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Cocaine-induced reduction of brain neuropeptide Y synthesis dependent on medial prefrontal cortex

CLAES WAHLESTEDT*†, FAROUK KAROUM‡, GEORGE JASKIW§, RICHARD JED WYATT‡, DAN LARHAMMAR¶, ROLF EKMAN||, AND DONALD J. REIS*

*Division of Neurobiology, Department of Neurology and Neuroscience, Cornell University Medical College, New York, NY; ‡Neuropsychiatry Branch and §Clinical Brain Disorders Branch, National Institutes of Mental Health Intramural Research Program, St. Elizabeth's Hospital, Washington, DC; ¶Department of Medical Genetics, University of Uppsala, Uppsala, Sweden; and ||Department of Psychiatry and Neurochemistry, University of Lund, Lund, Sweden

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ABSTRACT **Repeated administration of cocaine elicits substantial, long-lasting, but reversible reductions in neuropeptide Y (NPY) and NPY mRNA in the rat cerebral cortex and nucleus accumbens. The NPY reduction appears to be mediated through a decrease in NPY biosynthesis, occurring transneuronally, perhaps in response to changes in synaptic dopamine associated with mesolimbic and mesocortical dopamine neurons. The medial prefrontal cortex appears necessary for maintenance of cocaine's action on this neuronal network since excitotoxic lesions of this area prevented (lesion before cocaine) and reversed (lesion after cocaine) the reductions in NPY elicited by the cocaine. NPY may be a sensitive marker for chronic cocaine use. Its decrease may relate to the anxiety and depression associated with cocaine withdrawal in humans.**

Many of the acute euphoric effects of cocaine are thought to be a consequence of the augmented concentration of dopamine (DA) in regions of the limbic forebrain (1–6). The enhanced accumulation of DA presumably results from cocaine blocking presynaptic DA uptake (7, 8). Although DA may initiate self-reinforcing behaviors, the biochemical consequences of repeated cocaine administration and its termination are less well understood. Behaviorally, the end of a cocaine binge is often associated with intense anxiety (9–11) and an episode of major depression (12, 13). The dysphoria resulting from cocaine withdrawal can be lengthy and of importance for cocaine craving and recidivism (12, 13).

Conceivably, the behavioral changes associated with prolonged use and withdrawal from cocaine may reflect the unmasking of long-lasting central adaptive processes that act to oppose the immediate excitant actions of the drug (13). These long-term aftereffects may thereby share common anatomical and neurochemical features with those that are activated by acute administration of the drug. One candidate neurotransmitter that may relate to long-term cocaine use and withdrawal is neuropeptide Y (NPY), a 36-amino acid peptide that is widely distributed in the central and peripheral nervous systems (14, 15). NPY has been implicated in the expression of anxiety (16) and depression (17, 18) and its biosynthesis appears to be regulated by DA (19–21). We therefore sought to determine whether chronic cocaine administration and its withdrawal are associated with changes in the content and regulation of this neuropeptide in brain and, if so, whether such changes depend upon the integrity of the medial prefrontal cortex, a region thought to be critical for the expression of some of the reinforcing properties of the drug (5, 6).

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MATERIALS AND METHODS

Animals. *Study 1.* Sixty-four male Sprague–Dawley rats (200–275 g; Zivic–Miller) were housed in groups of four with free access to food and water and a constant light/dark schedule (8 a.m. to 6:00 p.m.). The rats were treated with either cocaine (10 mg/kg; Sigma) or saline i.p. twice daily for 1 week and sacrificed 1 hr, 2 weeks, 6 weeks, or 12 weeks after the last injection. The brains were dissected on wet ice after coronal sectioning. Starting with a slice extending from approximately AP +2.7 to +4.5 mm relative to bregma (22), a horizontal cut was made at the level of the rhinal sulcus and tissue superior to the cut was considered to be frontal cortex. Subsequently, this tissue (eight rats per group) was stored at −80°C and then subjected to either blot analysis for NPY mRNA or radioimmunoassay (RIA) for NPY-like immunoreactivity (NPY-LI). The NPY-LI measured at 2 weeks after cocaine withdrawal was pooled and analyzed by high-pressure liquid chromatography (HPLC) (16) and compared with control tissue.

Study 2. Forty-eight Sprague–Dawley rats (200–250 g) were divided into two groups and subjected to surgery. After induction of anesthesia with ketamine and xylazine i.p., rats were immobilized in a stereotactic apparatus with bite bar at 25 mm below the interaural line. Ibotenic acid (5 mg/0.5 ml over 3 min) or an equal volume of vehicle (phosphate-buffered saline) was administered bilaterally with an infusion pump at the coordinates: AP +3.5 mm, ML ±0.7 mm relative to bregma, and VP −3.5 mm from the dura. The cannulae remained in place for 8 min after the end of the injection. The lesion was verified in parallel experiments by staining cryostat sections with cresyl violet (23). Four weeks later, the two groups were subdivided further into four groups of 12 animals receiving saline or cocaine (10 mg/kg) i.p. twice daily for a week. Fourteen days later, the rats were killed and the frontal cortex was dissected as described above. The medial prefrontal portion was removed from the slice. The nucleus accumbens was dissected from a slice extending from AP +0.7 to +2.7 mm relative to bregma. Cingulate cortex and medial and lateral caudate nucleus were dissected from a slice extending from AP +0.7 to +1.3 mm (23).

Study 3. Forty-eight Sprague–Dawley rats were treated with cocaine (10 mg/kg twice daily) or saline for 1 week and then the prefrontal cortex was lesioned or sham-lesioned—i.e., yielding 12 animals in each group. They were sacrificed 2 weeks later. Other procedures were the same as in study 2.

Abbreviations: NPY, neuropeptide Y; DA, dopamine; LI, -like immunoreactivity; DOPAC, 3,4-dihydroxyphenylacetic acid.

†To whom reprint requests should be addressed at: Division of Neurobiology, Department of Neurology and Neuroscience, Cornell University Medical College, 411 East 69th Street, New York, NY 10021.

NPY mRNA. Tissue content of NPY mRNA was determined essentially as described (24). Briefly, poly(A)$^+$ RNA was prepared from frontal cortical tissue of saline- and cocaine-treated rats. The RNA was electrophoresed in 1% agarose gels containing 0.7% formaldehyde and transferred to nitrocellulose filters. The filter-bound RNA was hybridized to a nick-translated 287-base-pair *Xba* I–*Ava* I fragment containing the second exon of the rat NPY gene, encoding the signal peptide and most of the mature NPY peptide. After hybridization, the filters were washed in 15 mM NaCl/1.5 mM sodium citrate, pH 7.0/0.1% sodium dodecyl sulfate at 54°C. Subsequently, the filters were boiled in 1% glycerol and then probed with a nick-translated 1500-base-pair *Pst* I fragment encoding mouse α-actin. The hybridization signals were quantified by densitometric scanning of autoradiograms, using appropriate exposures. The concentration of NPY mRNA in each sample was normalized relative to the concentration of actin mRNA.

NPY RIA. For the RIA of NPY (see also ref. 17), a rabbit antiserum raised against porcine NPY conjugated to bovine serum albumin with carbodiimide was used. ^{125}I-labeled NPY (Bolton–Hunter labeled) used as tracer was purified by HPLC. The antiserum cross-reacted with peptide YY (PYY) to ≈33% but not with C-terminal fragments of NPY or PYY (NPY 13–36 and PYY 13–36) nor with pancreatic polypeptides. Peptides used in this study were obtained from either Peninsula Laboratories or Ferring Pharmaceuticals. The detection limit was 11.7 pmol/liter and the interassay variation was 7.5% (n = 16). For extraction, the tissue specimens were boiled in 500 ml of 0.5 M acetic acid for 10 min and then homogenized by a Polytron for 1 min. The homogenates were centrifuged at 4°C and 3000 × g for 10 min, reconstituted, and assayed for NPY-LI. For protein determination, homogenized tissue was analyzed by the method of Lowry.

Other Analyses. The metabolites of DA were measured by mass fragmentography as described (25). Somatostatin-LI (26), galanin-LI (27), and vasoactive intestinal peptide-LI (28) were determined by RIA.

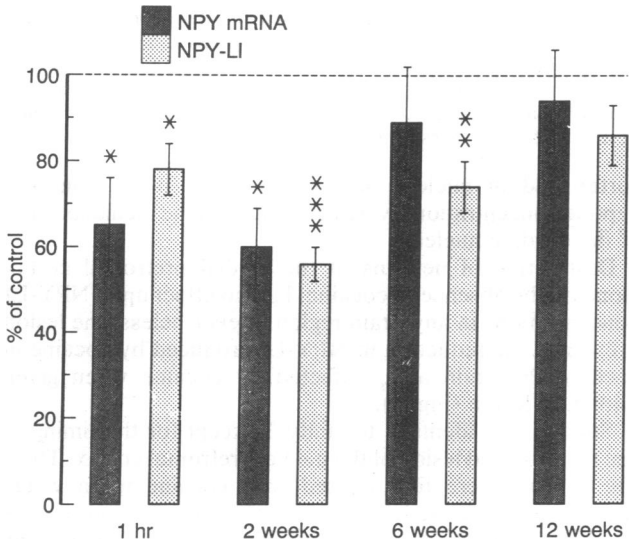

FIG. 1. Time course of the effect of cocaine on concentrations of NPY mRNA and NPY-LI in rat frontal cortex. Male Sprague–Dawley rats were treated with either cocaine (10 mg/kg) or saline i.p. twice daily for 1 week and sacrificed 1 hr, 2 weeks, 6 weeks, or 12 weeks after the last injection. Each bar represents mean percentage ± SEM of eight cocaine-treated animals compared with the mean of eight saline-treated animals set as 100%. NPY mRNA was normalized relative to actin mRNA, and the units used were arbitrary. Concentrations of NPY-LI in control cortices from saline-injected rats were 4139 ± 361 (1 hr), 3920 ± 330 (2 weeks), 4205 ± 372 (6 weeks), and 3862 ± 314 (12 weeks) pg/mg of protein (means ± SEM). Two-tailed t test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$.

RESULTS

Cocaine Reduces NPY Synthesis. In the first study we examined the effects of repeated cocaine administration and withdrawal on the content of NPY and its mRNA in the frontal cortex of adult male Sprague–Dawley rats. Cocaine (10 mg/kg i.p.) or saline was administered twice daily for 1 week. Thereafter groups of rats were sacrificed by decapitation at 1 hr, 2 weeks, 6 weeks, and 12 weeks following the last injection. The frontal cortex was removed bilaterally and samples were assayed by RIA for NPY-LI or by blot analysis for NPY mRNA.

The repeated administration of cocaine produced a prolonged and substantial reduction in NPY-LI in the frontal cortex (Fig. 1). The reduction was present 1 hr after treatment, reached a maximum of 57% of control at 2 weeks, and returned to control between 6 and 12 weeks following the discontinuation of cocaine administration. The NPY-LI measured in samples of frontal cortex 2 weeks after cessation of cocaine was characterized further by HPLC and was found to share a single major peak with NPY-LI that came from saline-treated controls coeluting with authentic (rat/human) NPY (Fig. 2). Thus, it is probably not a modified peptide and, therefore, not processed differently due to cocaine.

NPY mRNA was also measured in prefrontal cortex 1 hr following the last cocaine injections. As seen in Fig. 1, cocaine also reduced NPY mRNA although the time course and magnitude of change in the message were different from those of the peptide. This finding suggests that the mecha-

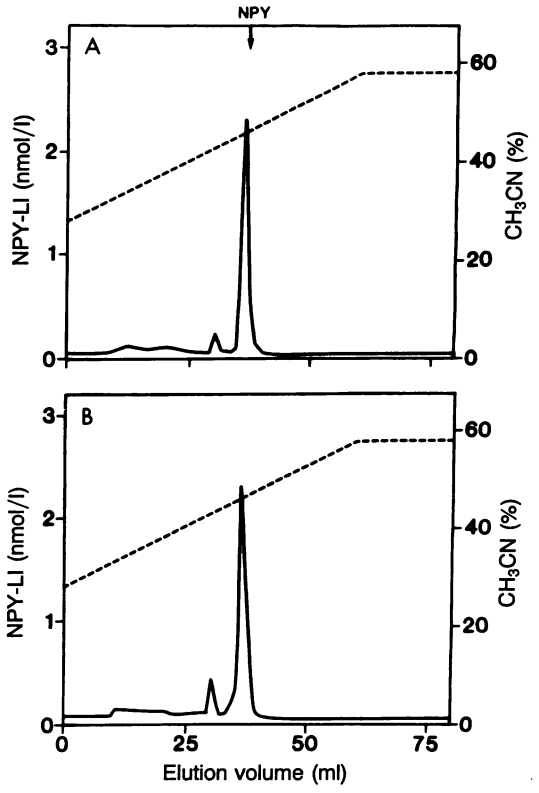

FIG. 2. HPLC elution patterns of NPY-LI of pooled (n = 8) extracts of frontal cortex from rats treated twice daily for a week with saline (control; *A*) or cocaine (10 mg/kg; *B*). Equal amounts of NPY-LI from the two pooled samples were injected in a volume of 1.7 ml. The elution position of synthetic rat/human NPY is indicated by an arrow, and the acetonitrile gradient is depicted by the broken line.

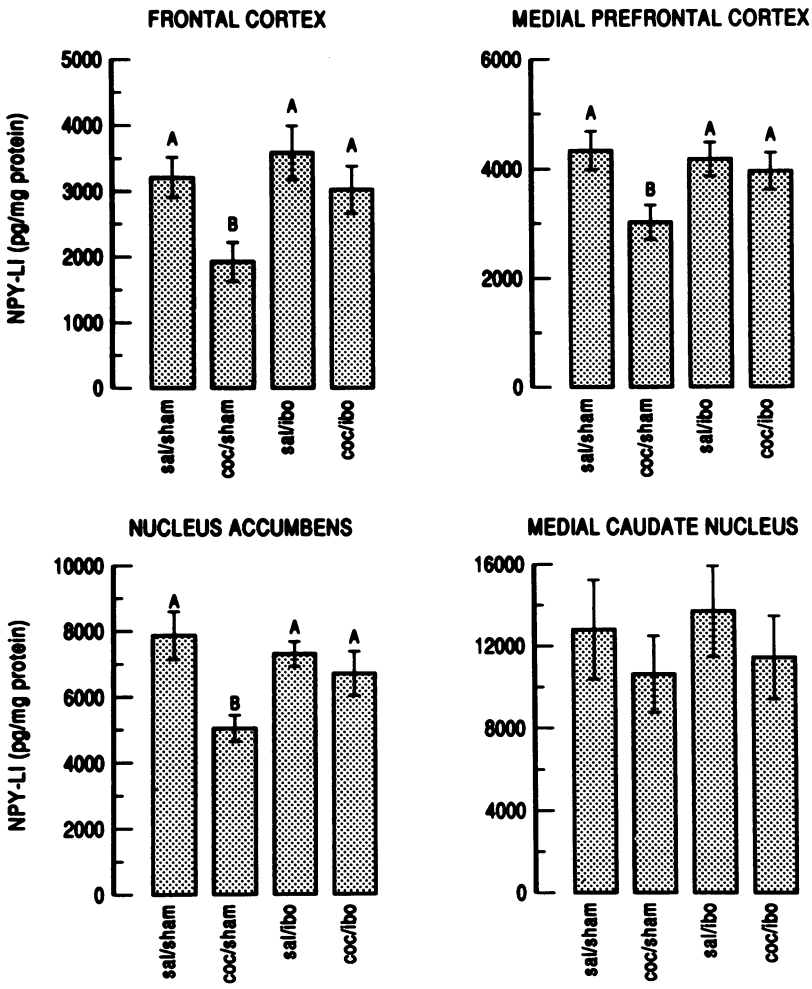

FIG. 3. Effect of lesioning the medial prefrontal cortex on cocaine-induced reduction of NPY-LI. The lesion (or sham-lesion) was performed by injecting ibotenic acid into medial prefrontal cortex 4 weeks prior to treatment with cocaine (10 mg/kg) or saline twice daily for a week. Two weeks after cessation of cocaine, rats were sacrificed. The four groups, each consisting of 12 rats, were as follows: group 1, saline and sham operation (sal/sham); group 2, cocaine and sham operation (coc/sham); group 3, saline and ibotenic acid lesion (sal/ibo); and group 4, cocaine and ibotenic acid lesion (coc/ibo). The four brain regions studied were frontal cortex (without medial prefrontal), medial prefrontal cortex, nucleus accumbens, and medial caudate nucleus. Concentrations of NPY-LI are given in pg/mg of protein. A and B represent Duncan's grouping of means; means labeled with different letters were significantly different at $\alpha = 0.05$; error bars represent SEM.

nism accounting for the reduced concentration of NPY-LI was decreased synthesis of the peptide and not depletion consequent to increased release.

That the changes in NPY-LI and NPY mRNA were a consequence of multiple exposures to cocaine was demonstrated by our observation that neither was modified when measured at 1 hr or 2 weeks after administration of a single injection of cocaine (10 mg/kg) (data not shown).

Involvement of Medial Prefrontal Cortex. Since the medial prefrontal cortex appears to be of importance for some of the behaviorally reinforcing effects of acute cocaine administration (5, 6), in a second series of experiments we examined the effects of excitotoxic lesions of the region on the cocaine-induced reduction in NPY-LI. Cocaine was administered to adult male Sprague–Dawley rats by the regimen described above (10 mg/kg of cocaine or an equal volume of saline i.p. twice daily for a week). Four weeks prior to cocaine treatment, however, the excitotoxin ibotenic acid (or vehicle) was microinjected into the medial prefrontal cortex. Equal numbers of cocaine and saline-treated rats were sham-operated. Two weeks after cessation of cocaine, the rats were sacrificed, and a number of brain regions was removed by dissection and NPY-LI was measured by RIA (Fig. 3).

In sham-operated rats cocaine, but not saline, significantly reduced NPY-LI not only in frontal but also in cingulate cortex and in nucleus accumbens (Fig. 3). In contrast, peptide concentrations were unchanged in the medial portion of the caudate nucleus.

Destruction of neurons of the medial prefrontal cortex alone (in the absence of cocaine) had no effect upon NPY-LI concentrations in any brain region. Nevertheless, the lesion prevented the reduction in NPY-LI produced by cocaine in each of the brain areas affected by cocaine when given without a lesion (Fig. 3).

Study 3 was identical to study 2 except for the timing of the ibotenic acid lesion of the medial prefrontal cortex. Thus, the animals were first treated with cocaine for a week, immediately followed by the lesion, and they were then sacrificed 2 weeks later. As shown in Fig. 4, certain brain regions showed a decrease in NPY-LI after cocaine but not after combined cocaine and lesion treatment.

Chemical Selectivity. *DA metabolites.* To assess the chemical selectivity of cocaine treatment as well as the effects of prefrontal lesions we measured the concentrations of the DA metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid, by mass fragmentography (25) in four groups of rats as described for study 3 (shown in Fig. 4; the numbers of animals in each group ranged from 8 to 10).

The only significant change found was for DOPAC in the prefrontal cortex (minus the medial prefrontal cortex). Ani-

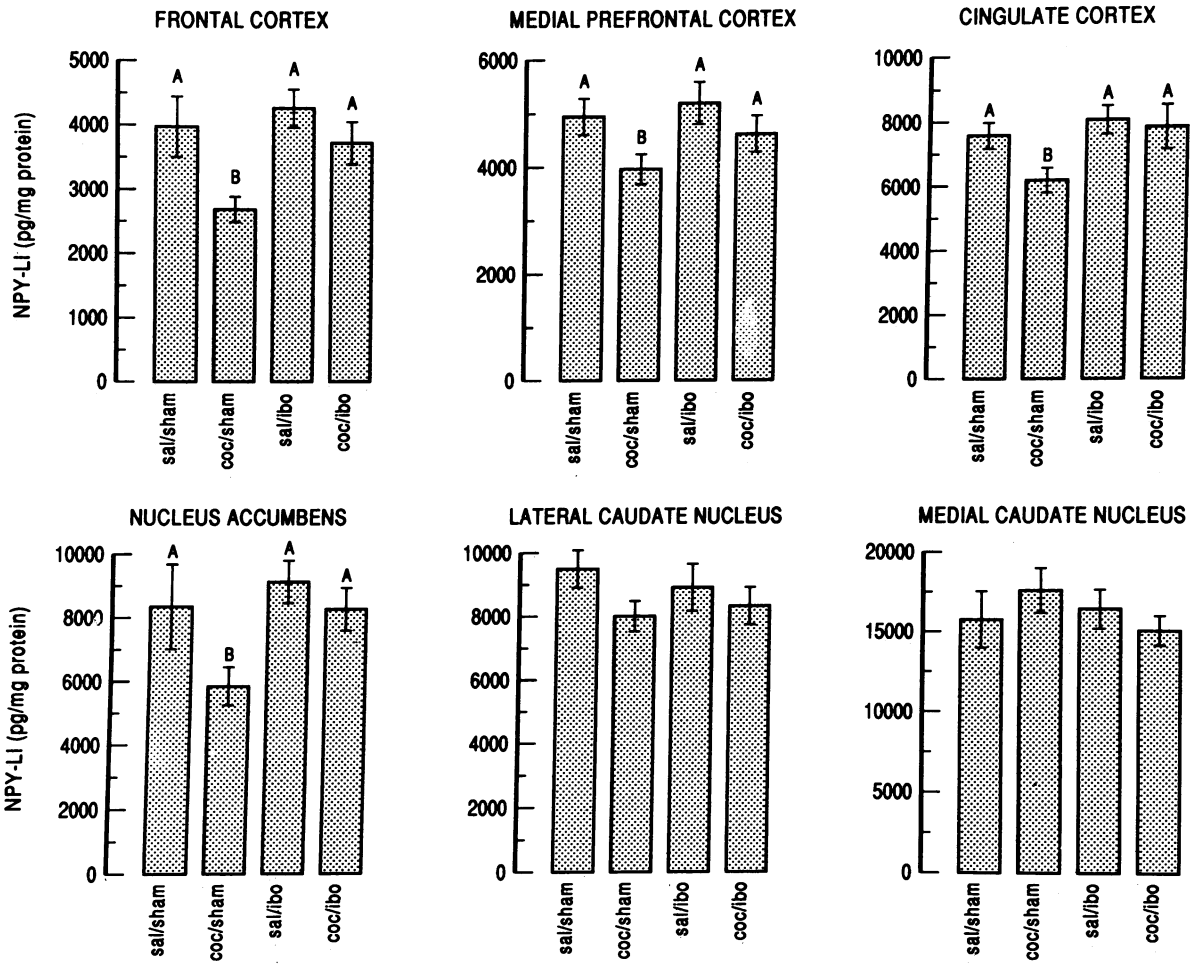

FIG. 4. Effect of lesioning the medial prefrontal cortex on cocaine-induced reduction of NPY-LI. Male Sprague–Dawley rats were treated with either cocaine (10 mg/kg) or saline twice daily for a week. The treatment was followed directly by lesioning the medial prefrontal cortex or by sham operation. The four groups, each consisting of 12 rats, were as follows: group 1, saline and sham operation (sal/sham); group 2, cocaine and sham operation (coc/sham); group 3, saline and ibotenic acid lesion (sal/ibo); and group 4, cocaine and ibotenic acid lesion (coc/ibo). The six brain regions studied were frontal cortex (without medial prefrontal), medial prefrontal cortex, cingulate cortex, nucleus accumbens, lateral caudate nucleus, and medial caudate nucleus. Concentrations of NPY-LI are given in pg/mg of protein. A and B represent Duncan's grouping of means; means labeled with different letters were significantly different at $\alpha = 0.05$; error bars represent SEM.

mals that received cocaine (sham-operated and lesioned) had reduced ($\alpha = 0.05$: Duncan's grouping of means) DOPAC concentrations. The DOPAC concentrations in the prefrontal cortex were 1.96 ± 0.22 (saline plus sham), 1.41 ± 0.13 (cocaine plus sham), 2.18 ± 0.24 (saline plus lesion), and 1.38 ± 0.11 (cocaine plus lesion), respectively (pmol/mg of protein; means ± SEM).

Somatostatin. In study 2, concentrations of somatostatin-LI showed changes similar to NPY-LI in the nucleus accumbens. Thus, the somatostatin-LI concentrations in this brain region were 650.3 ± 45.0 (saline plus sham), 508.7 ± 43.7 (cocaine plus sham), 672.8 ± 32.7 (saline plus lesion), and 700.2 ± 30.1 (cocaine plus lesion), respectively (ng/mg of protein; means ± SEM; $\alpha = 0.05$; Duncan's grouping of means). In study 3, the levels of somatostatin-LI were not significantly different between groups in any brain region.

Other peptides. Galanin and vasoactive intestinal peptide were measured by RIA in frontal cortex and nucleus accumbens from study 3. The concentrations of these two peptides were unchanged.

DISCUSSION

The present study demonstrates that repeated cocaine administration in rats results in topographically distinctive, widespread, prolonged, but reversible reductions in the concentration of NPY in rat brain. Since NPY-LI and NPY mRNA were reduced by the repeated administration of cocaine (Fig. 1), it is unlikely that the drug acts only to induce excessive NPY release. Rather, the data suggest that cocaine reduces NPY biosynthesis.

This assumption is supported also by the finding that the profile of the measured NPY-LI was unaffected by cocaine (Fig. 2) making it unlikely that central stimulation alters the processing of NPY.

Because the effects of cocaine on NPY mRNA and NPY-LI were apparent directly after treatment and showed a sustained unimodal change, we believe that the drug, rather than its withdrawal, was the cause of the phenomenon. Thus, unlike many other effects of centrally acting drugs, we found no evidence to support reciprocal changes with time. In addition, preliminary data indeed indicate that continuous 4-week cocaine treatment also results in reduced frontal cortical NPY-LI concentration at cessation (unpublished data), further supporting the unimodality of the change.

The areas showing reductions appear to be those innervated by mesocortical and mesolimbic DA neurons and involve regions of the cerebral cortex and nucleus accumbens (Figs. 3 and 4). This anatomy is in agreement with present views that dopaminergic transmission within these areas is essential for the behavioral actions of cocaine (1–6).

In previous studies (29–32) we demonstrated that prefrontal cortex DA and its metabolite DOPAC were decreased

following the same dose of cocaine given for the same period of time. In fact, at least for the prefrontal cortex, there is a rather striking parallel between the changes in NPY-LI, DA, and DOPAC. This suggests that whatever process is affecting one system may well be affecting the other.

The fact that cocaine enhances synaptic DA concentrations may be relevant to an understanding in the decrease of the biosynthesis of NPY elicited by the drug. Indeed, NPY and DA have been shown to interact reciprocally in rat brain: decreasing DA transmission increases tissue levels of NPY-LI (19–21), suggesting that under normal conditions DA may suppress the biosynthesis of NPY. The enhanced synaptic DA availability evoked by cocaine would be predicted, therefore, to be associated with diminished biosynthesis of the peptide. In addition, it is known that NPY enhances the concentrations of DA and DOPAC (33–36), suggesting dopaminergic involvement in a negative feedback loop by the peptide on its biosynthesis.

We have additionally discovered that the cocaine-induced reduction in NPY concentrations in various forebrain areas is prevented by excitotoxic destruction of neurons of the medial prefrontal cortex. Interestingly, the concentrations of NPY-LI (compare Figs. 3 and 4) were not changed within the lesioned cortex itself, supporting the evidence of others that many NPY-containing forebrain neurons are relatively resistant to excitotoxins and neurodegenerative processes (reviewed in ref. 18). The capacity of lesions of the medial prefrontal cortex to prevent (lesion before cocaine) as well as reverse (lesion after cocaine) the actions of cocaine elsewhere in brain is of interest in three respects. (*i*) It indicates that the reduction in NPY-LI in cortex is not due to a direct action of the drug but rather must be transneuronal and presumably secondary to excitation of mesocortical/mesolimbic fibers. (*ii*) It suggests that cocaine's actions, at least with respect to inhibiting NPY biosynthesis, must be mediated in part by activation of subcortical circuits. The identity of such trigger zones is unknown. (*iii*) The medial prefrontal cortex seems to be part of a circuit in control of processes, possibly adaptive, that are activated by repeated cocaine administration and can be probed by assaying tissue for NPY synthesis.

The effect of cocaine on NPY, as assessed 2 weeks after treatment, appeared to display some biochemical selectivity. Among three peptides studied in nucleus accumbens in addition to NPY, only one, somatostatin, appeared to behave like NPY. This parallel change in NPY and somatostatin is in accordance with the coexistence of the two peptides in many neurons of the accumbens (compare e.g., ref. 14). Interestingly, such neurons of this nucleus have been shown to display synaptic associations with tyrosine hydroxylase-immunoreactive terminals (37).

In this study, we were thus unable to detect significant effects of cocaine as well as of prefrontal cortical lesion on NPY-LI levels in the medial corpus striatum. This may indicate that the DA innervation of the medial striatum, which indeed seems to be anatomically and functionally related to prefrontal cortex (for references, see ref. 23), is less sensitive to the cortex-dependent cocaine mechanism reported here. Another peptide, dynorphin, has, however, been found to be elevated by the cocaine through a dopaminergic mechanism in the nigrostriatal system (38).

Based on the present animal experiments, it is tempting to consider that NPY (and possibly somatostatin), instead of being an epiphenomenal "marker," may be causally linked to the increased anxiety level and major depression-like symptoms that are associated with termination of cocaine abuse in humans (9–13). Depressed patients have, in fact, been found to exhibit reduced cerebrospinal fluid concentrations of NPY-LI, and in these studies NPY-LI correlated negatively to the level of anxiety (16, 17). Because the cocaine-induced changes observed in the rat were extraordinarily long-lasting, it appears relevant to examine cerebrospinal fluid and postmortem brain material from cocaine abusers with respect to NPY. It will be of importance to attempt to assess further the role of the prefrontal cortex in cocaine-related adaptive processes in humans.

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