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Neurochemical and behavioral evidence supporting (+)-AJ 76 as a potential pharmacotherapy for cocaine abuse

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Summary. In vivo microvoltammetry was used to detect synaptic concentrations of dopamine (DA) and serotonin (5-HT) from nucleus accumbens (NAcc) in awake, freely moving, male, Sprague Dawley laboratory rats, *while* their locomotor behavior was monitored, simultaneously, in an open-field paradigm; the purpose was to evaluate the pharmacology of the D_3-preferring, dopamine (DA) autoreceptor antagonist, (+)-AJ 76 [cis-(+)-1S, 2R-5-methoxy-1-methyl-2-(n-propylamino)-tetralin HCL] and its potential use as a pharmacotherapy for cocaine abuse. Results showed that **(1).** *(+)-AJ 76* significantly increased synaptic concentration of DA above baseline ($p < 0.001$); a small but significant decrease in synaptic concentration of 5-HT was seen ($p < 0.001$), although a significant increase occurred during the time course, at the 20 minute mark ($p < 0.05$). Analysis of the two hour data also showed that both locomotor and central locomotor activity were not affected; however, temporally related increases in both behaviors were significant at 10, 20 and 30 minutes ($p < 0.05$). In a second and separate study, **(2).** *cocaine* increased synaptic concentrations of DA ($p < 0.001$) and 5-HT ($p < 0.001$), and locomotor activity ($p < 0.001$) above baseline, but central locomotion was not affected, except for specific temporal enhancements at 10, 20, 30, 50, 60 and 90 min. ($p < 0.05$). In a third and separate study, **(3).** an *(+)-AJ 76/cocaine* study, (+)-AJ 76 was administered five minutes before cocaine. The results showed that synaptic DA concentration was significantly increased over baseline values ($p < 0.001$) but that synaptic DA was lower than cocaine-induced synaptic DA ($p < 0.001$). No significant difference in synaptic 5-HT occurred after (+)-AJ 76/cocaine treatment, but temporally related increases over baseline occurred from 10 to 40 min. ($p < 0.05$). Synaptic 5-HT concentrations after (+)-AJ 76/cocaine were not significantly different from those induced by cocaine per se. (+)-AJ 76/cocaine treatment significantly increased locomotor activity ($p < 0.001$); central locomotor

behavior was not affected, however, time course data showed significant increases at 10, 20, 40, 50 and 80 min. ($p < 0.05$). The major finding from the present studies, is that +(−) AJ 76/cocaine treatment produced synaptic concentrations of DA from NAcc which were lower than those due to cocaine per se, while no differential effect on synaptic 5-HT concentration, locomotor or central locomotor behavior occurred. Therefore, these data support the hypothesis that (+)-AJ 76 may be useful for the treatment of cocaine addiction or abuse.

Keywords: dopamine (DA), serotonin (5-HT), nucleus accumbens (NAcc), in vivo microvoltammetry, (+)-AJ 76, dopamine autoreceptor antagonist, cocaine, open-field behavior, locomotor (ambulatory) activity, central ambulatory activity, agoraphobia (thigmotaxis).

Introduction

Cocaine increases dopamine (DA) neurotransmission by inhibiting the DA reuptake transporter at the presynapse in DA nigrostriatal and mesolimbic neuronal pathways; increased DA neurotransmission occurs via DA reuptake inhibition, enhanced release of DA or a combination of DA reuptake inhibitory and enhanced release mechanisms (de Wit and Wise, 1977; Church et al., 1987; Ritz et al., 1987; Bradberry and Roth, 1989; Hurd and Ungerstedt, 1989; Kalivas and Duffy, 1990; Broderick, 1991a,b, 1992a,b; Broderick et al., 1993). Increased DA neurotransmission in mesolimbic and/or mesocortical DA reward pathways (Wise and Rompre, 1989) is thought to emanate from the ventral tegmental area (VTA) (Roberts and Koob, 1982; Goeders and Smith, 1983; Evenden and Ryan, 1988; Einhorn et al., 1988; Broderick and Phelix, 1991; Kalivas, 1993).

More recently, serotonin (5-HT) has also been implicated in cocaine's electrophysiological, neurochemical, transporter and reinforcing effects (Cunningham and Lakoski, 1988, 1990; Carroll et al., 1990; Broderick, 1991a, 1992a,b; Broderick et al., 1992, 1993, 1997; Bradberry et al., 1993; Carroll et al., 1993; Parsons et al., 1995). The advent of 5-HT to a study of cocaine effects is logical because both immunohistochemical studies (Steinbusch,1981) and immunocytochemical studies (Broderick and Phelix, 1997) show that DA cell bodies, ventral tegmentum, contain a dense network of 5-HT axonal varicosities. Neuroanatomic localization of tyrosine-containing (TH) and 5-HT-containing axons in NAcc, show a prominent overlap of DA and 5-HT axons in both core and shell (Phelix and Broderick, 1995). Ultrastructural evidence from light and electron microscopy have shown that 5-HT neurons innervate DA neurons synaptically (Herve et al., 1987). A cellular basis is evidenced for the 5-HT excitation of A_{10} DA neurons by the existence of asymmetric junctions formed by 5-HT labeled terminals in A_{10} projections to NAcc (Van Bockstaele and Pickel, 1993; Van Bockstaele et al. 1994; Broderick and Phelix, 1991). Moreover, 5-HT may be involved in the mechanism of cocaine abuse in a way directly related to movement behavior. Synaptic concentrations of 5-HT from the dopaminergic A_{10} pathway in behaving rats increase synchronously, within minutes, during each increase in natural loco-

motion but do not increase synchronously during cocaine-induced locomotion, thereby possibly causing motor dysfunction (Broderick and Phelix, 1997).

Cocaine is being abused in epidemic proportions. There is an obvious need for programs that effectively treat cocaine addiction (Jarvik, 1990). However, to date, the consensus remains similar to that spoken in the last decade, i.e., that effective uniform pharmacotherapy for cocaine is difficult to achieve (Kleber and Gawin, 1984). Should cocaine's addictive potential be mediated via DA release in reward pathways, DA antagonists might be expected to be useful treatments. However, neuroleptic agents appear to imitate the crash experienced after a cocaine binge, and exacerbate the anhedonia and craving experienced during cocaine withdrawal (Blum et al., 1989). For this reason, therapy which merely blocks the pleasurable effects of cocaine without attention to craving may meet with patient non-compliance (Kleber and Gawin, 1984). Also, for this reason, pharmacotherapeutic strategies for cocaine should include other neurotransmitters and a suitable candidate is 5-HT.

(+)-AJ 76 [cis-(+)-1S, 2R-5-methoxy-1-methyl-2-(n-propylamino)-tetralin HCL] is a DA-autoreceptor antagonist with a slightly higher affinity for the DA D_3 rather than the DA D_2 receptor (Sokoloff et al., 1990). Unlike neuroleptics though, which usually have sedative properties, (+)-AJ 76 has behavioral stimulant properties, given the caveat that they are weak, perhaps due to preferential DA autoreceptor antagonism (Svensson et al., 1986; Piercey and Lum, 1990). (+)-AJ 76 increased extracellular concentrations of DA in A_9, nigrostriatal DA terminals, an effect that was calcium dependent (Waters et al., 1993). The behavioral stimulant properties of (+)-AJ 76 may also be weak possibly due to (+)-AJ 76's antagonism of DA postsynaptic receptors. The suggestion is that the DA presynaptic receptor profile, as well as the DA postsynaptic profile of (+)-AJ 76, may be particularly useful for treating cocaine abuse (Piercey et al., 1992, Callahan et al., 1992; Richardson et al., 1993; Smith et al., 1995).

Only one previous study regarding the effects of (+)-AJ 76, has focussed on its reaction with 5-HT. This study showed that (+)-AJ 76 weakly depressed 5-HT (5-HT_{1A} subtype) neuronal cell firing. In addition, (+)-AJ 76 antagonized the hypothermic effects of the 5-HT_{1A} agonist, 8-OH-DPAT in mice (Piercey et al., 1989).

Previous studies, which have reported an interaction between (+)-AJ 76 and cocaine, show that (1) (+)-AJ 76 antagonized cocaine's effects on ventral tegmental neuron firing (Piercey et al., 1992), (2) (+)-AJ 76 antagonized cocaine's stimulation of brain energy metabolism without altering energy metabolism per se (Casey et al., 1996) and (3) (+)-AJ76 either partially blocks cocaine self-administration (Vanover et al., 1993) or (+)-AJ 76 fully blocks cocaine self-administration (Richardson et al., 1993).

Therefore, we studied, in vivo, the effects of (+)-AJ 76 on synaptic DA and 5-HT concentrations from A_{10} DA nerve terminals, NAcc, in behaving animals, while simultaneously monitoring its effects on locomotor (ambulatory) activity and central ambulatory (anxiolytic) activity. In addition, in separate experiments and following the same protocol, we studied cocaine

effects. Finally, using this same protocol and in separate experiments, we studied the effects of (+)-AJ 76/cocaine interactions, to decipher a possible antagonism of cocaine-induced neurochemical and behavioral properties by (+)-AJ 76.

Materials and methods

In vivo microvoltammetry

In the present studies, in vivo microvoltammetry with a semidifferential circuit was used; a clear separation of the biogenic amine neurotransmitters, DA and 5-HT was achieved. Dopamine and 5-HT were detected within seconds, concurrently and in separate waveforms. Oxidation peak potentials of $+0.14 \pm 0.015$ V and $+0.29 \pm 0.015$ V were characteristic for DA and 5-HT, respectively. Detailed methodology is published (Broderick, 1988, 1989, 1990, 1991a). The electrochemical signal for DA, was detected without interference at the same oxidation potential, from 3–4-dihydroxyphenylacetic acid (DOPAC) or ascorbic acid (AA). 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) or uric acid. 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. One voltammetric scan was completed in 60 seconds. Non-faradaic charging current was eliminated in the first 25 seconds. The neurotransmitters, DA and 5-HT, were detected in approximately 10–15 sec. and 10–12 sec., respectively, in a sequential manner during each voltammogram of the temporal series. The coulombic efficiency for the detection of 5-HT was two to three fold greater than that for DA (Broderick, 1987). Indicator microelectrodes were precalibrated and postcalibrated in vitro in a freshly prepared deoxygenated physiological saline-phosphate buffer solution, (0.01 M, pH = 7.4 containing DA (99% purity, Sigma, St. Louis, MO) and 5-HT (99% purity, Aldrich, Milwaukee, WI), as well as metabolites of the monoamines, ascorbic acid and uric acid (cf. Broderick, 1989). Detection limits for basal synaptic concentrations of DA and 5-HT in NAcc were 12 nM and 2 nM respectively. Histological placements of indicator microelectrodes in NAcc were confirmed by the potassium ferrocyanide blue dot method (specifications: current 50 μA, time in seconds, 40). Virtually no damage to brain tissue occurred. Recording characteristics of microelectrodes were stable.

Surgical procedures

Animals were purchased from Charles River Laboratories, Kingston, NY and were housed in our animal care facilities for two weeks before surgery was performed. The Animal Care Facility operates under the auspices of the CUNY, City College IACUC in compliance with NIH guidelines. The weight range for the animals, at the time of the studies, was 362–415 g.

Animals were group housed before surgery, individually housed after surgery and fed Purina Rat Chow and water ad libitum. A twelve hour dark-light cycle was maintained both in the housing of the animals and throughout the experimental studies. Each animal was anesthetized with pentobarbital Na, (50 mg/kg IP (dilute solution)) and was stereotaxically implanted (Kopf Stereotaxic, Tujunga, CA) with a stearate indicator electrode in ventrolateral (vl) NAcc (AP = +2.6, ML = +2.5, DV = −7.3) (Pellegrino et al., 1979). A Ag/AgCl reference electrode was placed in contact with dura, 7 mm anteriorally and contralaterally to the indicator microelectrode. A stainless steel auxiliary electrode, was placed in contact with dura.

Animals' body temperature was continuously monitored with a rectal probe and thermometer (Fisher Sci., Fadem, NJ). Body temperature was maintained at 37.5°C ± 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 two hours 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 three to four hours. The indicator, reference, and auxiliary microelectrodes were held in place with dental acrylic (Kadon Cavity Liner, Caulk, Becker Parkin Dental Supply Co. Inc., NY). Animals recovered in a bedded Plexiglas cage (dimensions: 12″ × 12″ × 18″) 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 one to two days after surgery.

In vivo microvoltammetric studies on conscious Sprague-Dawley laboratory rats were begun nine to fifteen days after the aseptic surgical operations were performed. On each experimental day, animals were 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 (1) (+)-AJ 76 (5 mg/kg), (2) cocaine (10 mg/kg) or (3) the combination of (+)-AJ 76 and cocaine (5 mg/kg and 10 mg/kg respectively) was administered intraperitoneally (IP). Therefore, there were three treatment groups with five animals in each group; each animal was used as its own control. During the first hour, animals underwent exploratory behavior in a novel chamber and during the second hour, habituated behavior occurred. Baseline (control) values were derived from each data point of the last forty minutes of the second hour data, i.e., during the time that the animals were habituated to the behavioral chamber. There were no movement or electrical artifacts.

(+)-AJ 76 was obtained from the Upjohn Company and dissolved in doubly deionized, organic free water (resistance = 5–10 MW) and sonicated in a Microtip Ultrasonicator (Sonifier® Cell Disrupter, Plainview, NY). Cocaine was obtained from Sigma Chemical Company, St. Louis, MO and dissolved in doubly deionized, organic free water (resistance = 5–10 MW).

Behavior

Locomotor and central locomotor activities were concurrently monitored with infrared photobeams which surrounded the faradaic chamber; a 16 by 16 array of infrared photobeams, held in place by an aluminum frame, was situated 3/4 inch above the Plexiglas floor of the chamber. Photobeams were sampled by an IBM computer to define the x-y position of the animal within a 1.5 inch resolution every 100 msec. When an x-y position was calculated, it was used to define an animal's position in one of sixteen equally sized sectors and one of nine unequally sized regions. These regions are used to define descriptive measures of entries spent in the center of the chamber, i.e., central locomotor activity, [thigmotaxic (agoraphobic) inhibition], is monitored in this way. Central locomotor activity may be equivalent to inhibition of fear or anxiolytic activity (Geyer et al., 1986). This system is a modified version of an Activity Pattern Monitor (APM) (San Diego Instruments, San Diego, CA). Behavioral data is presented in terms of Frequency of Events.

Statistics

Neurochemical and behavioral data, derived from the last forty minutes of the habituation period, provided the baseline (control/pre-drug) data. Statistically significant differences between pre-drug (baseline values) and post-injection (post-drug) values, for (1) DA (2) 5-HT, (3) locomotor and (4) central locomotor behavior were determined by subjecting the data to the following statistical analyses: (a) One Way Analysis of Variance

(ANOVA) (tested at $p = 0.001$ as criteria), with subsequent application of the post hoc test, Dunnet's Multiple Comparison Method for equal variances, or (b) Kruskal-Wallis One Way ANOVA on Ranks (tested at a $p = 0.001$ as criteria), with subsequent application of the post hoc test, Dunn's Multiple Comparison Method for unequal variances. Post hoc analyses were tested at a $p = 0.05$ as criteria. Each data point in the time course was subjected to 95% Confidence Limits (C.L.). Correlations between monoamines and behaviors were analyzed by Pearson Product Moment Coefficient of Correlation. Correlations were determined for the two hour period of study. Significance levels were $p < 0.05$ unless otherwise noted in text. (NS) = not significant.

Changes in synaptic concentrations of DA and 5-HT are presented as percent change (% of control) in order to minimize normal between-animal variations. Since the actual detection time for DA is 10–15 seconds and that for 5-HT is 10–12 seconds, the % change in synaptic concentration of DA or 5-HT, at each data point (pre vs. post drug), represents a second to second current change (pA) from baseline.

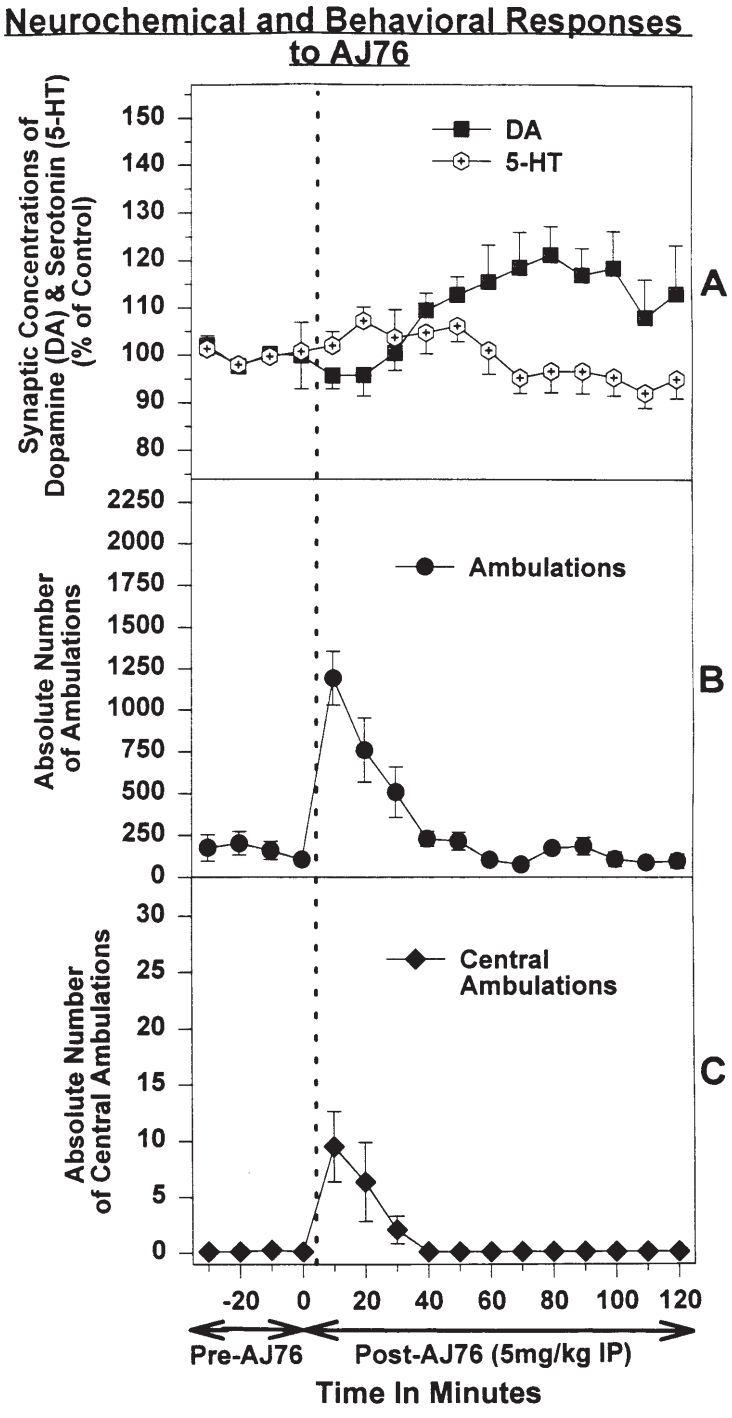
Results

(+)-AJ 76 (Fig. 1)

Figure 1A: (+)-AJ 76 significantly increased synaptic concentrations of *DA* over baseline values (One Way ANOVA; $p < 0.001$; $F = 28.905$; $df = 4,11$ (equal variance)). Post hoc analysis further showed that there were statistically significant differences from baseline in the second, third, and fourth half hour periods (Dunnett's Method: $p < 0.05$; $q' = 4.517$, $q' = 7.719$, $q' = 5.432$, second through fourth half hour respectively).

There was a small but significant decrease in synaptic concentration of 5-HT (One Way ANOVA; $p < 0.001$; $F = 14.127$; $df = 4,11$ (equal variance)). Further post hoc analysis showed that this decrease in 5-HT release occurred

Fig. 1. In vivo microvoltammetry was used to detect synaptic concentrations of dopamine (DA) and serotonin (5-HT) from nucleus accumbens (NAcc) in awake, freely moving, male, Sprague Dawley rats, while their locomotor and central locomotor behaviors were monitored, simultaneously, with infrared photobeams (same animal control) ($N = 5$). Animals were studied for 2 hours before drug injection; (first hour: exploratory behavior; second hour: habituated behavior) and for 2 hours after injection. The x axis denotes time in minutes. Time "−30 to 0 minutes" denotes the last forty minutes of the habituation period (baseline values). *(+)-AJ 76* was injected approximately 1 minute after time 0. Vertical bar separates *pre-(+)-AJ 76* data points from *post-(+)-AJ 76* data points. **A** The y axis represents change in oxidation current before and after injection of (+)-AJ 76 (5 mg/kg ip). (+)-AJ 76 significantly increased synaptic concentrations of *DA* above baseline ($p < 0.001$). Time course data showed that each point, in the latter hour and half of the study, was statistically significantly increased above baseline [$p < 0.05$: 95% C.L.]. Although (+)-AJ 76 produced a small but significant decrease in synaptic concentrations of *5-HT* when compared with baseline ($p < 0.001$) (2 hour data), a significant increase in 5-HT was seen 20 minutes after injection ($p < 0.05$) (95% C.L.). **B** The y axis represents change in *locomotor activity* before and after injection of (+)-AJ 76. No significant difference from baseline was noted ($p = 0.049$) (2 hour data), although temporally related increases above baseline, were significant at 10, 20 and 30 minutes after injection [$p < 0.05$: 95% C.L.]. **C** The y axis represents change in *central locomotor activity* before and after injection of (+)-AJ 76. No significant difference from baseline occurred ($p = 0.014$) (2 hour data), but temporally related increases above baseline, were significant at 10, 20 and 30 minutes after injection [$p < 0.05$: 95% C.L.]


Neurochemical and Behavioral Responses to AJ76
Synaptic Concentrations of Dopamine (DA) & Serotonin (5-HT) (% of Control)
150
140
130
120
110
100
90
80
DA
5-HT
A
Absolute Number of Ambulations
2250
2000
1750
1500
1250
1000
750
500
250
0
Ambulations
B
Absolute Number of Central Ambulations
30
25
20
15
10
5
0
Central Ambulations
C
-20
0
20
40
60
80
100
120
Pre-AJ76
Post-AJ76 (5mg/kg IP)
Time In Minutes

in the fourth half hour of the study (Dunnet's Method: $p < 0.05$, $q' = 3.978$). A small but significant increase was seen 20 minutes after injection ($p < 0.05$) (95% C.L.). Correlation value observed for DA versus 5-HT was ($r = -0.484$) (NS).

Figure 1B: (+)-AJ 76 did not affect *locomotor activity* in the two hour study (Kruskal-Wallis One Way Analysis of Variance on Ranks; $p = 0.049$; $H = 9.529$; $df = 4$ (unequal variance)), but did produce significant increases at 10, 20 and 30 minutes post-drug injection ($p < 0.05$) (95% C.L.). Correlation value for locomotion versus 5-HT was ($r = 0.512$) (NS); for DA was ($r = -0.582$).

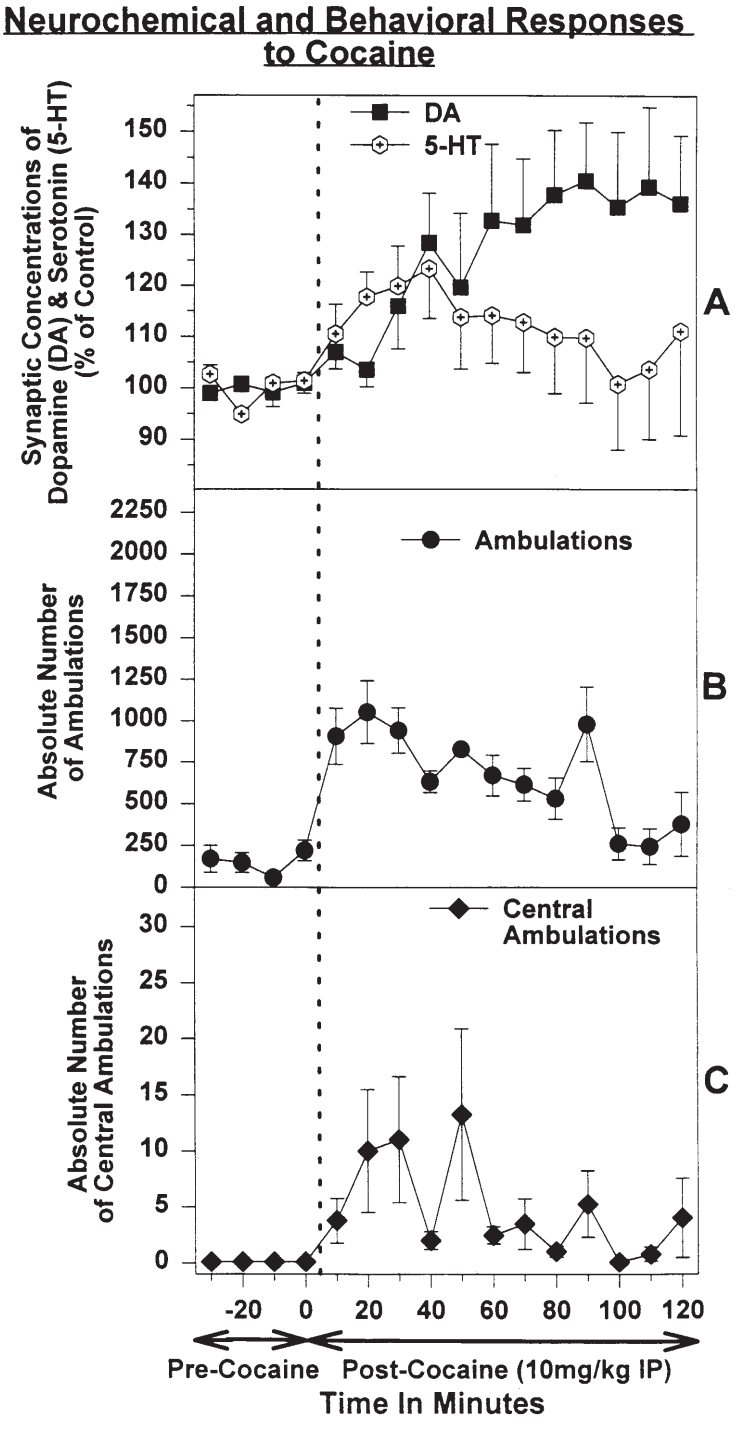
Figure 1C: (+)-AJ 76 did not affect *central locomotor activity* two hour period (Kruskal-Wallis One Way Analysis of Variance on Ranks; $p = 0.014$; $H = 12.435$; $df = 4$ (unequal variance)), although significant increases were seen at 10, 20 and 30 min. ($p < 0.05$) (95% C.L.). Correlation value for central locomotion versus 5-HT was ($r = -0.426$) (NS); for DA was ($r = -0.557$).

Cocaine (Fig. 2)

Figure 2A: Cocaine significantly increased synaptic concentrations of *DA* over baseline values (One Way ANOVA; $p < 0.001$; $F = 47.289$; $df = 4,11$ (equal variance)). Further post hoc analysis showed that significant increases in synaptic DA occurred in the second, third, and fourth half hours of the study (Dunnet's Method: $p < 0.05$, $q' = 7.851$, $q' = 10.712$, $q' = 10.769$ second through fourth half hour respectively).

Cocaine significantly increased the synaptic concentration of *5-HT* over baseline (One Way ANOVA; $p < 0.001$; $F = 17.165$; $df = 2,13$ (equal

Fig. 2. In vivo microvoltammetry was used to detect synaptic concentrations of DA and 5-HT from NAcc in awake, freely moving, male, Sprague Dawley rats, while their locomotor and central locomotor behaviors were monitored, simultaneously, with infrared photobeams (same animal control) (N = 5). Animals were studied for 2 hours before drug injection; (first hour: exploratory behavior; second hour: habituated behavior) and for 2 hours after drug injection. The x axis denotes time in minutes. Time "−30 to 0 minutes" denotes the last forty minutes of the habituation period (baseline values). *Cocaine* was injected approximately 1 minute after time 0. Vertical bar separates *pre-cocaine* data points from *post-cocaine* data points. **A** The y axis represents change in oxidation current before and after injection of cocaine (10 mg/kg ip). Cocaine significantly increased the synaptic concentrations of *DA* above baseline ($p < 0.001$). Time course data showed that every data point, with the exception of the 20 minute mark, was statistically increased over baseline [$p < 0.05$: 95% C.L.]. Cocaine significantly increased the synaptic concentration of *5-HT* above baseline values ($p < 0.001$). Time course data showed temporally related increases over baseline at 10, 20, 30, 40 and 60 minutes after injection [$p < 0.05$: 95% C.L.]. **B** Cocaine increased *locomotor activity* above baseline ($p < 0.001$). Time course data showed that each data point from the 10 minute mark through the 90 minutes was significantly increased [$p < 0.05$: 95% C.L.]. **C** Cocaine did not affect *central locomotor activity* ($p = 0.066$) (2 hour data), but temporally related increases were seen at 10, 20, 30, 50, 60 and 90 minutes after injection [$p < 0.05$: 95% C.L.]


Neurochemical and Behavioral Responses to Cocaine
Synaptic Concentrations of Dopamine (DA) & Serotonin (5-HT) (% of Control)
DA
5-HT
A
Absolute Number of Ambulations
Ambulations
B
Absolute Number of Central Ambulations
Central Ambulations
C
Pre-Cocaine
Post-Cocaine (10mg/kg IP)
Time In Minutes

variance)). Post hoc analysis showed that the significant increase in 5-HT synaptic concentration of 5-HT occurred in the first hour, post cocaine administration (Dunnett's Method: $p < 0.05$, $q' = 5.796$). Cocaine induced increases in synaptic DA and 5-HT exhibited a weak correlation ($r = -0.27$) (NS), but a positive and high correlation was seen during the first hour of study ($r = 0.615$).

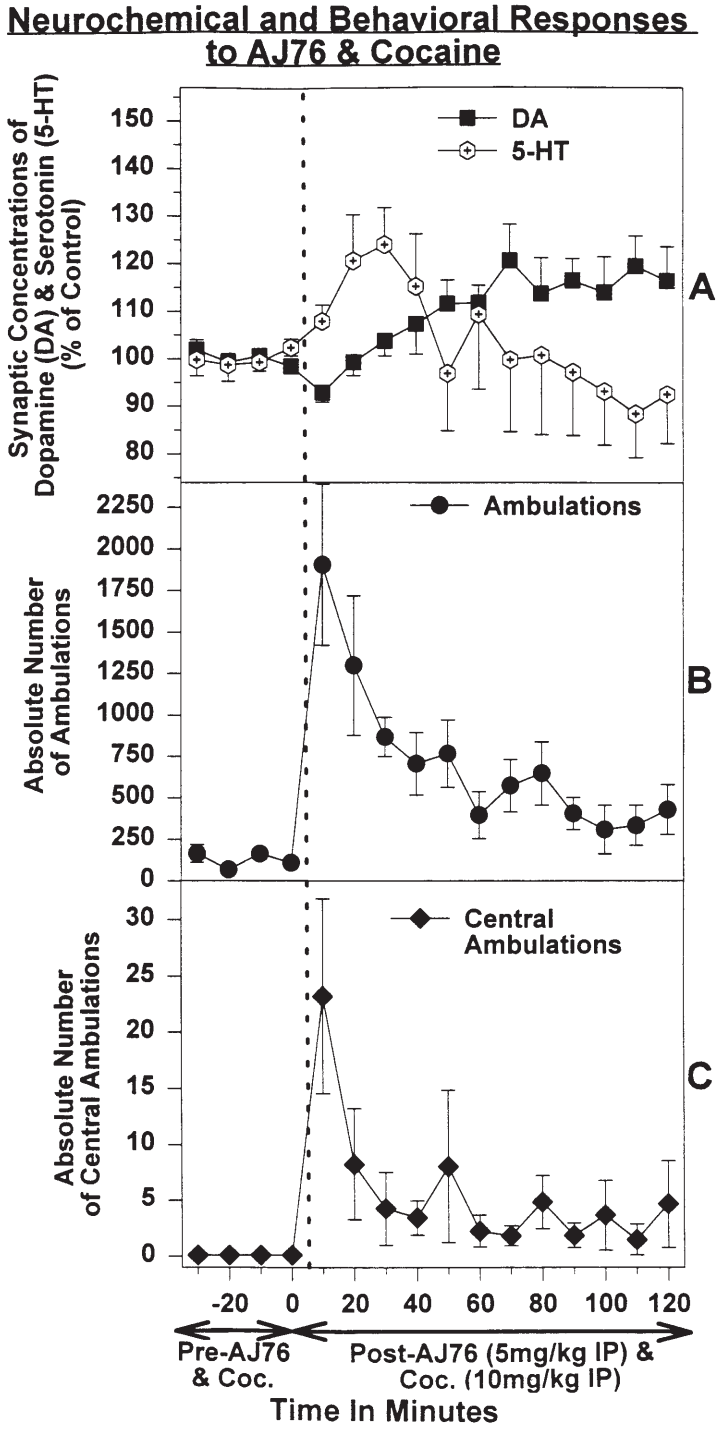
Figure 2B: A statistically significant increase in *locomotor activity* occurred (One Way ANOVA; $p < 0.001$; $F = 24.013$; df = 4,11 (equal variance). Post hoc analysis showed that increased locomotions were seen in the first, second, and third half hour post-cocaine administration periods (Dunnet's Method: $p < 0.05$, $q' - 8.541$, $q' = 5.845$, $q' = 5.832$ first through third hour respectively). Locomotor activity was highly correlated with synaptic 5-HT ($r = 0.729$) ($p < 0.01$) and DA was ($r = -0.567$).

Figure 2C: Cocaine did not affect *central locomotor activity* in the two hour analysis (One Way ANOVA; $p = 0.066$; $F = 3.024$; df = 4,11 (equal variance)), although there were significant increased data points at the 10, 20, 30, 50, 60 and 90 minute marks ($p < 0.05$) (95% C.L.). Central locomotor behavior was highly correlated with 5-HT ($r = 0.639$) and not with DA ($r = -0.141$) (NS).

(+)AJ 76/Cocaine (Fig. 3)

Figure 3A: (+)-AJ 76/cocaine treatment produced a significant increase in *DA* synaptic concentration from baseline (One Way ANOVA; $p < 0.001$; $F =$

Fig. 3. In vivo microvoltammetry was used to detect synaptic concentrations of DA and 5-HT from NAcc in awake, freely moving, male, Sprague Dawley rats, while their locomotor and central locomotor behaviors were monitored, simultaneously, with infrared photobeams (same animal control) (N = 5). Animals were studied for 2 hours before drug injection; (first hour: exploratory behavior; second hour: habituated behavior) and for 2 hours after drug injection. The x axis denotes time in minutes. Time "−30 to 0 minutes" denotes the last forty minutes of the habituation period (baseline values). (+)-AJ 76 was injected approximately 1 minute after time 0; cocaine was injected approximately 6 minutes after time 0. Vertical bar separates *pre-(+)-AJ 76/cocaine* data points from *post-(+)-AJ 76/cocaine* data points. **A** The y axis represents change in oxidation current before and after injections of (+)-AJ 76 (5 mg/kg ip) and cocaine (10 mg/kg ip). (+)-AJ 76/cocaine treatment increased synaptic concentrations of *DA* above baseline ($p < 0.001$). Increases in DA were delayed and began at 50 minutes; temporally related changes then remained significantly increased above baseline to the end of the study [$p < 0.05$: 95% C.L.]. There was no statistically significant change in synaptic concentrations of *5-HT* due to (+)-AJ 76/cocaine treatment ($p = 0.002$) (2 hour data), although significant increases were seen, immediately, at 10 minutes; these increases in 5-HT continued to the 40 minute mark [$p < 0.05$: 95% C.L.]. **B** (+)-AJ 76/cocaine increased *locomotor activity* ($p < 0.001$). Time course data showed that each point, during the two hour period of study, with the exception of the one-hundred minute mark, was significant. [$p < 0.05$: 95% C.L.]. **C** Significant differences in *central locomotor activity* did not occur ($p = 0.065$), but temporally related increases were significant at the 10, 20, 40, 50 and 80 minute marks [$p < 0.05$: 95% C.L.]


Neurochemical and Behavioral Responses to AJ76 & Cocaine
Synaptic Concentrations of Dopamine (DA) & Serotonin (5-HT) (% of Control)
DA
5-HT
A
Absolute Number of Ambulations
Ambulations
B
Absolute Number of Central Ambulations
Central Ambulations
C
Pre-AJ76 & Coc.
Post-AJ76 (5mg/kg IP) & Coc. (10mg/kg IP)
Time In Minutes

23.102; df = 4,11 (equal variance)). Post hoc analysis showed that the second, third, and fourth half hours were significant (Dunnet's Method: $p < 0.05$, $-q' = 4.061$, $q' = 6.754$, $q' = 6.614$ second through fourth half hour respectively). Comparison between (+)-AJ 76/cocaine treatment and cocaine effects per se, showed that the increase in synaptic concentration of DA from NAcc due to (+)-AJ 76/cocaine treatment, was significantly lower than cocaine-induced synaptic DA (One Way ANOVA; $p < 0.001$; $F = 19.434$; df = 2,21 (equal variance)) Post hoc tests showed that the difference between groups occurred in the second hour (Dunnet's Method: $p < 0.05$, $q' = 6.223$).

No statistically significant change in *5-HT* synaptic concentrations occurred (two hour data) (One Way ANOVA; $p = 0.002$; $F = 9.209$; df = 4,11 (equal variance)), but statistically significant increases occurred at 10, 20, 30, and 40 minutes post-injection ($p < 0.05$) (95% C.L.). Comparison between (+)-AJ 76/cocaine treatment and cocaine effects per se, showed that there was no difference between groups (One Way ANOVA; $p = 0.032$; $F = 4.061$; df = 2,21 (equal variance)). Correlation value for DA versus 5HT was ($r = -0.536$).

Figure 3B: (+)-AJ 76/cocaine treatment increased *locomotor activity* (One Way ANOVA; $p < 0.001$; $F = 11.520$; df = 4,11 (equal variance)). Post hoc analysis showed that the first half hour data, post-injection, showed significance (Dunnet's Method: $p < 0.05$, $q' = 6.547$). Temporally related increases were significant at each point during the study, with the exception of the 100 minute mark ($p < 0.05$) (95% C.L.). Comparison between (+)-AJ 76/cocaine treatment and cocaine effects per se, showed that no significant difference in locomotor behavior occurred between groups (One Way ANOVA; $p = 0.144$; $F = 2.127$; df = 2,21 (equal variance)).Correlation value for locomotion versus 5-HT was ($r = 0.536$); for DA was ($r = -0.341$) (NS).

Figure 3C shows that no statistically significant difference in *central locomotor activity* occurred (One Way ANOVA; $p = 0.065$; $F = 3.037$; df = 4,11 (equal variance)), although significant temporally-related increases occurred at 10, 20, 40, 50 and 80 minutes ($p < 0.05$) (95% C.L.). Comparison between (+)-AJ 76/cocaine treatment and cocaine effects per se, revealed no significant difference between groups. Correlation value observed for central locomotion versus 5-HT was ($r = 0.257$) (NS); for DA was ($r = -0.357$) (NS).

Discussion

Consistent with data derived from studies of (+)-AJ 76 per se on extracellular concentrations of DA in nigrostriatal systems (Hefti, 1987; Waters et al., 1993; Rayevsky et al., 1995), we found that (+)-AJ 76 significantly increased synaptic concentrations of DA within mesolimbic terminal fields, NAcc. Additionally, consistent with data showing that (+)-AJ 76 depressed 5-HT somatodendritic activity in a manner similar to a partial $5HT_{1A}$ agonist (Piercey et al., 1989), the present data show that (+)-AJ 76 produced a small but statistically significant decrease in synaptic 5-HT within the same synaptic

environment in which DA effects occurred. In addition though, since microvoltammetry has the advantages of providing a highly sensitive temporal, as well as spatial resolution, a significant increase in synaptic 5-HT was observed 20 minutes after injection of (+)-AJ 76. Therefore, the overall effect of (+)-AJ 76 was a biphasic one.

We did not observe the increases in locomotor activity previously reported for (+)-AJ 76 in strongly habituated rats (Svensson et al., 1986), but these are reported to be very mild effects. Central locomotor activity (anxiolytic activity) was not statistically different from baseline values after the intraperitoneal administration of (+)-AJ 76. This is the first report of the effect of (+)-AJ 76 on central locomotor activity; (+)-AJ 76, per se, does appear to have some anxiolytic activity in this open-field paradigm. However, in the present studies, we monitored behavior at specific data points and there were time-related increases in central locomotor activity due to (+)-AJ 76 per se.

Cocaine produced its expected increases in synaptic DA and 5-HT concentrations from NAcc, as well as its expected increases in locomotor behavior. The present results show that cocaine did not exhibit anxiolytic activity as measured by central locomotor activity when two hour analysis was done at $p = 0.001$; the present results are in agreement with results derived from the plus-maze paradigm, used in behavioral studies, by other investigators (Yang et al., 1992). It is noteworthy, however, that temporally-related increases in central locomotor behavior were seen during the time course of the present study. In previous studies from this laboratory, we have observed statistically significant increases in central ambulatory activity following cocaine; however, a statistical criteria of $p = 0.05$ was used (Broderick et al., 1993).

It has been suggested that (+)-AJ 76 could be a useful cocaine pharmacotherapy because its ability to increase mesolimbic DA release presynaptically may allay craving whereas postsynaptic blockade at mesolimbic DA receptors, may be useful in blocking euphoria (Piercey et al., 1992; Callahan et al., 1992; Richardson et al., 1993; Casey et al., 1996). The present data show that (+)-AJ 76 may block some cocaine effects by down-modulating the known cocaine-induced increase in synaptic DA which occurs from A_{10} nerve terminals, NAcc. However, the ability of (+)-AJ 76 to decrease cocaine-induced mesolimbic synaptic concentrations of DA is not readily explicable in terms of D_3 receptor or DA autoreceptor pharmacology. At first glance, it might appear that (+)-AJ 76 interacts directly with the DA transporter to interfere with cocaine's actions. If this suggestion were the case, the suggestion raises the possibility that (+)-AJ 76, like cocaine, could owe some of its DA releasing properties to interference with the transporter molecule. However, since (+)-AJ 76 does not compete for the cocaine-binding site (Sethy, unpublished results), any direct modulation of transporter function would have to be allosteric to the cocaine site.

A role for the effects of (+)-AJ 76 on cocaine-induced synaptic 5-HT from NAcc as these relate to the locomotor behavior produced by (+)-AJ 76/cocaine treatment is not clear. It is important, though, that (+)-AJ 76 did not block the cocaine-induced increase in synaptic 5-HT from NAcc; nor did it

block the stimulant behavior produced by cocaine. Indeed, synaptic concentrations of 5-HT from NAcc were highly correlated with locomotor behavior after the (+)-AJ 76/cocaine administration over the two hour study. Consequently, the data show that (+)-AJ 76, in its interaction with cocaine, did not produce sedation. These findings concomitant with the DA profile of (+)-AJ76/cocaine treatment may be positive aspects for a possible cocaine pharmacotherapy.

Previous studies have shown that (+)-AJ 76 blocked cocaine self-administration by producing a delay in the onset of cocaine intake (Richardson et al., 1993). The dimethyl analog of (+)-AJ 76, a compound known as (+)-UH 232, and another compound (−)-DS 121, a phenylpiperidine structurally related to the partial DA receptor antagonist (−)-PPP (Svennson et al., 1986; Sonesson et al., 1993), have pharmacological profiles similar to (+)-AJ 76. These include the ability to delay and decrease the intake of cocaine (Smith et al., 1995). The data in the present studies show that the increase in synaptic concentrations of DA after the (+)-AJ 76 treatment, undergoes a delay and is observed 50 minutes after administration, as opposed to the immediate increase in DA observed after cocaine was administered. Perhaps the present data, then, lend an explanatory note to the reported reduction in the self-administration of cocaine.

Although all of the above compounds bind slightly better to DA D_3 receptors than to DA D_2 receptors, it is not clear which of these receptors is responsible for the reduction in cocaine self-administration. The literature reports various other properties for (+)-AJ 76: (+)-AJ 76, e.g., blocked the discriminative stimulus effects of the D_2/D_3 agonist, quinpirole (Gui-Hua and Woolverton, 1992); in addition, (+)-AJ 76 blocked apomorphine-induced yawning (Dourish et al., 1989). Gifford and Johnson (1993) report, in studies specifically using (+)-AJ 76, that DA release-controlling receptors, are most similar to DA D_2 receptors. Millan et al. (1995) report that (+)-AJ 76's blockade of clozapine-induced hypothermia is due to its interaction with DA D_3 receptors.

Vanover et al. (1993) postulate that the reduction in self-administration of cocaine caused by (+)-AJ 76, is thought to be more influenced by DA D_2 postsynaptic receptors than by DA D_3 autoreceptors because the rate-increasing effects of (+)-AJ 76 were comparable to the increase in cocaine administration produced by other DA D_2 antagonists (e.g., haloperidol). However, the fixed ratio interval (FRI) schedule used, differed from the progressive ratio protocol used by Richardson et al. (1993), where the effects of (+)-AJ 76 differed dramatically from haloperidol, which decreased the onset time for cocaine self-administration. Moreover, Richardson et al. (1993) have reported that (+)-AJ 76 produced a dose-dependent preference for the compartment associated with its administration. Furthermore, (+)-AJ 76 partially generalized to subjective cues produced by cocaine (Callahan et al., 1992). Taken together, these data suggest that (+)-AJ 76 is a compound which is not analagous to the typical neuroleptics, the DA D_2 receptor antagonists, which produce anhedonia and which have only rarely been used to treat cocaine abuse (Gawin and Kleber, 1986).

Because the DA D_3 receptor has its highest distribution in the mesolimbic area (Sokoloff et al., 1990), the DA D_3 receptor subtype is a candidate receptor for mediating reward signals associated with these neuroanatomic sites. One postulate regarding the mechanism of action of the interaction between (+)-AJ 76 and cocaine, is that DA $D_{3,}$ acts in concert with DA D_2 receptors. The partially reinforcing effects of (+)-AJ 76, which may ameliorate craving, could act in this way. Although the exact mechanism remains to be elucidated, another postulate is that postsynaptic blockade of DA D_3 receptors may alone account for to the antagonism of the reinforcing effects of cocaine.

In conclusion, (+)-AJ 76 is a weak behavioral stimulant, which produces a significant increase in synaptic concentrations of DA, within seconds of release from NAcc and down-modulates the known increase in cocaine-induced release within the same neuroanatomic substrate. (+)-AJ 76 and other compounds, which belong to the "autoreceptor antagonist" class, have interesting profiles suggesting that they could be useful as pharmacotherapies for cocaine abuse and/or addiction. It is likely that these compounds owe at least part of their ability to block cocaine self administration to postsynaptic receptor blockade, possibly the DA D_3 receptor subtype. However, the present observation that (+)-AJ 76 reduces cocaine's ability to increase synaptic DA from NAcc provides another clear mechanism that likely contributes to the ability of these "autoreceptor antagonist" compounds to interfere with cocaine's reinforcing effects. Finally, it is interesting that (+)-AJ 76 did not diminish either the 5-HT or the locomotor behavior produced by cocaine; its stabilization of 5-HT and increased locomotor behavior present other positive aspects for the suggestion that (+)-AJ 76 may be a potential cocaine pharmacotherapy.

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