Chronic Continuous or Intermittent Infusion of Cocaine Differentially Alter the Concentration of Neurotensin-like Immunoreactivity in Specific Rat Brain Regions

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Neurotensin (NT) is an endogenous brain tridecapeptide that exhibits selective anatomic and neurochemical interactions with rat brain dopaminergic systems. Because modulation of dopaminergic neurotransmission may underlie many of the behavioral properties of cocaine, the effects of both acute and chronic administration of cocaine on the concentration of NT-like immunoreactivity (NT-LI) in specific brain regions was determined. Adult male rats were treated with cocaine for 14 days at a dose of 40 mg/kg/day (0.118 mmoles/kg/day) administered as either 1 subcutaneous injection per day, or infused continuously using subcutaneously implanted minipumps. Neurotensin-like immunoreactivity in specific brain regions was then measured 24 hours or 8 days following drug administration. After 24 hours of withdrawal from daily subcutaneous injection, the concentration of NT-LI was significantly increased in the substantia nigra (SN) and frontal cortex. After 24 hours of withdrawal from continuous infusion with cocaine, NT-LI was increased only in the SN. After 8 days of withdrawal, NT-LI was increased in the SN of rats treated with daily subcutaneous injections of cocaine, but not in the group treated with continuous infusion. Twenty-four hours following a single acute injection of 40 mg/kg of cocaine, NT-LI was increased in the SN and nucleus accumbens. These results provide evidence consistent with a neuroanatomically selective involvement of NT systems in the behavioral and/or addictive properties of cocaine. ***[Neuropsychopharmacology 8:259–265, 1993]***

KEY WORDS: *Neurotensin; Cocaine; Radioimmunoassay; Substantia nigra; Nucleus accumbens; Frontal cortex*

The potential involvement of central nervous system (CNS) dopaminergic (DAergic) systems in the behavioral effects of the psychostimulant, cocaine, has been intensively investigated (Kleven et al. 1990; Ng et al. 1991; Peris et al. 1990). However, it is only recently that attention has been directed to possible cocaine-induced alterations in neuropeptide-containing systems. For example, chronic cocaine administration results in increased concentrations of dynorphin in the striatum and substantia nigra (SN) (Sivam 1989), but decreased concentrations of neuropeptide Y and its messenger ribonucleic acid in the medial prefrontal cortex (Wahlestedt et al. 1991). In addition, somatostatin receptors are decreased in density in the hippocampus and olfactory bulb of rats treated chronically with cocaine (Rodriguez-Sanchez and Arilla 1990), as are recep-

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tors for corticotropin-releasing factor in terminal areas of the mesolimbic/mesocortical DA pathway (Goeders et al. 1990).

Our laboratory sought to test the hypothesis that neurotensin (NT), an endogenous brain tridecapeptide that exhibits a number of well-documented, selective interactions with DAergic systems within the mammalian CNS (Nemeroff and Cain 1985), may mediate, at least in part, the behavioral effects of cocaine. In previous studies, we demonstrated that intracisternal injection of NT attenuated the locomotor hyperactivity induced by a single acute injection of cocaine (Nemeroff et al. 1983). More recently, chronic cocaine treatment has been reported to increase NT concentrations in the striatum and SN; however, following a single dose of cocaine, NT increases were observed only in the SN (Hanson et al. 1989). These increases in NT concentration were attenuated by blockade of N-methyl-D-aspartate receptors, suggesting glutaminergic mediation of cocaine-induced alterations in NT concentrations (Johnson et al. 1991). In addition, 10 days following chronic cocaine administration, NT binding was increased in the SN compacta and prefrontal cortex, but decreased in the ventral tegmental area (VTA) (Pilotte et al. 1991). Therefore, in view of the evidence implicating CNS NT systems as an important component of the neurochemical response to cocaine, we evaluated alterations in NT concentrations in specific rat brain regions at both 24 hours and 8 days following 14 days of continuous or intermittent exposure to cocaine.

The two methods of chronic cocaine administration have been shown by both our group (King et al. 1992) and others (Reith et al. 1987) to result in different residual behavioral profiles. Continuous exposure to cocaine induces behavioral tolerance whereas intermittent administration yields behavioral sensitization. We believe that the continuous-infusion paradigm represents an animal model of "binge-like" cocaine abuse in which chronic users maintain a sustained plasma level of cocaine over extended periods by readministering the drug every 1/2 hour or less for days (Gawin and Ellinwood 1988; Gawin 1991). In contrast, the intermittent-dosing paradigm models the recreational user in whom sensitization develops to the reinforcing effect of the drug, a necessary step in the development of heavy abuse. Thus, the current experiments represent an examination of the possible role of CNS NT systems in the neurochemical mechanisms underlying chronic cocaine-induced behavioral tolerance and sensitization.

METHODS

Animals

Adult male Sprague-Dawley rats weighing between 100 and 125 g (Charles River Laboratories) were acclimated to the vivarium for 1 week prior to treatment. They were housed in pairs under controlled conditions of temperature and humidity and given free access to laboratory chow and water.

Drug and Minipump Preparation

Cocaine HCl (generously provided by National Institute on Drug Abuse) was dissolved in 0.9% sterile saline, and used for either injection or minipump preparation. Alzet osmotic minipumps (model 2ML2, Alza Corporation) were filled with either 2 ml of 100 mg/ml (0.294 mmoles/ml) of cocaine HCl or saline. The infusion rate was 5 μl/hr resulting in an overall average dose of 40 mg/kg/day (0.118 mmoles/kg/day).

Surgery

The animals were shaved and injected locally with lidocaine at the dorsal midline incision site prior to the administration of methoxyflurane inhalation anesthesia. A 2-cm midline incision was made and the minipump inserted into a subcutaneous pocket with the portal toward the head. The wound was closed with metal autoclips. On day 14, selected pumps were surgically removed and the residual amount of cocaine determined. Results were consistent with both the expected infusion rate and with stability of the cocaine solution under conditions of physiologic pH and temperature for the 14-day treatment period.

Cocaine Treatment

On day 1 of treatment, animals were divided into groups receiving (1) implants of cocaine-containing minipumps delivering an average of 40 mg/kg of cocaine per day; (2) a once-daily subcutaneous injection of 40 mg/kg of cocaine; or (3) once-daily subcutaneous injections of saline or implants of saline-containing minipumps. In our initial experiments, both saline-injection and saline-minipump groups were used as controls. However, because there were no statistically significant differences in the NT-LI concentration in any brain region between the two types of vehicle treatments, in subsequent experiments we used only the vehicle-injection group. Treatment was continued for 14 days. At either 24 hours or 8 days following the final treatment with cocaine or vehicle, the rats were killed by decapitation and brains rapidly removed and frozen. From the frozen brains, the frontal cortex (FC), caudate nucleus (CN), nucleus accumbens (NA), SN, and VTA were dissected. For the dissections, frozen brains were placed ventral side up in a brain matrix and anchored using a razor blade 1 mm behind the optic chiasm. The brain was stabilized in the matrix using a razor blade through the FC and successive 1-mm sec-

tions were made until the cerebellum was reached. The FC was cut out of the first slice, the NA was dissected from the section including the anterior commissure, and the CN was cut from the slice succeeding the one containing the NA. The last slice normally contained the VTA and SN.

Neurotensin Radioimmunoassay

Frozen brain tissue was extracted by sonication in ice cold 1 N HCl in polypropylene centrifuge tubes and centrifuged at 10,000 × *g* for 15 minutes at 4°C. Duplicate aliquots of supernatant in borosilicate glass tubes were lyophilized, reconstituted in phosphate-buffered saline (PBS [0.01 mol/L NaH_2PO_4, 0.15 mol/L NaCl, 0.01% sodium azide, 0.01 mol/L ethylenediaminetetraacetic acid, 0.05% Triton X-100, and 0.1% gelatin, pH 7.6]), and assayed for NT-like immunoreactivity (NT-LI) using a sensitive and specific radioimmunoassay according to previously described methods (Bissette et al. 1984). Synthetic NT_{1-13} was used as standard and was iodinated by the chloramine-T method (Hunter and Greenwood 1962). Our NT antiserum recognizes the 6-8 midportion of intact NT and was used at a final dilution of 1:8000. Goat anti-rabbit antiserum (Arnel Products, New York, NY) was used as second antibody.

Precipitates were dissolved in 1 N NaOH and assayed for protein concentration according to the method of Lowry et al. (1951) using bovine serum albumin as standard. Neurotensin concentrations were expressed as picograms NT-LI per milligram protein.

Statistics

Data were analyzed for statistical significance using one-way analysis of variance followed by Scheffé's (1953) test to compare differences between individual means. Significance was assumed if $p < 0.05$.

RESULTS

The concentration of NT-LI in specific brain regions was measured 24 hours following a single subcutaneous injection of vehicle or 40 mg/kg of cocaine. Following cocaine injection, NT-LI was significantly increased in the NA (↑94% ± 9.1%, Fig. 1a) and SN (↑44% ± 8.3%, Fig. 1b). In contrast, acute injection with cocaine did not significantly alter the concentration of NT-LI in the FC (Fig. 1c), CN (Fig. 1d), or VTA (Fig. 1e).

Rats were treated with vehicle or 40 mg/kg of cocaine (subcutaneous injection or continuous infusion) for 14 days, and the concentration of NT-LI was determined after 24 hours and 8 days of withdrawal. In contrast to the effects of a single injection of cocaine, NT-LI was not altered in the NA after either period of withdrawal (Fig. 1a). However, in the SN, NT-LI was significantly increased both 24 hours (↑61% ± 8.6%) and 8 days (↑26% ± 8.6) following 14 daily injections of cocaine. In rats implanted with cocaine-containing minipumps, NT-LI was significantly increased in the SN after 24 hours (↑35% ± 6.9%), but not 8 days of withdrawal (Fig. 1b). The increase in SN NT-LI was significantly larger in the cocaine-injected group than in the minipump group at both the 24-hour and 8-day time points.

In the FC, NT-LI was significantly increased, (↑33% ± 7.9%) 24 hours following withdrawal from 14 days of cocaine injections. This increase was no longer present after 8 days of withdrawal and, furthermore, NT-LI in the FC was not altered at either time point after continuous treatment with cocaine via implanted minipump (Fig. 1c). In the CN, although NT-LI was not statistically altered at 24 hours or 8 days of withdrawal following either daily injections or minipump infusion (Fig. 1d), there was a clear trend toward an increase in CN NT-LI in rats treated with single or chronic injections of cocaine. In the VTA, chronic cocaine treatment had no effect on the concentration of NT-LI at 24 hours or 8 days of withdrawal using either injection paradigm (Fig. 1e).

DISCUSSION

We have shown that both acute and chronic exposure to cocaine induce time-dependent, anatomically selective alterations in the concentration of NT in specific brain regions. Confirming an earlier report (Hanson et al. 1989), we observed that NT systems within the SN are exquisitely sensitive to acute and chronic cocaine injections, with a significant increase in NT concentration that persists for at least 8 days after 14 days of treatment. It is noteworthy, however, that SN NT was increased after 24 hours, but not after 8 days of withdrawal from 14 days of continuous cocaine exposure, and that even at 24 hours, the injection group had significantly greater SN–NT concentrations than the minipump-treated group. Within the SN, NT has been localized to both axon terminals and neuronal perikarya (Hökfelt et al. 1984; Jennes et al. 1982), with a high density of NT receptors localized on DA perikarya and dendrites (Palacios et al. 1982; Szigethy and Beaudet 1989). This anatomic distribution of NT content and receptors is consistent with the ability of the peptide to exert an excitatory effect on SN DA neurons (Pinnock 1985).

The large increase in NA–NT concentration following an acute injection of cocaine was not observed after either mode of chronic cocaine treatment, indicating that the basal activity of NT systems within the NA develop a tolerance to repeated exposure to the drug. Because there is a great deal of evidence implicating

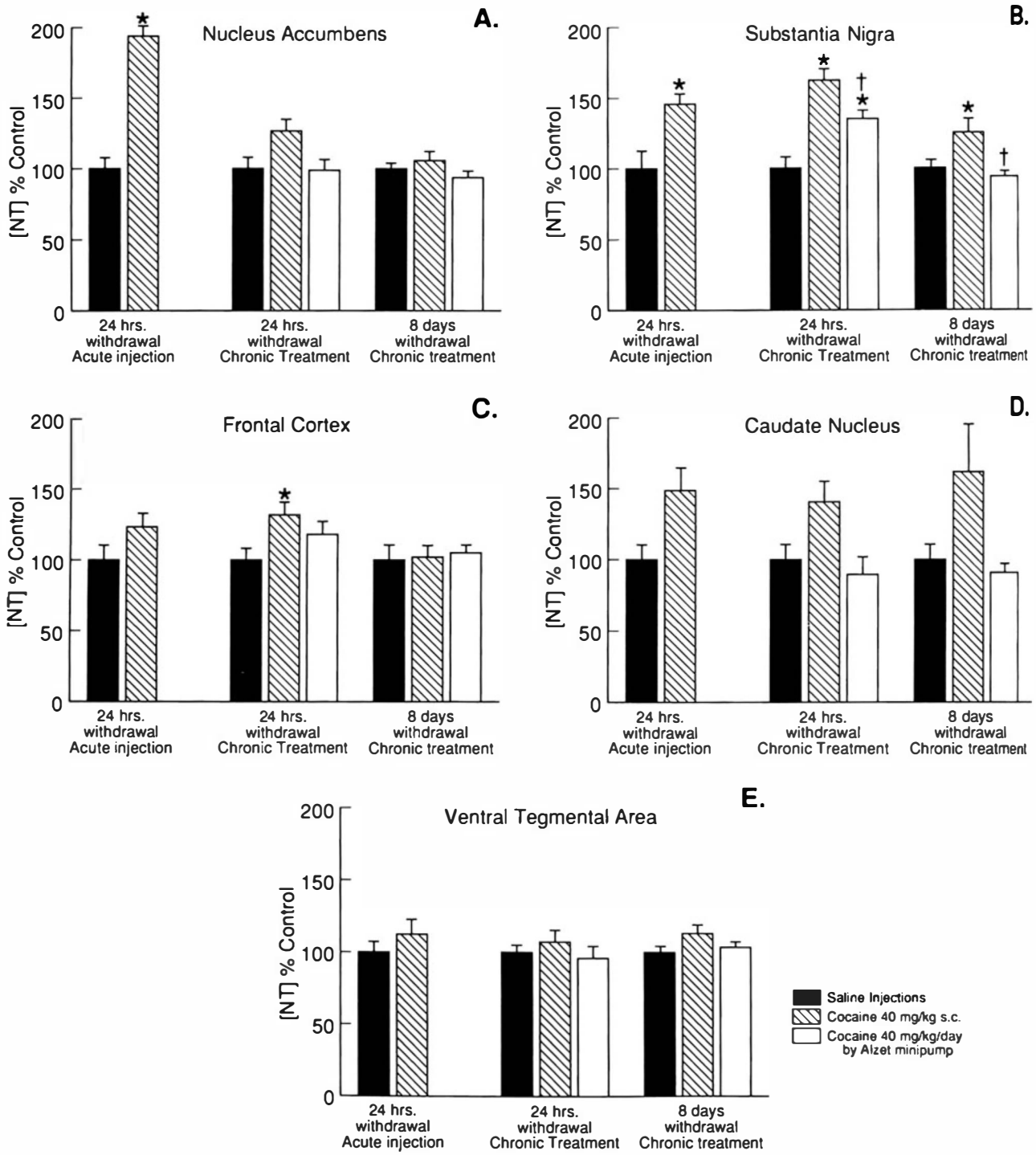

Figure 1. Rats were sacrificed 24 hours following a single subcutaneous (SC) injection of 40 mg/kg cocaine or vehicle (n = 6 to 8) and 24 hours and 8 days after 14 days of SC vehicle injections, once-daily 40 mg/kg SC cocaine injections or 14 days of 40 mg/kg/day continuous infusion of cocaine using minipumps implanted subcutaneously (n = 13 to 16). After the designated period of withdrawal, the rats were killed by decapitation and the brain removed and rapidly frozen. The NA (Fig. 1a), SN (Fig. 1b), FC (Fig. 1c), CN (Fig. 1d), and VTA (Fig. 1e) were dissected from the frozen brain. Samples were extracted in 1 N HCl and the radioimmunoassay for NT performed as described in Methods. Two separate experiments were run for both the 24-hour and 8-day withdrawal groups. All brain regions within a particular experiment were assayed simultaneously. Results from both experiments were expressed as percent of control NT-LI and combined for statistical analysis. Absolute amounts of NT-LI in picogram per milligram protein for each of the saline control groups are provided below.

Acute injection and 24-hour withdrawal (experiment 1). NA: 239.9 ± 28.6; SN: 392 ± 47; FC: 63.6 ± 5.9; CN: 111.3 ± 11.1; and VTA: 800.6 ± 60.9.

NA-DA systems in the physiologic response to cocaine (Delfs et al. 1990; Henry et al. 1989) as well as evidence linking intra-NA NT to the modulation of DA-mediated behaviors (Kalivas et al. 1984), it will be of interest to determine the significance of this apparent tolerance of the NA–NT system that develops during chronic cocaine exposure. In contrast to NA NT, there is an apparent sensitization of FC NT to chronic cocaine injections, but not to continuous infusion. Rats will self-administer cocaine into the medial prefrontal cortex (Goeders and Smith 1983); a phenomenon that is dependent upon intact DAergic innervation of the cortex (Goeders and Smith 1986). Although information concerning the functional significance of frontal cortical NT is sketchy, a mixed mesocortical NT–DA pathway in the rodent has been reported (Seroogy et al. 1988; Studler et al. 1988) and NT and DA are coreleased upon afferent stimulation (Bean et al. 1989; Bean and Roth 1991). Neither acute nor chronic treatment with cocaine significantly altered the NT content of CN or VTA. The lack of cocaine-induced NT concentration changes in the CN is somewhat discrepant with the report of Hanson et al. (1989) in which an increase in striatal NT content up to 24 hours following multiple doses of cocaine was reported. However, perusal of our results (Fig. 1d) indicates that there was a clear trend toward an increase in CN NT following both acute and chronic injections of cocaine. We noted that Hanson et al. (1989) also observed significant experimental variability in striatal NT content 24 hours following an acute intraperitoneal injection of 30 mg/kg cocaine. A possible explanation for the observed treatment-induced variability in NT content is the neurochemical heterogeneity of NT within the basal ganglia. For example, in the cat, NT is enriched in the striosomal compartment of the striatum (Goedert et al. 1983, 1984). Thus, to reliably observe cocaine-induced changes in striatal NT content, it may be necessary to evaluate the response of NT subsystems within this brain region.

It is important to recognize that the two chronic cocaine-treatment protocols yield opposite behavioral profiles. Daily injections result in behavioral sensitization, whereas, in contrast, continuous infusion induces behavioral tolerance to a subsequent challenge dose of cocaine (King et al. 1992; Reith et al. 1987). It is possible that behavioral and neurochemical differentiation between the intermittent- and continuous-treatment paradigms results from differential rates of cocaine metabolism following the challenge injection. However, although the rate of cocaine metabolism was not determined in our experiments, Reith et al. (1987) monitored plasma and brain levels of cocaine and a metabolite, benzoylecgonine, 12 minutes after a challenge dose of cocaine in their sensitized and tolerant animals, but could discern no difference between the groups. An alternative explanation for the observed differentiation between continuous and intermittent paradigms is that the behavioral and neurochemical sequelae are the reflection of a low (continuous) versus high (intermittent) dose-response effect. Two points must be addressed in this regard. First, the animals undergoing continuous administration are clearly receiving a pharmacologically active dose of cocaine. This is evidenced by the development of behavioral tolerance in rats treated identically to those used in the experiments described in this report (King et al. 1992). Second, the majority of reports evaluating the effects of low-dose (10 to 15 mg/kg) intermittent cocaine injection have noted behavioral sensitization in the injected animals (e.g., Segal and Kuczenski 1992), indicating that the temporal basis of the treatment regimen is a critical factor in the development of tolerance or sensitization. Therefore, although it is still not possible to definitively conclude that the NT alterations we observed were unrelated to a dose-response phenomenon, we believe it likely that continuous versus intermittent exposure to cocaine engenders separable neurochemical states that mediate the opposing behavioral responses.

In summary, our findings are consistent with the notion that cocaine-induced changes in CNS–NT systems may underlie, at least in part, the pronounced behavioral sensitization/tolerance effects of cocaine. We have shown that the method of cocaine exposure selectively alters NT concentration within particular DA cell body and terminal regions. The differential responsiveness of nigrostriatal NT and mesolimbic NT is particularly interesting. To further evaluate the behavioral and functional significance of cocaine-induced alterations in NT neurobiology, it will be critical to refine the anatomic and neurochemical analysis of the NT response by analyzing cocaine-induced changes in NT gene expression as well as NT receptor function and expression.

Figure 1. (*Continued*)

Twenty-four-hour withdrawal (experiment 2). NA: 449 ± 77; SN: 480 ± 28; FC: 67 ± 5.8; CN: 130 ± 23.2; and VTA: 791 ± 129.

Eight-day withdrawal (experiment A). NA: 318.9 ± 15.2; SN: 489.5 ± 36; FC: 80.2 ± 10.9; CN: 63.3 ± 10.2; and VTA: 1121.6 ± 98.3.

Eight-day withdrawal (experiment B). NA: 232.8 ± 18.6; SN: 338.4 ± 24; FC: 86.6 ± 12; CN: 67 ± 7.9; and VTA: 872.6 ± 67.3.

$^{*}p < 0.05$ relative to control; $^{\dagger}p < 0.05$ cocaine injection versus cocaine minipump.

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