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Clozapine and cocaine effects on dopamine and serotonin release in nucleus accumbens during psychostimulant behavior and withdrawal

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Abstract

There is an increasing awareness that a psychosis, similar to that of schizophrenic psychosis, can be derived from cocaine addiction. Thus, the prototypical atypical antipsychotic medication, clozapine, a 5-HT_2/DA_2 antagonist, was studied for its effects on cocaine-induced dopamine (DA) and serotonin (5-HT) release in nucleus accumbens (NAcc) of behaving male Sprague-Dawley laboratory rats with In Vivo Microvoltammetry, while animals' locomotor (forward ambulations), an A_{10} behavior, was monitored at the same time with infrared photobeams. Release mechanisms for monoamines were determined by using a depolarization blocker, gamma-butyrolactone (yBL). BRODERICK PROBE® microelectrodes selectively detected release of DA and 5-HT within seconds and sequentially in A_{10} nerve terminals, NAcc. Acute and subacute studies were performed for each treatment group. Acute studies are defined as single injection of drug(s) after a stable baseline of each monoamine and locomotor behavior has been achieved. Subacute studies are defined as 24-h follow-up studies on each monoamine and locomotor behavior, in the same animal at which time, no further drug was administered. Results showed that (1) acute administration of cocaine (10 mg/kg ip) (n = 5) significantly increased both DA and 5-HT release above baseline (P < .001) while locomotion was also significantly increased above baseline (P < .001). In subacute studies, DA release decreased significantly below baseline (P<.001) and significant decreases in 5-HT release occurred at the 15-min mark and at each time point during the second part of the hour (P < .05); the maximum decrease in 5-HT was 40% below baseline. Locomotor behavior, on the other hand, increased significantly above baseline (P< .05). (2) Acute administration of clozapine/cocaine (20 and 10 mg/kg ip, respectively; n = 6) produced a significant block of the cocaine-induced increase in DA (P<.001) and 5-HT release (P<.001). Cocaine-induced locomotion was blocked simultaneously with each monoamine by clozapine as well (P < .001). In subacute studies, DA release continued to be blocked presumably via clozapine by exhibiting a statistically significant decrease (P<.001), but 5-HT release increased significantly (P< .001), while cocaine-induced locomotor activity also continued to be antagonized by clozapine, i.e., locomotor activity exhibited no difference from baseline (P> .05). In summary, acute studies (a) support previous data from this laboratory and others that cocaine acts as a stimulant on the monoamines, DA and 5-HT and on locomotor behavior as well and (b) show that clozapine, 5-HT_2/DA_2

Abbreviations: APM, activity pattern monitor; AA, ascorbic acid; BSA, bovine serum albumin; DOPAC, 3,4dihydroxyphenylacetic acid; DOI, [(±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride]; DA, dopamine; DR, Dorsal Raphe; EPS, Extrapyramidal Symptoms; "yBL, gamma-butyrolactone; HVA, homovanillic acid; R(+)-8-OH-DPAT, [R(+)-8-hydroxy-2-(di-n-propylamino)-tetralin]; 5-HIAA, 5-hydroxyindoleacetic acid; IACUC, Institutional Animal Care and Use Committee; A_{10}, mesolimbic pathway/mesocorticolimbic neuronal pathway; A_9, nigrostriatal neuronal pathway; NE, norepinephrine; NAcc, Nucleus Accumbens; PEA, phosphotidylethanolamine; pA, picoamperes; PFC, Prefrontal Cortex; RR, Raman Resonance; 5-HT, serotonin; Ag/Ag/Cl, silver/silver chloride; 24-h follow-up studies, subacute studies; SERS, Surface Enhanced Raman Spectroscopy; TH, tyrosine hydroxylase; UA, uric acid; VTA, Ventral Tegmental Area; vlNAcc, ventrolateral Nucleus Accumbens.

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antagonist, blocked enhanced DA, 5-HT and psychomotor stimulant behavior induced by cocaine. Subacute studies (a) suggest that withdrawal responses occurred in the cocaine group, based on recorded deficiencies in monoamine neurotransmitters (b) show that withdrawal effects in the cocaine group likely presynaptic, were distinguished from locomotor behavior, classically known to be mediated postsynaptically, and finally, (c) suggest that clozapine, with longer lived pharmacokinetic properties, reversed 5-HT cocaine-related withdrawal effects, but was unable to reverse DA cocaine-related withdrawal responses. Taken together with data from this laboratory, in which the 5-$HT_{2A/2C}$ antagonist, ketanserin, affected cocaine neurochemistry in much the same way as did clozapine, a mediation by either separate or combined 5-HT_{2A}/2c receptors for these clozapine/cocaine interactions, is suggested. Further studies, designed to tease out the responses of selective 5-HT_{2A} and 5-HT_{2C} receptor compounds to cocaine and clozapine/cocaine, are underway.

Keywords: A_9; Aj_0; Acute; Animal models of psychosis; Autoreceptors; BRODERICK PROBE® microelectrodes; 5-HT2/DA2 antagonists; Clozapine; Clozapine/cocaine combination; Cocaine; Cocaine withdrawal; Depression; Dopamine (DA); In Vivo Microvoltammetry; Locomotor (ambulatory) activity; Negative symptoms; Nucleus accumbens (NAcc); Open-field behavior; Positive symptoms; Psychostimulants; Raman Resonance (RR); Schizophrenia; Serotonin (5-HT); Subacute; Surface Enhanced Raman Spectroscopy (SERS); Withdrawal

1. Introduction

1.1. Addiction and psychoses

We continue to strategize possible pharmacotherapies for cocaine-induced psychosis by studying the effects of a variety of typical and atypical antipsychotic medications on cocaine-induced neurochemistry and psychomotor stimulant behavior. The hypothesis derives from similarities between schizophrenic and cocaine psychosis, similarities which are being reported in the clinical literature at an alarming rate (Brady et al., 1991; Harris and Batki, 2000; Lysaker et al., 1994; Mendoza et al., 1992; Miller et al., 1992; Mitchell and Vierkant, 1991; Nambudin and Young, 1991; Rosenthal and Miner, 1997; Rosse et al., 1994; Satel and Edell, 1991; Serper et al., 1999; Sherer et al., 1988; Taylor and Staby, 1992; Tueth, 1993). Cocaine psychosis is a major psychopathology (Satel et al., 1991) and hyperfunction of DA (dopamine)-ergic systems is a critical element in cocaine-induced psychosis (Lieberman et al., 1990). Too, what complicates the situation further, are data which show that about 50% of the patients who suffer from schizophrenia have also been substance abusers at some time during their illness. Actually, schizophrenic patients are reported to feel the need to alleviate their psychosis by self-treating with reinforcing drugs (Buckley, 1998; Mueser et al., 1995).

1.2. Psychomotor stimulant animal model of addiction and psychosis

One way to strategize such treatments for cocaine addiction and psychosis is to reverse certain elements of the disorders by utilizing laboratory studies in animals (McKinney, 1989). The strategy is reasonable especially since data from animal studies of stimulant psychosis and human schizophrenic psychosis share the same neurochemical and behavioral manifestations (Gawin et al., 1989; Margolin et al., 1995; Wise, 1995; Wise and Bozarth, 1987). Also, because cocaine is self-administered universally across species (Fischman, 1984; Risner and Jones, 1980), it is highly likely that similar universal underlying reward mechanisms and mechanisms of consequent adverse symptomatology are similar from species to species.

The animal model of psychomotor stimulant behavior for cocaine addiction and psychosis has been validated by using this model to correlate antipsychotic medications and DA-ergic neuroanatomic pathways, for example, but not exclusively, typical antipsychotics act through DA within nigrostriatal pathways and atypical antipsychotics act through DA and 5-HT within mesolimbic and mesocorticolimbic pathways (Cools and van Rossum, 1970; Costall and Naylor, 1973; Kelly et al., 1975; Pijnenburg et al., 1975; Wise and Bozarth, 1987). Human data support these animal data (Gawin and Kleber, 1986a,b; Gawin et al., 1989; Meltzer, 1989). Interestingly, it is currently thought that both typical and atypical antipsychotic agents alleviate schizophrenic psychosis via DA_2 blockade in mesocorticolimbic areas, whereas DA_2 blockade in nigrostriatal areas is thought to produce motor side effects, extrapyramidal symptoms (EPS). Nonetheless, the psychomotor stimulant animal model has become an accepted model to study cocaine psychosis, albeit limited to certain aspects of the disease. An accepted neuroanatomic site for testing reversal of positive symptoms of psychosis is within nucleus accumbens (NAcc), mesolimbic nerve terminals (Weinberger et al., 1992).

1.3. Cocaine, monoamine transporters and release mechanisms

Cocaine has a high affinity for monoamine transporters, and via these transporters, re-uptake of monoamines into presynaptic nerve terminals is inhibited (Izenwasser et al., 1990; Koe, 1976); interestingly, certain subjective reward and jittery effects from cocaine have recently been associated with these monoamine transporters (Hall et al., 2002). In addition, cocaine has been shown to be dependent on

stimulated release mechanisms (Ng et al., 1991) and on basal release mechanism by using the DA impulse flow inhibitor, gamma butyrolactone (yBL) (Broderick, 1991b). Although cocaine is not a direct receptor acting agonist, enhancement of DA neurotransmission may also be provided adjunctly through indirect activation of DA receptors, i.e., Dj and D_2 (Spealman et al., 1992; Wise, 1995).

1.4. Cocaine, monoamine concentrations and reward mechanisms

Cocaine increases DA concentrations in mesolimbic neuronal circuits and the evidence suggests that the mechanism underlying cocaine's rewarding effect involves hyperfunction of the mesolimbic DA system, particularly in Aio nerve terminals, NAcc (Broderick, 1991a,b; Brown et al., 1991; Hernandez andHoebel, 1988; Kalivas andNemeroff, 1988) and in A_{i0} somatodendrites, ventral tegmental area (VTA) (Bradberry and Roth, 1989; Broderick, 1992a; Einhorn et al., 1988; Kalivas, 1993; Kalivas and Duffy, 1990). There is a general consensus from both clinical and preclinical studies that DA mediates the rewarding effects of cocaine (de Wit and Wise, 1977; Gawin et al., 1989; Lieberman et al., 1990; Roberts and Koob, 1982; Roberts et al., 1977; Tsibulsky et al., 1998; Wise, 1995; Wise and Bozarth, 1987; Wise and Rompre, 1989).

Cocaine increases 5-HT concentrations in A_{Io} terminals, NAcc, after single administration (Bradberry et al., 1993; Broderick et al., 1993). Sensitized 5-HT efflux in NAcc occurs after repeated cocaine administration (Parsons and Justice, 1993) and cocaine-increases 5-HT release induced by electrical stimulation of A_{Io} neurons, in vitro (Chen and Reith, 1993). Importantly, when 5-HT concentrations are deficient, such as in the Fawn-Hooded laboratory rat, cocaine-induced increases in 5-HT release are attenuated (Hope et al., 1995). Consistent with increased concentrations of 5-HT after cocaine, cocaine inhibits 5-HT re-uptake in vitro (Ross and Renyi, 1969) and more recent studies have shown that cocaine inhibits 5-HT re-uptake specifically in NAcc (Galloway, 1990). Also consistent with increased concentrations of 5-HT after cocaine, cocaine represses impulse frequency rates in vivo and in vitro in 5-HT somatodendrites, DR (Cunningham and Lakoski, 1988; Pan and William, 1989). Furthermore, 5-HT-cocaine interactions have been associated with transporter mechanisms (Carroll et al., 1993; Hall et al., 2002; Reith et al., 1983).

Nonetheless, a precise association between 5-HT and brain reward remains to be determined. Dietary 1-tryptophan, a 5-HT precursor, and fluoxetine, a 5-HT re-uptake inhibitor, have been reported to reduce cocaine self-administration (Carroll et al., 1990a,b; McGregor et al., 1993; Peltier et al., 1994) and depletion of forebrain 5-HT with parachlorophenylalanine (PCPA) facilitates cocaine self-administration (Loh and Roberts, 1990; Richardson and Roberts, 1991). However, there are studies that are discrepant from these previous studies (Porrino et al., 1989). Also, self-stimulation studies, using 5-HT_{2a} antagonists and mixed DA_2/5-HT_{2A} antagonists, have suggested no involvement between 5-HT_{2A}, brain stimulation and cocaine stimulation reward (Frank et al., 1995; Moser et al., 1995; Ramana and Desiraju, 1989; Tsibulsky et al., 1998). Particularly relevant is a possible interpretation from the latter studies that atypical antipsychotics may not affect the regulation of positive affect while still blocking neurochemical and behavioral effects of cocaine, which may lead to psychosis.

1.5. Cocaine, monoamines and psychomotor stimulant behavior

Intra-NAcc infusions of cocaine mimics the hyperlocomotor effects of cocaine (Delfs et al., 1990) and the DA mesolimbic pathway has been directly implicated in the behavioral effects of cocaine (Kalivas and Nemeroff, 1988). Manipulations of 5-HT modulate the locomotor stimulant effects of cocaine (Walsh and Cunningham, 1997). Cocaine increases 5-HT in DA mesolimbic pathways simultaneously with increased locomotion, but the temporal pattern is disrupted compared with 5-HT increases with exploratory activity (Broderick, 2001). Specific 5-HT receptor mediation has been shown to correlate with open-field locomotion, e.g., local application of 5-HT and 5-HT_{1A} agonist, 8-OH-DPAT into median raphe nuclei causes hyperactivity (Hillegaart et al., 1989) and 8-OH-DPAT, has been shown to up-modulate cocaine-induced psychostimulant behavior (De La Garza and Cunningham, 2000). Specific 5-HT_{2A} and 5-HT_{2C} receptor mediation has been shown to correlate with cocaine-induced hyperactivity (Filip and Cunningham, 2002; McMahon and Cunningham, 2001; McMahon et al., 2001).

1.6. Monoamine interactions, basis for cocaine mechanisms

Turnover of DA is altered in NAcc when the 5-HT somatodendrites, DR, are electrolytically lesioned and these interactions modulate locomotion (Costall et al., 1976, 1990; Herve et al., 1979). Somatodendrites for DA, VTA, contain dense networks of 5-HT axonal varicosities (Broderick and Phelix, 1997; Herve et al., 1987; Steinbusch, 1981; Van Bockstaele et al., 1994) and axons in NAcc core and shell exhibit overlapping of tyrosine hydroxylase (TH) and 5-HT (Phelix and Broderick, 1995; Van Bockstaele and Pickel, 1993).

1.7. Pharmacotherapies for psychoses

Pharmacotherapies for cocaine psychosis are virtually nonexistent. Thus far, clinicians are relying for therapy on antipsychotic medications and reasonably so because, as mentioned previously, neurochemical and behavioral similarities exist between schizophrenic and cocaine psychosis. Our main focus, then, is also in treatment strategies for

cocaine based on antipsychotics, particularly in the area of atypical antipsychotic medications due to their dual interactions on dopamine (DA) and serotonin (5-HT) in DA neuronal pathways primarily in the mesolimbic/mesocorticolimbic A_{lo} neuronal circuitry. This now well-known 5-HT_2/DA_2 receptor affinity in the Ai_0 circuit helps to alleviate both positive and negative symptoms of psychosis in addition to being mood enhancers (Meltzer, 1989, 1992). The leading hypothesis for the mechanism of action of these newer generation, atypical antipsychotic agents, is the presence of a high 5-HT-DA receptor blockade ratio in mesolimbic and mesocorticolimbic neural circuits. When 5-HT-ergic activity is blocked as is the case with many atypical antipsychotics, DA inhibition of DA release is also blocked, consequently, increasing presynaptic DA release and balancing DA blockade at postsynaptic receptor sites. The final result is less risk for EPS (Glazer, 2000).

1.8. Clozapine

Clozapine is considered to be the prototype of the atypical antipsychotics as it was the first to be recognized as having few if any EPS, not causing tardive dyskinesia or Parkinson's side effects including dystonia (Lieberman et al., 1989; Parsa et al., 1991). It is interesting that clozapine is not generally a first line defense drug against schizophrenia, but clozapine is especially effective for treating drug-resistant schizophrenia, when typical antipsychotics have failed the patient (Kane et al., 1988; Ranjan and Meltzer, 1996). Clozapine does not produce catalepsy (Kruzich and See, 2000). On the other hand, it is well known that clozapine may produce agranulocytosis in .0.5-2% of patients; blood serum levels must be monitored weekly for the first 6 months. Sedation and weight gain are limiting factors in clozapine treatment (Stahl, 2000).

The varied effects of clozapine may come about because the receptor-binding profile for clozapine is complex. Clozapine binds to the following receptors: 5-HTi_A, 5-HT_2a, 5-HT_{2C}, 5-HT3, 5-HT_6, 5-HT_7, DA_b DA_2, DA_3, DA4, M_b H_b a_b and a_2 (Brunello et al., 1995; Pere, 1995; Schotte et al., 1993, 1996; Stahl, 2000). Clozapine has high affinity for 5HT_2a receptors and low affinity for DA_2 receptors (Meltzer, 1991, 1999; Meltzer and Nash, 1991; Meltzer et al., 1992). Of the occupancy ratios for atypical antipsychotic medication, clozapine has the lowest occupancy for DA_2 receptors (Kapur and Remington, 2001; Meltzer et al., 1992).

1.9. Clozapine/cocaine

Clozapine is an excellent candidate to test reversal of cocaine's effect, not only because of low DA receptor occupancy which is thought to reduce EPS, but also because clozapine is prescribed for cocaine addiction with reasonable success, i.e., clozapine pretreatment diminishes subjective responses to cocaine, including expected high and rush responses (Farren et al., 2000). In another study, pretreatment with clozapine has been shown to alleviate cocaine abuse in more than 85% of active substance (cocaine) abusers (Zimmet et al., 2000).

2. Methods

2.1. Drugs

Clozapine was obtained from Sigma Aldrich, St. Louis, MO, dissolved in distilled water, and the pH of the solution was adjusted to 2.7 with citric acid powder. Cocaine was obtained from Sigma Aldrich, and dissolved in distilled water.

2.2. Animals

Animals were purchased from Charles River Laboratories, Kingston, NY, and were housed in our animal care facilities for 1 to 2 weeks before surgery was performed. The Animal Care Facility operates under the auspices of the CUNY, City College Institutional Animal Care and Use Committee (IACUC) in compliance with National Institute of Health (NIH) guidelines. The weight range for the animals, at the time of the studies, was 350-475 g. Animals were group housed before surgery, individually housed after surgery and fed Purina Rat Chow and water ad libitum. A 12-h dark-light cycle was maintained both in the housing of the animals and throughout the experimental studies.

2.3. Surgical procedures and implantation of microelectrodes

2.3.1. Protocols follow paradigm described in Broderick et al. (2003)

Each animal was anesthetized with pentobarbital Na, {50 mg/kg ip [dilute (6%) solution]} and stereotaxically implanted with a BRODERICK PROBE® indicator microelectrode in ventrolateral (vl) NAcc (AP + 2.6, ML + 2.5, DV — 7.3) (Pellegrino et al., 1979). The stereotaxic equipment was purchased from David Kopf Instruments, Tujunga, CA. A Ag/AgCl reference electrode was placed in contact with dura, 7 mm anteriorally and contralaterally to the indicator microelectrode. A stainless steel auxiliary microelectrode was placed in contact with dura. BRODERICK PROBE® microelectrodes were manufactured on site.

Animals' body temperature was continuously monitored with a rectal probe and thermometer (Fisher Sci., Fadem, NJ). Body temperature was maintained at 37.5 ± 0.5 °C with an aquamatic K module heating pad (Amer. Hosp. Supply, Edison, NJ). Booster injections of pentobarbital Na were administered once after the first 2 h of surgery (0.10 cc) and once every subsequent hour (0.05 cc) to maintain an adequate level of anesthesia throughout surgery. The total

time for surgery was 3-4 h. The indicator, reference, and auxiliary microelectrodes were held in place with dental acrylic (Jet Line, Lang Dental, CA). Animals recovered in a bedded Plexiglas cage [dimensions: 12x12x18 in. (width x depth x height)] after surgery and before the experimental studies began, with food and water ad libitum. The animals were treated with physiological saline (0.5 cc) immediately and for 1-2 days after surgery as needed. The antibiotic, chloramphenicol (50 mg/kg ip) was administered if needed.

In vivo microvoltammetric studies on conscious Sprague-Dawley laboratory rats were begun 9-15 days after the aseptic surgical operations were performed. On each experimental day, the animal was placed in a Plexiglas-copper faradaic chamber. The three-microelectrode assembly, enclosed within the animal's prosthetic acrylic cap, was connected to a CV37 detector by means of a mercury commutator (Br. Res. Instr., Princeton, NJ), a flexible cable, and a mating connector (BJM Electronics, Staten Island, NY). The CV37 detector was electrically connected to a Minigard surge suppresser (Jefferson Electric, Magnetek, NY), which was then connected to an electrical ground in isolation. Stable electrochemical signals for DA and 5-HT were evident before either (i) clozapine (20 mg/kg ip), (ii) cocaine (10 mg/kg ip) or (iii) combination of clozapine and cocaine (20 and 10 mg/kg ip, respectively) were administered. Each animal was used as its own control. Changes in synaptic concentrations of DA and 5-HT are presented as percent change (percent of control) to minimize normal between-animal variations. Currents recorded were in the order of magnitude of pA or nA. In vivo microvoltammetric scans were recorded in seconds and repeated every 5 min for a period of 2 h before each treatment and a period of 2 h after each treatment.

2.4. In vivo microvoltammetry: technology

In Vivo Microvoltammetry with a semidifferential (semiderivative) circuit was used; a clear separation of the monoamine neurotransmitters, DA and 5-HT was achieved. Dopamine and 5-HT were detected within seconds in separate signals. Oxidation peak potentials (half-wave potentials) of +0.14 ±0.015 and +0.29 ±0.015 V were characteristic for DA and 5-HT. Detailed methodology is published (Broderick, 1988, 1989, 1990, 1991b, 1999, 2001, 2002; Broderick and Pacia, 2003; Broderick et al., 1993, 2000). The electrochemical signal for DA, was detected without interference at the same oxidation potential, from 3-4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and ascorbic acid (AA). Indeed, clear and separated signals are routinely achieved with BRODERICK PROBE® microelectrodes for AA, HVA and DOPAC. The electrochemical signal for 5-HT was detected without interference at the same oxidation potential, from the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA) and uric acid (UA). Potentials were applied with a CV37 detector (BAS, West Lafayette, IN). Potentials were applied from —0.2 V to +0.4 V with respect to a Ag/AgCl (1 M NaCl) electrode, at a scan rate of 10 mV/s at time constants of 5 and 1 s tau. One scan was completed in 60 s. Nonfaradaic charging current was eliminated in the first 25 s. The neurotransmitters, DA and 5-HT, were detected in approximately 10-15 and 10-12 s, respectively, with BRODERICK PROBE® stearic scid microelectrodes and 10-12 and 8-10 s, respectively, with BRODERICK PROBE® lauric acid microelectrodes. The coulombic efficiency for the detection of 5-HT was two- to threefold greater than that for DA (Broderick, 1987).

Calibration curves were determined experimentally, in vitro, in a freshly prepared deoxygenated physiological saline-phosphate buffer solution (0.01 M, pH = 7.4). We use ultra pure nitrogen (N_2) (T.W. Smith Corp., Brooklyn, NY) to deoxygenate the buffer solution. Solutions of DA and 5-HT (99% purity, Sigma Aldrich) as well as metabolites of the monoamines, were aliquoted into the buffer and the peak height of the electrochemical signals were correlated with specific nM and pM concentrations. Calibration studies were also performed in freshly prepared deoxygenated buffer solutions containing phosphotidylethanolamine (PEA), combined with bovine serum albumin (BSA) (Sigma Aldrich), a solution which closely mimics brain constituents. These studies showed that lipid constituents of brain and not proteins, amplify the detection sensitivity of the indicator microelectrodes, supporting previous data which show that lipids amplify electrochemical signals detected by BRODERICK PROBE® microelectrodes; the phenomenon is termed The Lipid Amplification Number (LAN) (Broderick, 1999; Broderick et al., 2000). Surface Enhanced Raman Spectroscopy (SERS) and Raman Resonance (RR) techniques have correlated our findings on signal amplification by lipids (Foucault et al., 2002). Detection limits for basal synaptic concentrations of DA and 5-HT in NAcc were 12 and 2 nM, respectively. Placement of indicator microelectrodes in NAcc of each animal, was confirmed by the potassium ferrocyanide blue dot method, using a current of 50 mA for period of 30 s. Virtually no damage to brain tissue occurred. Recording characteristics of microelectrodes were stable.

2.5. Behavior

Locomotor activity (ambulation) was monitored with infrared photobeams at the same time as DA and 5-HT release in NAcc was detected with the BRODERICK PROBE® in conjunction with In Vivo Microvoltammetry. The chamber was faradaic, covered with copper to refract possible electrical artifacts [dimensions: 24 x 18 x 23.5 in. (width x depth x height)]. A 16 x 16 array of these infrared photobeams, were held in place by an aluminum frame which was situated 3/4 in above the Plexiglas floor of the chamber to detect locomotor activity. Photobeams were sampled by a Pentium computer to define the x-y position

of the animal within a 1.5 in resolution every 100 ms. When an x-y position was calculated, it was used to define the frequency of locomotor activity in counts. The locomotor activity system is a modified version of an Activity Pattern Monitor (APM) (San Diego Instruments, San Diego, CA). Behavioral data are presented in absolute frequency, i.e., number of counts recorded.

The first hour predrug allowed exploratory behavior. Exploratory behavior is defined as open-field behavior of ambulations (forward locomotion) wherein animals respond to the stimuli of a novel environment with high frequency of behavioral counts. The second hour predrug allowed the animal to become habituated before treatment. Habituation behavior is defined as a behavioral state in which behavior exhibits reduced responses to novel stimuli; animals cease exploring or searching in their novel environment and maintain a steady-state response to novel stimuli.

In the acute studies, each drug was administered at the end of the habituation period. Baseline (control) values were taken every 5 min for the last 30 min of the habituation period at which time drug injection(s) took place. Electrochemical recordings for DA and 5-HT release in NAcc, were continued for 2 h; at the same time, locomotor behavior continued to be monitored and recorded with infrared photobeams. At the end of the 2 h drug(s) study, animals were then placed back in their home cages.

In the subacute studies, which took place 24 h later, the animals were again placed in the faradaic behavioral chamber and no further drug was administered. Recordings of separate electrochemical signals for DA and 5-HT release in NAcc, were taken for 1 h; at the same time, locomotor behavior was monitored and recorded with infrared photobeams.

2.6. Data analysis

Neurochemical and behavioral data, derived from the last 30 min of the habituation period, provided the baseline data. Statistically significant differences between baseline and postdrug injection(s) for (1) DA (2) 5-HT and (3) locomotor behavior were determined by subjecting the data to one-way analysis of variance (ANOVA) (tested at criteria Z^3 =.05), with subsequent application of the post hoc test, Tukey's Multiple Comparison Test; where appropriate, data points in the time course were subjected to 95% confidence limits (C.L.).

3. Results

3.1. Day 1: acute studies: Effects of cocaine or clozapine/cocaine combination on DA release in NAcc

Cocaine (open circles): Cocaine significantly increased DA release over baseline (habituation) values (one-way ANOVA: P<.0001; F=51.17; #=3,56). Post hoc analysis showed that significant differences between precocaine (baseline) and postcocaine (same animal control) occurred as well (Tukey's Multiple Comparison Test: P<.001, q = 9.498).

Clozapine/cocaine (closed circles): Clozapine significantly blocked the effects of cocaine on DA release (one-way ANOVA: P<.0001; F=51.17; #=3,56). Post hoc analysis showed significant differences between cocaine and clozapine/cocaine groups (Tukey's Multiple Comparison Test: P< .001, q= 16.43) (seeFig. 1A).

3.2. Day 1: acute studies: effects of cocaine and clozapine/cocaine combination on 5-HT release in NAcc

Cocaine (open circles): Cocaine significantly increased 5-HT release over baseline (habituation) values (one-way ANOVA: P<.0001; F= 154.2; #=3,56). Post hoc analysis showed that significant differences between precocaine (baseline) and postcocaine (same animal control) occurred as well (Tukey's Multiple Comparison Test: P<.001, q= 16.19).

Clozapine/cocaine (closed circles): Clozapine significantly blocked the effects of cocaine on 5-HT release (one-way ANOVA: P<.0001; F= 154.2; #=3,56). Post hoc analysis showed significant differences between cocaine and clozapine/cocaine groups (Tukey's Multiple Comparison Test: P<.001, q = 28.98) (see Fig. IB).

3.3. Day 1: acute studies: effects of cocaine and clozapine/cocaine combination on locomotion (ambulations)

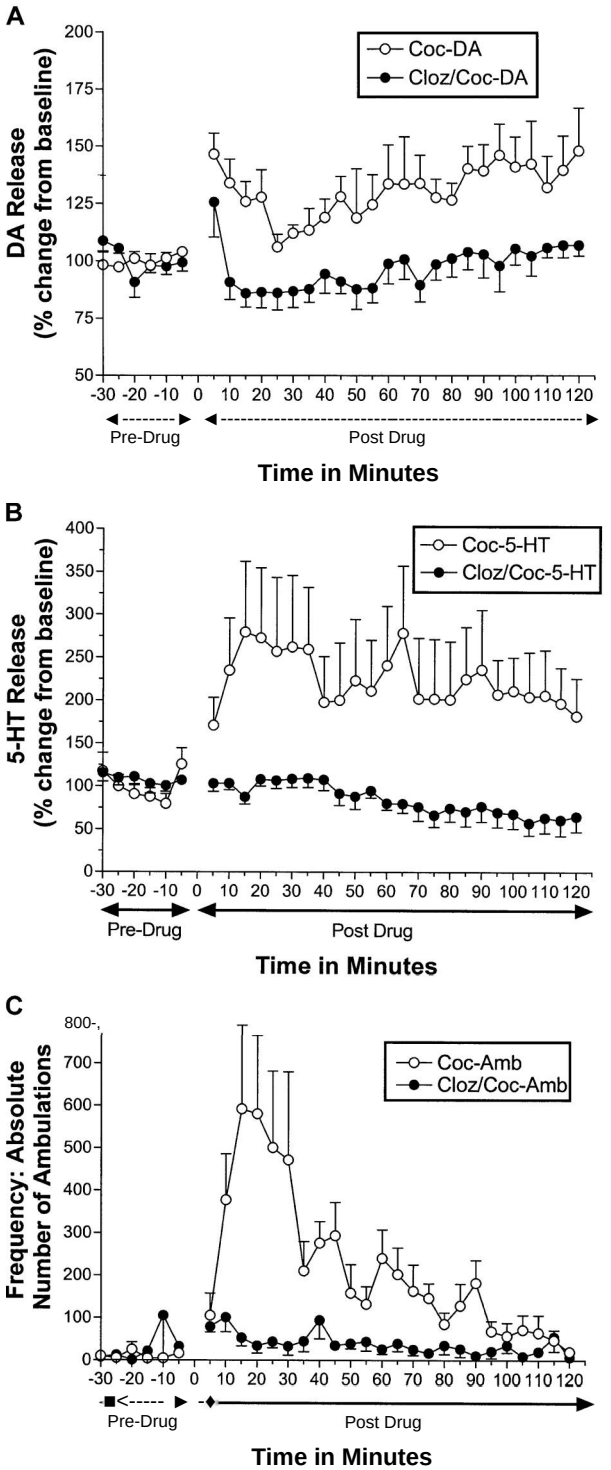
Cocaine (open circles): Cocaine significantly increased locomotor activity (ambulations) over baseline (habituation) values (one-way ANOVA: P<.0001; F= 13.06; #=3,56). Post hoc analysis showed that significant differences between precocaine (baseline) and postcocaine (same animal control) occurred as well (Tukey's Multiple Comparison Test: P<.01, q = 5.688).

Clozapine/cocaine (closed circles): Clozapine significantly blocked the effects of cocaine on locomotor activity (one-way ANOVA: P<.0001, F= 13.06; #=3,56). Post hoc analysis showed significant differences between cocaine and clozapine/cocaine groups (Tukey's Multiple Comparison Test: P<.001, $_?$ = 7.784) (Fig. 1C).

3.4. Day 2: subacute studies: effects of cocaine or clozapine/cocaine combination on DA release in NAcc

Cocaine (open circles): During the subacute studies, when no further cocaine was administered, DA release in NAcc significantly decreased from baseline (habituation) values (from a significant increase) (one-way ANOVA: P<.0001; F= 106.3; #=3,30). Post hoc analysis showed that significant differences occurred between baseline (Day 1) and (Day 2) values (same animal control) (Tukey's

Multiple Comparison Test: P<.001, <7 =18.99). Compared to drug effect on Day 1, DA release was decreased dramatically by about 80% during the hour period of study.



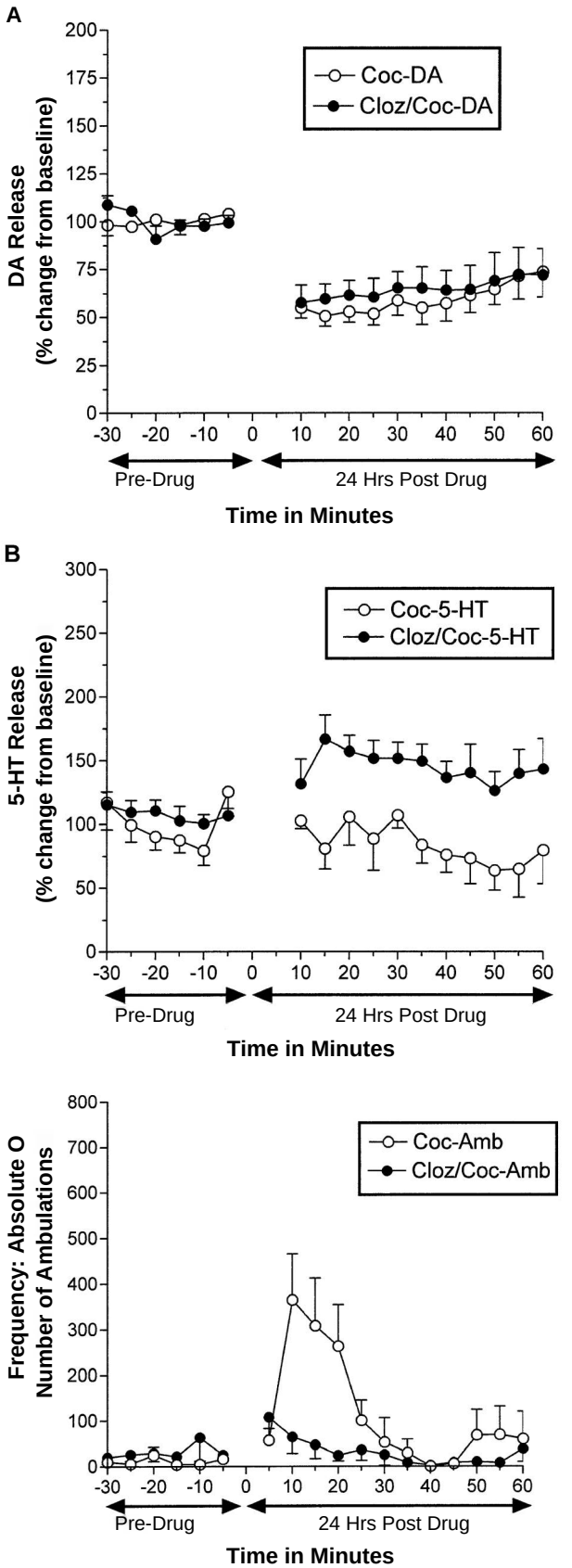
Clozapine/cocaine (closed circles): During the subacute studies, when no further drug(s) were administered, DA release in NAcc significantly decreased from baseline (habituation) values (one-way ANOVA: P<.0001; F= 106.3; #=3,30). Post hoc analysis showed that a significant difference occurred between baseline (Dayl) and (Day 2) values (same animal control). (Tukey's Multiple Comparison Test: P<.001, <7=16.48). A significant difference between cocaine and clozapine/cocaine (Day 2) groups did not occur (Tukey's Multiple Comparison Test: P>.05, q = 2.985) (Fig. 2A).

3.5. Day 2: subacute studies: effects of cocaine or clozapine/cocaine combination on 5-HT release in NAcc

Cocaine (open circles): During the subacute studies, when no further cocaine was administered, 5-HT release in NAcc was decreased below baseline (Day 1) values compared to (Day 2) values, at specific time points during the time course of the 1-h study, i.e., at the 15-min mark and at each time point in second part of the hour (P< .05); (one-way ANOVA: P<.0001; F= 38.99; #=3,30), although the post hoc analysis did not show statistical significance for the hour (Tukey's Multiple Comparison Test: P>.05, <7 = 3.225). Compared to drug effect on Day 1, 5-HT release was decreased dramatically by approximately 150% in the second half hour of the study.

Clozapine/cocaine (closed circles): During the subacute studies, when no further drug(s) were administered, 5-HT release in NAcc was significantly increased above (Day 1)

Fig. 1. (A) Day 1: Acute studies: Effects of cocaine (Coc) and clozapine/cocaine (Cloz/Coc) combination on DA release in NAcc of freely moving and behaving, Sprague-Dawley Rattus norvegicus. Line graphs show acute responses for DA. Axes: x axis, predrug denotes time for baseline values for DA, postdrug denotes time after drug injection(s); y axis represents % change in DA produced by drug injection(s). Cocaine (n = 5) increased DA (P<.001). Results from administration of clozapine/cocaine combined (n = 6), show that clozapine blocked cocaine-induced DA during the 2-h time course study (P<.001). (B) Day 1: Acute studies: Effects of cocaine (Coc) and clozapine/cocaine (Cloz/Coc) combination on 5-HT release in NAcc of freely moving and behaving, Sprague-Dawley R. norvegicus. Line graphs show acute responses for 5-HT. Axes: x axis, predrug denotes time for baseline values for 5-HT, postdrug denotes time after drug injection(s); y axis represents % change in 5-HT produced by drug injection(s). Cocaine (w = 5) increased 5-HT {P <.001). Results from administration of clozapine/cocaine combined (« = 6), show that clozapine blocked cocaine-induced 5-HT release during the 2-h time course study (P <.001). (C) Day 1: Acute studies: Effects of cocaine (Coc) and clozapine/cocaine (Cloz/Coc) combination on Locomotion (Ambulations) in freely moving and behaving, Sprague-Dawley R. norvegicus. Line graphs show acute responses for locomotion. Axes: x axis, predrug denotes time for baseline values for locomotion, postdrug denotes time after drug injection(s); y axis represents change in frequency for locomotion produced by drug injection(s). Cocaine (n = 5) increased locomotion over baseline (P<.001). Results from clozapine/cocaine combined (n = 6), show that clozapine blocked cocaine-induced locomotion during the 2-h time course study (P<.001).



baseline values (one-way ANOVA: P<.0001; F= 38.99; <^f=3,30), post hoc analysis showed significant differences between baseline (Day 1) and (Day 2) values (same animal control) (Tukey's Multiple Comparison Test: P<.001, </ = 7.704). Significant differences occurred between cocaine (Day 2) and clozapine/cocaine groups (Day 2) (Tukey's Multiple Comparison Test: P<.001, q= 14.90) (Fig. 2B).

3.6. Day 2: subacute studies: effects of cocaine or clozapine/cocaine combination on Locomotion (ambulations)

Cocaine (open circles): During the subacute studies, when no further cocaine was administered, locomotor activity on (Day 2) was significantly increased over baseline (Day 1) values (one-way ANOVA: P<.0186; F=3.843; 6//'= 3,32). Post hoc analysis showed that significant differences occurred between baseline (Day 1) and (Day 2) values

Fig. 2. (A) Day 2: Subacute studies: Effects of cocaine (Coc) and clozapine/cocaine (Cloz/Coc) combination on DA release in NAcc of freely moving and behaving, Sprague-Dawley R. norvegicus. Line graphs show subacute responses for DA. Axes: x axis, predrug denotes time for baseline values for DA from Day 1 studies (acute), postdrug denotes time for Day 2, DA values (subacute), when no further drug was administered to drug groups (same animal control); y axis, % change in DA compared with baseline. On Day 2, in the cocaine group (h = 5), DA decreased from baseline (P<.001), likely withdrawal related. Similarly, in the clozapine/cocaine group (n = 6), DA decreased from baseline (P<.001). There was no significant difference in DA effects between subacute cocaine and clozapine/cocaine groups (P>.05). Thus, the data suggest that DA-related cocaine withdrawal responses, subacutely, is not reversed by clozapine. (B) Day 2: Subacute studies: Effects of cocaine (Coc) and clozapine/cocaine (Cloz/Coc) combination on 5-HT release in NAcc of freely moving and behaving, Sprague-Dawley R. norvegicus. Line graphs show subacute responses for 5-HT. Axes: x axis, predrug denotes time for baseline values for 5-HT from Day 1 studies (acute), postdrug denotes time for Day 2, 5-HT values (subacute), when no further drug was administered to drug groups (same animal control); y axis, % change in 5-HT from baseline. On Day 2, in the cocaine group (n = 5), 5-HT decreased from baseline at the 15-min mark and during the second part of the 1-h time course (P< .05), likely reflecting 5-HT-related cocaine withdrawal effects. However, in the clozapine/cocaine group (n = 6), 5-HT increased above baseline (P<.001). There was a significant difference between subacute cocaine versus clozapine/cocaine groups (P<.001). The data suggest that clozapine, which has a longer pharmacokinetic half-life than does cocaine, may have reversed the 5-HT-related withdrawal effects of cocaine. (C) Day 2: Subacute studies: Effects of cocaine (Coc) and clozapine/cocaine (Cloz/Coc) combination on locomotion (ambulation) in freely moving and behaving, Sprague-Dawley R. norvegicus. Line graphs show subacute responses for locomotion. Axes: x axis, predrug denotes time for baseline values for locomotion from Day 1 studies (acute), postdrug denotes time for Day 2, locomotor values (subacute), when no further drug was administered to drug groups (same animal control); y axis represents change in frequency of locomotor counts compared with baseline. On Day 2, in the cocaine group (n = 5), locomotion was increased over baseline values (P<.05). In the clozapine/cocaine group (« = 6), locomotor counts showed no change from (Day 1) baseline (P>.05). There was a significant difference between subacute values in the cocaine group versus the clozapine/cocaine group (P<.05). Due to clozapine's longer lived pharmacokinetic properties, clozapine-induced sedation may be the mechanism for continued, diminished locomotion during subacute (Day 2) studies.

(same animal control) (Tukey's Multiple Comparison Test: P<.05, 7 = 3.925). Nonetheless, locomotor activity decreased by 250 counts when compared with (Day 1) effects of cocaine-induced psychomotor stimulation.

Clozapine/cocaine (closed circles): During the subacute studies, when no further cocaine or clozapine were administered, locomotor activity remained significantly decreased (one-way ANOVA: P<.0186; F= 3.843; #=3,32). Post hoc analysis showed that no significant differences occurred between baseline (Day 1) and (Day 2) values (same animal control) (Tukey's Multiple Comparison Test: P>.05, 7 = 0.03647). Moreover, (Day 2) cocaine and clozapine/cocaine groups did significantly differ (Tukey's Multiple Comparison Test: P<.05, 7 = 3.846) (Fig. 2C).

In all groups, additional saline controls had no effect.

4. Discussion

4.1. Cocaine, monoamines and psychomotor stimulant behavior: acute studies

We have extended our work from two recent articles on the effects of cocaine on DA and 5-HT release in NAcc of freely moving and behaving laboratory rats WHILE monitoring cocaine-induced psychomotor stimulant behavior simultaneously. Comparing the present data to the first of these recent papers (Broderick et al., 2003), we have simply added animals to our cocaine group. Comparing the present data to the second of these recent papers (Broderick and Piercey, 1998b), an entirely different group of animals was utilized. The results from all three studies from our laboratory were equivalent, i.e., increased DA, 5-HT release in NAcc occurred with increased psychomotor stimulant behavior (Broderick, 2001; Broderick and Piercey, 1998b; Broderick et al., 2003).

4.2. Cocaine, monoamines and psychomotor stimulant behavior: subacute studies

Cocaine studies in the subacute group were also extended by increasing the number of animals above what was used in our previous studies (Broderick et al., 2003); again, the results were equivalent. When no further drug was administered, there was a significant decrease in DA release in NAcc and significant decreases in 5-HT release during specific points in the time course data. The data are in agreement with several reports (Broderick et al., 1997; Parsons et al., 1995,1996). In addition, the data agree with a previous report showing long-lasting effects after a single moderate dose of cocaine (Zahniser et al., 1988). Behavioral activity maintained an increase at the same time that accumbens DA and 5-HT release were decreased, thereby suggesting dissociative function between behavior classically known to be mediated via DA_2 postsynaptically and monoamine release and reuptake inhibitory mechanisms, presynaptically.

The subacute data suggest that these monoamine deficiencies may be associated with symptoms of withdrawal and the data agree with clinical reports of DA-ergic systems and craving (Dackis and Gold, 1985; Gawin and Kleber, 1986a,b; Lieberman et al., 1990; Margolin et al., 1995). Neuroadaptation may be occurring as reported in animal studies (Broderick, 2001; Koob and Nestler, 1997) because neither the short pharmacokinetic half-life of cocaine nor that of its metabolites, provides a rational explanation (Misra et al., 1974a,b; Nayak et al., 1976; Mets et al., 1999; Sun and Lau, 2001). Of course, another plausible explanation though, is one provided by others, that transient compensatory changes take place a day after cocaine cessation (Zahniser et al., 1988).

4.3. Cocaine, monoamine interactions, possible mechanisms

Classical cocaine mechanisms point to a postsynaptic DA_2 release with additional DA release derived presynaptically from DA somatodendrites, VTA. Current thinking on the mechanism of action of cocaine points to a DA/5-HT interaction in DA mesolimbic circuits. A postsynaptic 5-HT-ergic up-modulation of DA in NAcc has been implicated; the $5\text{-}HT_{2A/2C}$ receptor has been shown to up-modulate DA release in NAcc after intermittent cocaine (Yan et al., 2000) and endogenously, as well (Yan, 2000). Local application (infusion) of [(±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride] (DOI), a $5\text{-}HT_{2A}$/2c agonist, was infused into NAcc to increase DA which was subsequently blocked by ketanserin, a $5\text{-}HT_{2A/2C}$ antagonist. Furthermore, infusion of DOI in NAcc was antagonized by the selective $5\text{-}HT_{2C}/_{2}b$ receptor antagonist, SB 206553, but not by the selective $5\text{-}HT_{2}a$ antagonist, SR 46349B (Lucas and Spampinato, 2000), suggesting that DA increases in NAcc after infusion of DOI may be due to a mediation by the $5\text{-}HT_{2}c$ receptor.

However, prematurely pointing specifically to the $5\text{-}HT_{2}c$ receptor for cocaine's mechanism of action may present a limitation because local application (infusion into NAcc) of $5\text{-}HT_{2}c$ receptor agonists did not alter basal locomotor activity nor mimic the stimulus effects of cocaine (Filip and Cunningham, 2002; McMahon et al., 2001). Also, a recent report shows that a $5\text{-}HT_{2A}$ receptor mediation is prominent in blocking cocaine-induced locomotor activity (McMahon and Cunningham, 2001). Therefore, the mechanism of action of cocaine is probably dually directed, i.e., via classical DA_2 and currently explored $5\text{-}HT_{2A/2C}/DA_2$ receptor circuits.

Although studies by Di Matteo et al. (1999), do not address cocaine, the data do importantly show the direct autoreceptor properties of selective $5\text{-}HT_{2C}$ receptor compounds vis-a-vis the effects of these compounds when locally applied by infusion. Thus, the selective $5\text{-}HT_{2C}$ antagonist, SB 242084, increased and the selective $5\text{-}HT_{2}c$ agonist, RO 60-0175, decreased DA release in NAcc (Di Matteo et al., 1999).

4.4. Clozapine/cocaine: acute studies

Clozapine significantly reduced cocaine-induced increases in DA release in NAcc by an average of 40% in the first hour of study and 50% in the second hour of study. Simultaneously, clozapine significantly reduced cocaine-induced increases in 5-HT release in NAcc by an average of 138% in the first hour of study and average of 113% in the second hour of study. Also, at the same time, locomotor activity (ambulation counts) produced by cocaine, were reduced by an average of 500 counts in the first half hour and by an average of 150 counts in the next hour of study. Since these are the first studies of this kind ever performed, direct comparisons cannot be made. Nonetheless, the present studies are in general agreement with preclinical studies in which clozapine was shown to antagonize cocaine-induced place preference in animals (Kosten and Nestler, 1994) and to block reinforcement by intravenous cocaine in animals (Loh et al., 1992).

4.5. Clozapine/cocaine: possible mechanisms of action: acute studies

The data suggest that increased 5-HT by cocaine leads to an increase in DA release perhaps via either a separate or combined 5-HT_{2A}/2c receptor mediation, which is subsequently blocked by clozapine. This suggestion is made because of previous reports of the importance of 5-HT_2a/2c receptors either alone or combined in cocaine mechanisms (Filip and Cunningham, 2002; McMahon and Cunningham, 2001; Yan, 2000; Yan et al., 2000) and because clozapine is, in fact, the prototypical atypical 5-HT_2a/DA_2 receptor antagonist, although not exclusively bound to these two types of receptors. Clozapine has high antagonist affinity for the 5-HT_{2C} receptor and indeed, phosphoinositol inverse agonist activity at the 5-HT_{2C} receptor (Herrick-Davis et al., 1999, 2000; Kuoppamaki et al., 1995) has been shown specifically in NAcc, in the action of clozapine (Di Matteo et al., 2002). Classical DA_2 postsynaptic antagonism of cocaine-induced psychomotor stimulant behavior by clozapine probably accounts, at least in part, for the complete blockade of motor activity observed. Interestingly, in the apomorphine-induced hypomotility test, clozapine did not antagonize this presynaptic response, unlike the $DA_{2/3}$ antipsychotic agent, sulpiride (Robertson and MacDonald, 1986).

A mediation by 5-HT_{2A}/2c receptors in the mechanism of action for clozapine's blockade of cocaine, is also further suggested. In acute studies performed in this laboratory, we substituted (3 mg/kg sc) ketanserin, a 5-HT_{2A}/2c antagonist, for clozapine and the results were remarkably similar to clozapine in blocking cocaine-induced monoamine release and psychomotor stimulant behavior, although ketanserin blockade of cocaine was actually weaker than that of clozapine in all three parameters studied (Broderick et al., 2001).

Ketanserin and clozapine do not have similar receptor profiles, in general, but ketanserin is similar to clozapine in that both are direct receptor antagonists which bind with high affinity to 5-HT_{2A}, 5-HT_{2C}, adrenergic (cti) and histamine (HJ receptors; ketanserin does not have bind to DA receptors (Duffy et al., 2000; Lutje Hulsik, 2002). The strong ct | influence is a concern, but there is good evidence that only 5-HT_{2A}/2c receptors are involved and ct) adrenoreceptors are not involved in the mechanism of cocaine's stimulant activity (Filip et al., 2001). The high antagonist affinity for Hi receptors on the part of both ketanserin and clozapine, is probably not a concern. Studies on H! and even H_2 promoter polymorphisms conclude that participation of these receptors have an unlikely influence in the clinical response to clozapine treatment (Mancama et al., 2002) . Thus, 5-HT_{2A}/2c properties for clozapine are highly likely mechanisms for clozapine's antagonism of cocaine effects.

It is interesting that studies on risperidone's effects on cocaine-induced stimulant monoamine neurochemistry and locomotor behavior, showed that risperidone completely blocked 5-HT release in NAcc and simultaneous locomotor activity but did not completely block DA release on NAcc. Indeed, risperidone significantly increased DA release in the second hour of the study, given the caveat that high dose risperidone (2 mg/kg sc) was tested (Broderick et al., 2003).

4.6. Clozapine/cocaine: subacute studies

Results showed that DA release in NAcc, at this time, decreased by an average of 60% during the hour of study, while 5-HT release increased by 50% above baseline for the hour of study and locomotor activity remained reduced by an average of 250 ambulatory counts in the first 20 min.

It is important to note that these long lasting effects of clozapine are supported by pharmacokinetics. The half-life of clozapine is 8 h at lower doses and 4-66 h at higher doses; the hydroxlated and N-oxide derivatives are reported to be inactive (Clinical Pharmacology of Clozapine, 2002). Interestingly, preliminary data from our laboratory have shown that significantly increased 5-HT release after combined clozapine/cocaine administration, does not begin to diminish until the fifth day of recovery after drug administration.

4.7. Clozapine/cocaine: possible mechanisms of action: subacute studies

The occurrence of increased 5-HT release in subacute studies may be explained by clozapine's mechanism than by cocaine's mechanism. If we look at clozapine, 5-HT presynaptic autoreceptors, as studied in synaptosomes, may lend an explanatory note (Drescher and Hetey, 1988). Also, presumably by autoreceptors, clozapine increased DA efflux in NAcc (Kuroki et al., 1999; Volonte et al., 1997), DA and

5-HT release in NAcc in the behaving animal (Broderick et al., unpublished data; Ichikawa et al., 1998) and DA and 5-HT release in NAcc in the anesthetized animal (Broderick and Piercey, 1998a). Therefore, increased 5-HT release, as shown in these subacute studies, may be mediated by inhibitory presynaptic autoreceptors. The explanation for decreased DA release is not apparent, unless this DA decrease is simply compensatory (Beart and McDonald, 1982; Herve et al., 1979).

Moreover, increased 5-HT release on the second day of study might possibly have been derived from increased cocaine serum levels due to the longer lasting effects of clozapine since a clinical study reported enhanced cocaine serum levels after administration of both clozapine and cocaine to cocaine addicts (Farren et al., 2000). It is noteworthy that increased 5-HT release on the second day of study, did not occur in a risperidone/cocaine group (Broderick et al., 2003).

Thus far, research in our laboratory suggests that clozapine's action on cocaine is mediated at least partly, via 5-HT_{2A}/2C receptors because the results presented here, from clozapine/cocaine combination studies, resemble those obtained during subacute studies when substituting the 5-HT_{2}a/2c antagonist, ketanserin for clozapine. In subacute studies with ketanserin/cocaine, when no further ketanserin or cocaine was administered, DA release in NAcc decreased and 5-HT increased to a statistically significant level, just as did results, subacutely, in the clozapine/cocaine group.

There were some differences in monoamine reactions during the subacute studies between the ketanserin/cocaine group and the clozapine/cocaine group, e.g., the DA response was weaker and the 5-HT response was somewhat stronger. Notably, locomotor activity was significantly higher on the second day for the ketanserin/cocaine group vis-a-vis the clozapine/cocaine group. The clozapine/cocaine group continued to exhibit sedation, possibly through residual potent, muscarinic anticholinergic receptor mediation (Richelson and Souder, 2000; Broderick et al., 2001).

On the other hand, the atypical antipsychotic medication, the 5-HT_{2A}/DA_{2} receptor antagonist, risperidone, did not exhibit subacute responses to cocaine as did clozapine. In fact, DA and 5-HT release returned to baseline and locomotor activity increased insignificantly above baseline (Broderick et al., 2003). It is noteworthy however, that studies with the high dose of risperidone (2 mg/kg sc) were performed (Broderick et al., 2003). High dose risperidone exhibits more typical than atypical antipsychotic properties (Williams, 2001).

Not to be neglected, though, is a possible mediation by the 5-HTi_{A} receptor because clozapine exhibits moderate receptor binding for the 5-HT_{iA} receptor (Schotte et al., 1993, 1996; Sumiyoshi et al., 1995). Importantly, the 5-HT_{1a} receptor has been shown clinically to mediate schizophrenic psychosis (Chou et al., 2003) and preclinically, to mediate the action of DA in NAcc (Ichikawa and Meltzer, 2000). Finally, cti antagonism has been shown to mediate inhibition of dorsal raphe (DR) firing by clozapine through 5-HT_{1A} receptors (Sprouse et al., 1999).

5. Conclusions

Acute studies showed that clozapine blocked accumbens DA, 5-HT and locomotor effects of cocaine. These studies are the first of their kind. The subacute studies are also unique; the subacute studies allowed us to study withdrawal effects of cocaine in addition to the unexpected long-lasting effects of clozapine/cocaine treatment on accumbens DA and 5-HT release in the freely moving and behaving animal. Enhanced 5-HT release may help alleviate clinical depression associated with cocaine withdrawal (Price et al., 2001), although decreased DA release could be a disadvantage, possibly leading to craving (Dackis and Gold, 1985). Nonetheless, critical treatment strategies for cocaine addiction and psychosis could be derived from these results.

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