Center for Laboratory Animals, which houses all animals under experimental investigation on the campus.

Rats were randomly assigned into two groups and injected once daily for 10 days at 1700 h with vehicle or PEA. PEA was obtained from Sigma (St. Louis, MO, USA) and was injected intraperitoneally in a volume of 0.5 ml dissolved in 0.9% saline at 50 mg/kg body weight [3,41]. The first injection was given 10 days before the stress procedure or dexamethasone suppression test. Five days before the stress procedure or dexamethasone suppression test, a chronic cannula was inserted in the right carotid of the rat using a previously described method [11] to allow sequential blood sampling and subsequent plasma hormone assay.

2.2. Ether stress

Rats were placed for 2 min in a closed cylindrical box containing ether vapor, after which time they had usually lost their righting reflex. They were then moved back to their home cage. Blood samples were collected 30 min before stress, and 15, 30, 60, 120 min after the start of ether stress. Blood was immediately centrifuged and the resulting plasma was kept at $-20°C$.

2.3. Dexamethasone suppression test

Blood was collected on day 1 at 0900, 1200, and 1800 h and immediately after this last blood sample, rats were injected subcutaneously with dexamethasone (either 1 μg/kg or 10 μg/kg). The day after, blood was again collected at 0900, 1200 and 1800 h. Blood samples were immediately centrifuged and the resulting plasma was kept at $-20°C$.

2.4. Radioimmunoassay (RIA)

Corticosterone concentrations were determined by radioimmunological assay, validated in the laboratory for 20 μl aliquots of plasma. The detection limit was to 5 ng/ml; intra- and inter-assay coefficients of variations were below 6% and 8%, respectively.

Rat plasma adreno-corticotrophin hormone (ACTH) was measured in 50 μl samples using RIA kits (CEA-ORIS, Sarclay, France). Intra- and inter-assay coefficients of variation were 5% and 9%, respectively. The lowest detectable plasma ACTH concentration was 25 pg/ml.

2.5. Corticosteroid receptor binding assays

Rats were adrenalectomized 12–14 h prior to being sacrificed using ether anesthesia, allowing ample time for clearance of the endogenous steroid. Animals were sacrificed between 0900 and 1100 h, by decapitation, within 2 min of their removal from their cages.

An in vitro cytosolic binding assay was used to measure adrenal steroid receptor levels [33,36]. Tissue samples (hippocampus, hypophyse, hypothalamus) were homogenized and centrifuged in 30 mM Tris, 1 mM EDTA, 10 mM sodium molybdate, 10% v/v glycerol and 1 mM dithiothreitol (TEDGM, pH 7.4) containing radiolabeled steroids with or without unlabeled competitors. For saturation binding assays, six different concentrations (0.2–6 nM) of (^{3}H) dexamethasone (47 Ci/mmol; NEN) or (^{3}H) aldosterone (52 Ci/mmol; NEN) were used in duplicate. Glucocorticoid receptor (GR) binding was derived by subtracting (^{3}H) dexamethasone binding in the presence of a selective GR competitor, RU 29362. Mineralocorticoids receptor (MR) binding was obtained by subtracting non-specific binding (in the presence of 0.5 μM corticosterone) from (^{3}H) aldosterone binding (in the presence of 0.5 μM RU 28362). After overnight incubation (18–22 h, 4°C), cytosol and incubation solutions were filtered to separate bound from unbound steroid using dextran-coated charcoal absorption. Samples were treated with 500 μl of a charcoal–dextran–gelatin mixture (1%:0.3%:0.1%) for 10 min at 4°C and centrifuged at $4000 \times g$ for 15 min. The supernatant, containing bound steroid, was collected, added to scintillation cocktail and radioactivity was counted in a Packard β-counter with 55% efficiency for tritium. For the single point assay, cytosol aliquots were incubated with TEDGM containing, for GR binding: (^{3}H) dexamethasone saturated with or without RU28362 (0.5 μM), and for MR binding: (^{3}H) aldosterone-RU28362 (0.5 μM) with or without 5 μM corticosterone. After incubation, samples were applied to sephadex LH-20 columns (Pharmacia Fine Che-

micals) equilibrated with TEDGM and washed 5 min later in the same buffer. The eluate, containing bound steroid, was collected into scintillation vials and radioactivity counted as described previously. Protein content was determined by the method of Bradford [4] and specific binding was expressed as fmol per mg of cytosol protein. Maximal numbers of binding sites (B_{max}) and dissociation constants (K_d) were derived from saturation experiments using the LIGAND program previously described [20].

2.6. Immunocytochemistry

2.6.1. Tissue preparation

Rats were perfused intracardially with phosphate-buffered saline (PBS) followed by a freshly prepared solution of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 6.9) containing 0.2% picric acid for immunocytological fluorescence microscopy. Brains were rapidly dissected and immersed in the same fixative for 24–48 h 4°C, rinsed overnight in PBS containing 10% sucrose, frozen on dry ice and 12 μm frontal cryosections were cut. Sections were mounted on gelatin-coated glass slides and kept at 20°C for 4 h. They were then treated for indirect immunofluorescence.

Sections were stained for corticotrophin releasing hormone (CRH) and incubated overnight at 4°C in a humid atmosphere with a rabbit polyclonal anti-CRH antibody (a kind gift by Dr. G. Barbanel et al. [1]) diluted 1/1000. Sections were then rinsed in PBS, incubated with fluorescent isothiocyanate-conjugated goat rabbit antiserum (diluted 1/1000) for 4 h at room temperature, rinsed in PBS, mounted on slides in Mowiol and examined under a Leica fluorescence microscope.

Specificity of immunostaining was assessed by incubating the primary antiserum with its specific antigen (i.e. 0.1–0.5 mg synthetic CRH/ml of diluted antiserum) and then incubating the pre-adsorbed antiserum with control sections for 4 h at room temperature.

The difference in the intensity of immunostaining between the two groups was first estimated visually and then quantified from the original film negatives. Immunofluorescent sections were photographed on Tris-X Kodak film under constant fluorescence illumination conditions (HBO 200 high-pressure mercury lamp; excitation filter 12 450–490 nm; selection filter 515 nm), magnification ($\times 16$ objective) and exposure (45 s). The negatives were placed on a viewing box under constant illumination and the intensity of fluorescence quantified using a semiautomatic image analyzer (Biocom 200, Biocom, Les Ulis, France) coupled to a computer.

For each axon, immunostaining was assessed in five to seven sections covering various areas of the median eminence (ME). On each negative analyzed, the area of tissue labeled per section and the intensity of axon labeling were quantified. ME areas were classed as labeled if they had gray-scale values higher than those measured outside the ME in an unlabelled zone, used as background. The extent of the ME that was labeled was then expressed as a percentage of the total area of the ME. Labeling intensity was estimated from the mean gray-scale value for the labeled zones of the internal ME. It was corrected for non-specific variations in fluorescence by subtracting the mean gray-scale value of the unlabelled zones on the same section.

2.7. In situ hybridization

2.7.1. Tissue preparation

Rats were decapitated and brains were carefully removed, immediately frozen on dry ice, and stored at −80°C until use. Cryosections (12 μm) through the hypothalamic paraventricular nucleus (PVN) were mounted on slides coated with two layers of gelatin, dried with a slide warmer and kept at −80°C.

Sections were warmed to room temperature and fixed with 4% formaldehyde in PBS, pH 7.2, for 5 min. They were washed twice in PBS N-acetylated with 0.25% acetic anhydride in 0.1 M triethanolamine, 0.9% NaCl (pH 8) for 10 min and delipidated in ethanol and chloroform. A synthetic oligonucleotide directed against to bases 496–543 (48 bases) of the rat CRH mRNA was used as a probe. The CRH probe was labeled with (^{35}S) dATP (1300 Ci/mmol; New England Nuclear, Paris, France). The specific activity of the radioactive probe was about 12,000 Ci/nmol.

Sections from the hypothalamic PVN sections were hybridized overnight at 37°C in 4× SSC (1× SSC = 0.15 M NaCl and 0.015 M sodium citrate, pH 7.2), 50% formamide, 10% dextran sulfate, 50 ng/ml sheared single-stranded salmon sperm DNA, 25 mg/ml yeast tRNA, 1× Denhardt's solution, 0.1 M dithiothreitol with 1×10^6 dpm (^{35}S) CRH probe under a parafilm coverslip. The coverslip was removed by immersion in 1× SSC and slides were washed four times in 2× SSC. The slides were placed against X-ray film (Biomax-MR Kodak) for 24 h and autoradiographs were used to quantify CRH mRNA levels throughout the PVN. Corresponding darkfield photomicrographs were taken in the PVN.

2.7.2. Quantification of the hybridization signal

Brain paste standards were used to calculate the number of CRH mRNA copies detected as described by Young et al. [42]. The standards were made by homogeneously mixing different amounts of γ-(^{35}S)adenosine 5′-(γ-thio) triphosphate (New England Nuclear, >1000 Ci/mmol) into aliquots of brain paste (260 to 10^6 dpm/mg). The paste was frozen and 12-μm sections were cut, each containing the nine standards. Variation in section thickness was ±1 μm (S.D.). These sections were exposed concurrently with the experimental sections. Grain reflectances were determined over each standard and plotted against dpm/mg corrected for the half-life decay of ^{35}S. The greatest densities of autoradiographic grains in experimental sections were always within the non-saturated portion of the calibration curve that allowed interpolation of dpm/mg values from the grain

densities. The dpm/mg values were then converted to copies per μm^3 of tissue according to the formula [42]: copies/μm^3 = dpm/mg $\times$ 1.0 $\times$ 10^{-9} mg/μm^3 $\times$ (1/SA) $\times$ A (where SA = probe specific activity in dpm/mol, and A = Avogadro's number 6.02 $\times$ 10^{23} copies/mol).

All sections hybridized were analyzed. The hybridization signal was quantified from autoradiographs and all images were concurrently digitized. The optical density of the labeled areas was determined with a Biocom 200 (Biocom, Les Ulis, France) image analysis system.

2.8. Statistical methods

Data are given as means ± S.E.M. One way ANOVA was therefore performed on all data to detect significant ($P < .05$ or $P < .01$) differences between the two groups.

3. Results

3.1. Effect of subchronic PEA treatment on transcription of the gene encoding CRH in the PVN

As illustrated by light microscope darkfield microphotographs (Fig. 1), the labeling obtained after in situ hybridization with the radioactive probe was distributed throughout the medial parvocellular area of the hypothalamic PVN while the magnocellular area only showed non-specific labeling. Subchronic PEA treatment resulted in a higher signal in the PVN: as assessed by autoradiography and densitometric scanning (Fig. 2), the signal was 136% higher in PEA-treated than vehicle-treated animals ($P < .01$).

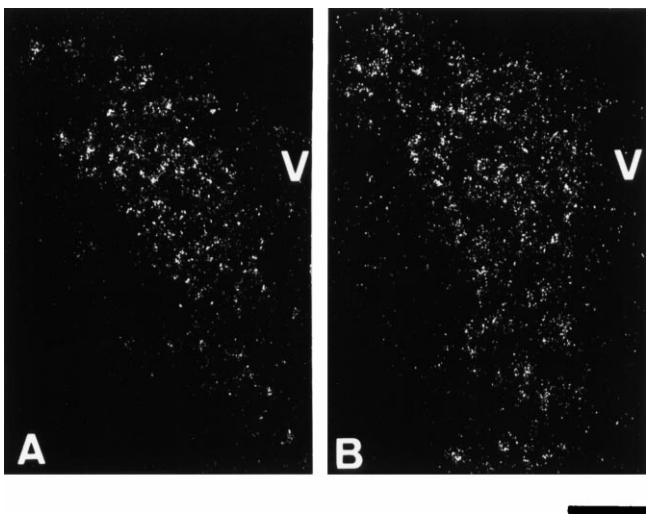

Fig. 1. Darkfield view of the effect of chronic PEA or vehicle intraperitoneal treatment for 10 days on CRH gene expression in the parvocellular part of hypothalamic PVN. (A) Rats injected with vehicle ($n = 6$). (B) Rats injected with PEA (50 mg/kg), ($n = 6$). Bar equals 100 μm. V: third ventricle.

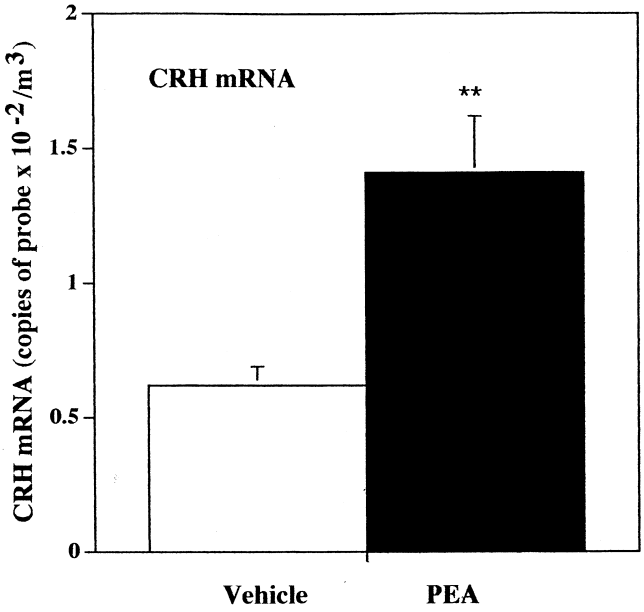

Fig. 2. CRH mRNA levels in the parvocellular part of hypothalamic PVN after vehicle (white bars; $n = 6$) or chronic PEA (black bars; $n = 6$) intraperitoneal treatment for 10 day. Areas hybridized with the CRH probe were quantified on the film autoradiograms as described in Materials and methods. Values are means ± S.E.M. ** $P < .01$ versus vehicle-injected rats.

3.2. Effect of subchronic PEA treatment on CRH-41 immunostaining in the ME

Quantitative evaluation of CRH-41 immunostaining in the ME confirmed the visual analysis of the immunofluorescence pictures. Irrespective of the treatment, a dense aggregation of nerve processes and terminals was stained with antisera against CRH-41 throughout the EME. However, CRH-41 was always more abundant in PEA-treated rats (Fig. 3). Quantitative evaluation of the CRH-41 immunostained the ME (Fig. 4) giving the following formula: intensity $\times$ area of the CRH immunolabeling in EME/total area of the ME, provided an estimation of both the relative labeling of the (percentage of the whole ME explored and corresponding to the amount of immunoreactive ME terminals, i.e., the number of labeled fibers), and the labeling intensity of the immunostained ME area (i.e., the immunoreactive content of the stained nerve fibers, expressed arbitrary units). This ratio was much higher in PEA-treated rats than in controls (+138%, $P < .01$) (Fig. 4).

3.3. Effects of subchronic PEA treatment on plasma ACTH and corticosterone concentrations during stress and normal conditions

The plasma concentrations of ACTH and corticosterone before and after stress in rats injected with vehicle (Fig. 5A–B), were consistent with those in the literature [11]: 15 min after the initiation of stress, the ACTH concentration reached a maximum, and stayed high for 30 min. By 45 min, the concentration had fallen to about 30 of the peak

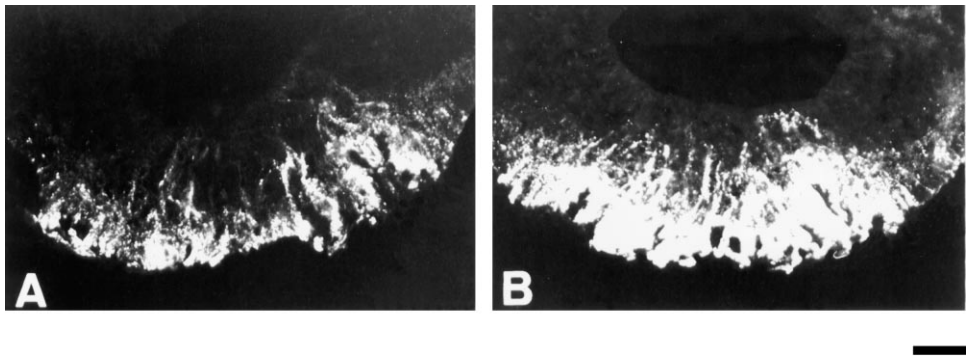

Fig. 3. Immunostaining with a rat CRH41 antiserum of the ME of (A) rats injected with vehicle, $n=6$ and (B) rats injected with PEA (50 mg/kg), $n=5$. Sections (12 μm) were stained simultaneously. CRH-41 immunolabeling was higher in the rats treated with PEA than in rats treated with vehicle. Bar equals 100 μm.

value and by 60 min, it had returned to baseline. The corticosterone concentration paralleled that of ACTH. In animals given PEA, plasma concentration of ACTH from the pituitary between 15 and 60 min after stress was significantly higher than in the vehicle-treated animals (Fig. 5A) (15 min: +154%, $P<.01$; 30 min: +101%, $P<.01$; 45 min: +173%, $P<.01$; 60 min: +251%, $P<.01$). Similarly the corticosterone concentration was also higher in PEA-treated animals (Fig. 5B), the difference being significant after 45 (+196%, $P<.01$) and 60 min (+273.14%, $P<.01$).

3.4. Effects of subchronic PEA treatment on the negative control of the hypothalamo–pituitary–adrenal (HPA) axis by dexamethasone

In vehicle-treated rats, the plasma concentration of ACTH and corticosterone were higher in the evening than in the

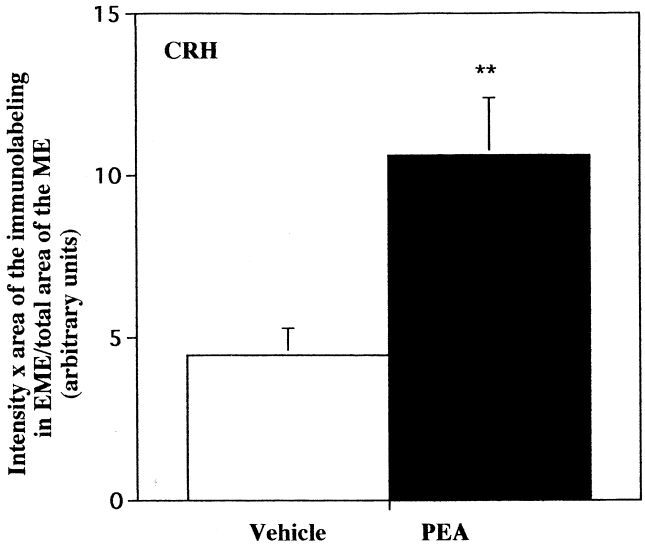

Fig. 4. Quantification of the relative CRH-41 immunolabeled areas in the ME of rats treated with vehicle (white bars; $n=6$) or PEA (black bars; $n=5$). Values are means ± S.E.M. $**P<.01$ compared to vehicle-injected rats.

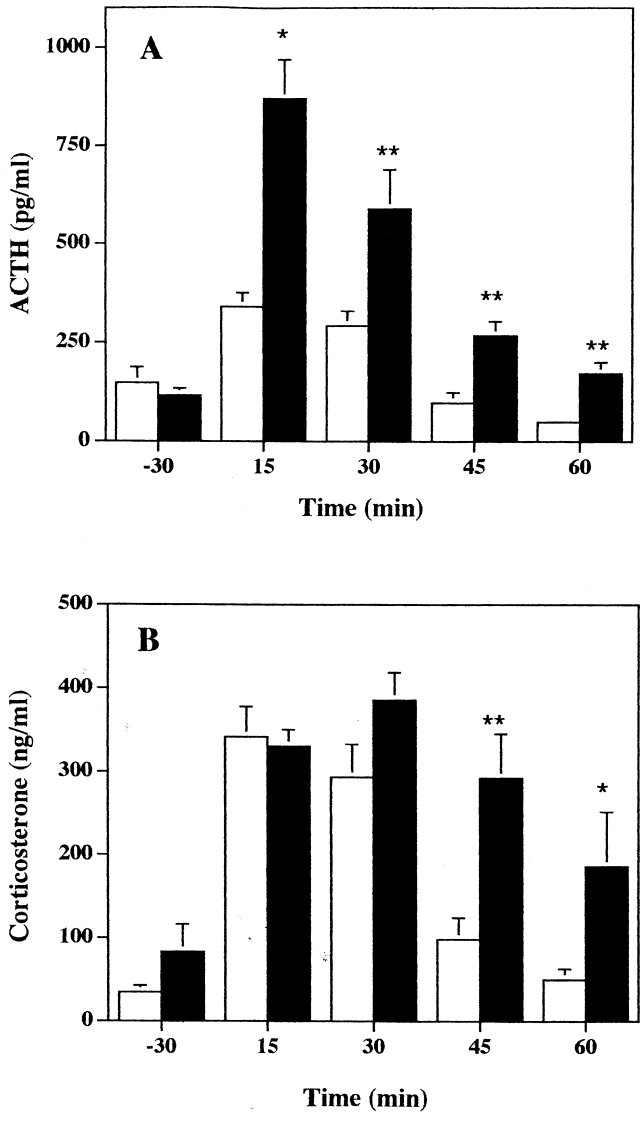

Fig. 5. Effect of chronic PEA (50 mg/kg) intraperitoneal treatment for 10 days on plasma ACTH (A) and corticosterone (B) levels under basal conditions (− 30 min before stress) and after ether stress (+15, 30, 45, 60 min). Values are means ± S.E.M. $*=P<.05$, $**P<.01$ compared to controls rats. Vehicle (white bars; $n=6$), PEA (black bars; $n=8$).

morning (ACTH: +88%, $P<.01$; corticosterone: +128%, $P<.01$) (Fig. 6A–B). The concentrations of ACTH and corticosterone in PEA-treated animals were indistinguishable from those in vehicle-treated animals at both time points. The circadian rhythms of ACTH and corticosterone secretion are thus not affected by PEA (Fig. 6A–B).

In PEA- and vehicle-treated animals administered 1 μg/kg dexamethasone (Fig. 6C), plasma ACTH was undetectable at 0900 and 1200 h (14/14 vehicle-treated rats and 8/13 PEA-treated rats were undetectable). At 1800 h, the ACTH concentration was similar in the two groups, and significantly lower than that in the control group when compared to the concentrations measured before dexamethasone injection (rats treated with vehicle: −64%, $P<.01$; rats treated with PEA: −54%, $P<.01$).

The concentrations of corticosterone in all groups of animals treated with 1 μg/kg dexamethasone were indistinguishable from each other and from those before injection with dexamethasone (Fig. 6D).

Administration of 10 μg/kg dexamethasone (Fig. 6E–F) caused plasma ACTH and corticosterone concentrations in both PEA- and vehicle-treated animals to fall below the detection limit of the assays used (25 pg/ml and 5 ng/ml, respectively).

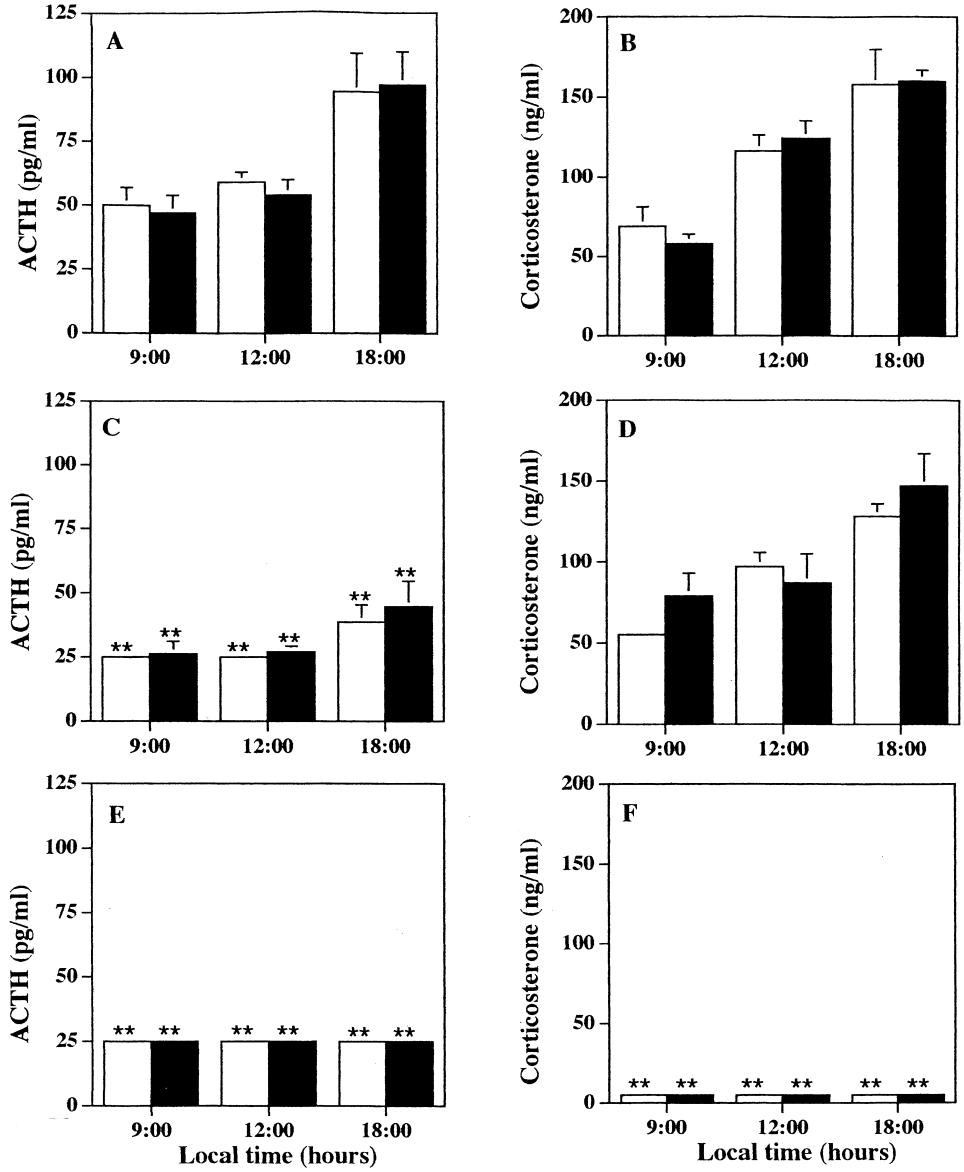

Fig. 6. Effect on plasma ACTH (A, C, E) and corticosterone (B, D, F) of dexamethasone injections after a chronic PEA (50 mg/kg) intraperitoneal treatment for 10 days. (A–B) Before injection of dexamethasone on the first day of blood sample at 0900, 1200, and 1800 h. (C–D) After injection of dexamethasone at dose 1 μg/kg on the second day of blood sample at 0900, 1200, and 1800 h. (E–F) After injection of dexamethasone at dose 10 μg/kg on the second day of blood sample at 0900, 1200, and 1800 h. Vehicle (white bars; $n=28$), PEA (black bars; $n=25$). Values are means ± S.E.M. * $P<.01$ compared within the same group of rats and at the same times on the first day before injection of dexamethasone.

Table 1
Effects of subchronic PEA treatment on hippocampus, hypothalamus, and pituitary type I and type II glucocorticoid receptor densities (B_{max}: maximal numbers of binding sites) and affinities (K_d: dissociation constants)

		Type I		Type II	
		K_d (nM)	B_{max} (fmol/mg P)	K_d (nM)	B_{max} (fmol/mg P)
Hippocampus	Vehicle	1.00 ± 0.14	125 ± 16	0.40 ± 0.01	273 ± 33
	PEA	1.00 ± 0.12	127 ± 17	0.40 ± 0.03	281 ± 25
Hypothalamus	Vehicle	0.70 ± 0.20	13 ± 2	0.50 ± 0.10	190 ± 13
	PEA	1.60 ± 0.60	10 ± 2	0.40 ± 0.10	195 ± 17
Pituitary	Vehicle	0.90 ± 0.40	49 ± 12	0.80 ± 0.30	289 ± 46
	PEA	0.80 ± 0.40	57 ± 15	0.50 ± 0.20	300 ± 48

3.5. Effects of subchronic treatment with PEA on the physiology of glucocorticoid receptors

There was no difference between the numbers of type I and type II receptors in control and PEA-treated rats in any of the anatomical structures studied. Similarly, there was no difference in K_d of binding for either type I (1 nM) or type II (0.5 nM) receptors (Table 1).

4. Discussion

Our study shows that subchronic treatment with PEA increases the response of the corticotroph axis to stress. This activating effect appears to involve increased expression of the CRH gene in hypothalamic neurons of the PVN and storage of CRH in nerve endings of the ME under basal conditions. This leads to an increase in the release of CRH into the circulation in response to stress. Activation is not associated with reduced feedback regulation by glucocorticoids, as assessed by the dexamethasone test. Indeed, the injection of 10 μg/kg dexamethasone strongly decreased plasma ACTH and corticosterone concentrations in both vehicle- and PEA-treated rats. In contrast, the injection of 1 mg/kg dexamethasone reduced the plasma ACTH concentrations measured during the course of the day, abolishing the morning/evening variations observed in the two groups of animals, without affecting plasma corticosterone concentration. It has been known for some time that dexamethasone induces a decrease in plasma ACTH concentration [2,6,17,31] and the uncoupling of plasma concentrations of ACTH and corticosterone [34]. These effects of dexamethasone on HPA axis seem to be associated with a decrease in the release of ACTH by the adenohypophysis. This is also consistent with the absence of change in the number or affinity of type I and type II receptors in the central nervous system and pituitary. Although PEA stimulates the corticotroph axis, and thus has amphetamine-type activity [5,37], it differs from amphetamines by not affecting glucocorticoid receptors [22,35]. Injection of PEA into the rat has various effects including increasing both the activity of the hypothalamic neurons synthesizing CRH, and the plasma concentrations of ACTH and corticosterone. Activation of the corticotroph axis is only detectable, in terms of hormone production, in response to stress. Indeed, treatment with PEA increased the plasma concentrations of ACTH and corticosterone in response to stress. In contrast, in the basal state, the injection of PEA caused no significant change in the plasma concentrations of ACTH and corticosterone, with animals treated with vehicle used as the reference group. However, plasma ACTH concentration before stress was slightly higher in the group of animals treated with vehicle than the baseline values generally recorded in unstressed animals. This may be due to the intraperitoneal injections, which may cause abdominal inflammation [19].

These effects of PEA on the HPA axis could be the result of stimulation of the catecholaminergic system. Indeed, PEA stimulates the release of catecholamines and inhibits their uptake [30]. PEA thereby increases the concentrations of catecholamines in the synaptic gap, and this may explain the hypersecretion of ACTH and corticosterone following stress. Intracerebroventricular (icv) injections into rats of moderate doses of noradrenaline or adrenaline results in significant, dose-dependent increases in CRH concentration in the blood in pituitary portal blood vessels and circulating ACTH levels [1,28], similar to those associated with stress [35]. The release of CRH into the pituitary portal blood following stress is reduced by bilateral ventral noradrenergic bundle lesions [13] and is increased after stimulation of this nerve bundle [28]. Part of the catecholaminergic innervation of the hypothalamus originates in the *locus coeruleus* (noradrenergic nucleus A6) [32]. Intravenous injection of PEA increases the frequency of neuron firing in the *locus coeruleus*, and this effect is amplified by simultaneous treatment with inhibitors of monoamine oxidase or an α2 antagonist [15]. Thus, the effects of PEA may be mediated by the ascending noradrenergic pathway ending in the PVN. Iontophoretic injections of PEA and of noradrenaline into the cortex have similar effects on the cortical neurons. This supports the view that some of the effects of PEA are mediated by noradrenaline. Stimulation of the electrical activity of the cortical neurons is abolished by ipsilateral lesions of the locus [24]. If catecholamines stimulate the activity of the corticotroph axis, PEA may facilitate regulation at the noradrenergic nerve endings afferent to the CRH neurons.

In addition, a pharmacological study of adrenergic receptors thought to be involved in the catecholaminergic control of the HPA axis has shown that the stimulatory effect on ether stress-induced ACTH release was reduced by 80% by intracerebroventricular pre-treatment with prazosin or propanolol [38]. Catecholaminergic innervation of the hypothalamus plays a major stimulatory role in the CRH/ACTH system and this effect is mediated by $\alpha 1$ receptors. In addition, autoradiography has shown that $\alpha 2$ receptors are present on neuron membranes that synthesize CRH41 and AVP, along with $\alpha 1$ receptors [8,18]. These postsynaptic $\alpha 2$ receptors act as mediators of stimulation by NA or clonidine (an $\alpha 2$ agonist).

Chronic PEA treatment decreases the density of adrenergic receptors [23]. This decrease may be due to down-regulation induced by the increase in catecholamine concentration in the synaptic gap with chronic PEA treatment. Chronic treatment with PEA also potentiates the action of dopamine [25]. PEA may act either directly on the MAO-B, which is found predominantly in dopaminergic neurons in the brain, or on the dopamine receptors, which may be modulated by increases in PEA levels. Evidence for a relationship between dopamine and the stress response system is provided by reports demonstrating specific interactions of the dopamine system with the HPA axis [7,9,27]. Indeed, dopamine increases central HPA drive and corticosteroid feedback resistance. Thus, PEA may stimulate stress-induced neurohormonal secretions of the HPA axis, by synergism with the catecholaminergic system.

PEA, an endogenous molecule, may be an important regulatory element in the monoaminergic systems affecting the ability of the organism to adapt to its environment, as we have shown for HPA axis functioning. Studies are currently underway in our laboratory to determine the precise role of the interactions between PEA and the catecholamines or their receptors on the secretory activity of the HPA axis in the hypothalamus.

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