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8-OH DPAT Can Restore the Locomotor Stimulant Effects of Cocaine Blocked by Haloperidol

ROBERT CAREY, ERNEST DAMIANOPOULOS AND GAIL DE PALMA

VA Medical Center and SUNY Health Science Center, Syracuse, NY

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CAREY, R., E. DAMIANOPOULOS AND G. DE PALMA. *8-OH DPAT can restore the locomotor stimulant effects of cocaine blocked by haloperidol.* PHARMACOL BIOCHEM BEHAV **66**(4) 863–872, 2000.—In the first experiment, separate groups of rats ($n = 7$) were treated with either saline, cocaine (10 mg/kg), haloperidol (0.1 mg/kg), or cocaine (10 mg/kg) plus haloperidol (0.1 mg/kg). Locomotor behavior was measured in an open-field environment, and cocaine induced a reliable locomotor stimulant effect compared to saline-treated animals. Haloperidol produced a progressive decline in locomotion over the 5 test days. Haloperidol also blocked cocaine stimulant effects compared to cocaine-treated animals. In the second experiment, five groups ($n = 7$) of animals were treated either with saline, cocaine (10 mg/kg), 8-OH DPAT (0.2 mg/kg), 8-OH DPAT (0.2 mg/kg) plus haloperidol (0.1 mg/kg), or 8-OH DPAT (0.2 mg/kg) plus haloperidol 0.1 mg/kg plus cocaine (10 mg/kg). Over the course of 5 days of treatment, cocaine induced a locomotor stimulant effect. Saline and 8-OH DPAT animals did not differ in terms of locomotion. The 0.1 mg/kg haloperidol plus 0.2 mg/kg 8-OH DPAT treatment decreased locomotion compared to the saline group, but the group given 0.2 mg/kg 8-OH DPAT plus 0.1 mg/kg haloperidol plus cocaine (10 mg/kg) exhibited a locomotor stimulant effect equivalent to the cocaine group. In a third experiment, it was found that the 0.2 mg/kg 8-OH DPAT treatment did not enhance the locomotor stimulant effect of cocaine. Thus, the 8-OH DPAT treatment was able to restore a cocaine locomotor stimulant effect in animals treated with haloperidol without directly enhancing the locomotor stimulant effects of cocaine. In Experiments 2 and 3, entries into the central zone of the open field were measured. Cocaine reliably increased central zone entries. The 8-OH DPAT treatment, however, selectively blocked this behavioral effect of cocaine suggesting a qualitative influence of 5-HT_{1A} receptors upon cocaine, independent of locomotion activation by cocaine. Ex vivo measurements of dopamine and 5-hydroxytryptamine metabolism in limbic tissue were consistent with the established effects of cocaine, haloperidol, and 8-OH DPAT upon dopamine and 5-hydroxytryptamine neurotransmission. In addition, measurement of cocaine brain concentration indicated that neither haloperidol or 8-OH DPAT affected cocaine concentration in brain. © 2000 Elsevier Science Inc.

Cocaine Locomotion Haloperidol 8-OH DPAT Central zone

COCAINE is a potent inhibitor of dopamine transport (18,35,36), and this effect upon dopamine is considered to contribute substantially to the locomotor stimulant effects of cocaine (19,46). It is not surprising, therefore, that dopamine antagonists can attenuate a variety of cocaine effects including locomotor stimulation. In particular, the D_2 preferring antagonist, haloperidol has been shown to be effective in the blockade of cocaine locomotor stimulant effects with acute or short-term treatments (3,42,45). A major difficulty with using dopaminergic antagonists such as haloperidol to study cocaine effects expressed in motoric behavior is that the haloperidol treatment can alter the behavioral baseline (27,43). Thus, dose levels of haloperidol that attenuate cocaine behavioral responses also may attenuate behavioral responses in saline-treated animals. Furthermore, using a dose level of haloperidol that does not affect locomotion is not necessarily a solution, in that the receptor antagonism may be inadequate to test the hypothesis. Alternatively, the higher the dose, the greater the receptor blockade and the greater the impact upon baseline locomotion. In the present study, we used a dose of haloperidol which, with acute administration, has modest and subtle effects upon locomotion (24,25), but which biochemical indices have demonstrated that substantial receptor blockade occurs (26,41). In the first study, we report

Requests for reprints should be addressed to R. Carey, 800 Irving Avenue, Research (151), VA Medical Center, Syracuse, NY 13210.

the effects of acute and repeated low-dose haloperidol treatment upon the locomotion stimulant effect of cocaine. In the second experiment, we evaluated whether the 5-HT_{1A} agonist, (±)-8-hydroxy-dipropylaminotetralin (8-OH DPAT), which has been shown to reverse the effects of haloperidol upon catalepsy (1,23), could also reverse the suppressive effects of haloperidol upon spontaneous locomotor behavior as well as cocaine induced locomotion. The final experiment evaluates whether the 8-OH DPAT treatment alone modifies cocaine locomotor stimulant effects.

EXPERIMENT 1

Method

Animals. Twenty-eight naive male Sprague–Dawley rats from Taconic Farms (Germantown, NY), 6 months old and weighing approximately 450 g at the start of the experiments, were used. Upon arrival, the animals were housed in individual 25 × 17 × 17-cm wire-mesh cages in a climate-controlled room at 22°C, with a 12 L:12 D cycle. During the first week after arrival, all animals were handled and weighed daily for 7 days. During the second week the animals received three injections (IP) of 0.9% saline (1 ml/kg) to acclimate the animals to the injection procedure. All experiments occurred during the 12-h light cycle (0600–1800h).

Drugs. Cocaine hydrochloride (Sigma Chemical Co., St. Louis, MO) was dissolved in sterile distilled H_20 to a concentration of 10 mg/ml. (±)-8-Hydroxy-dipropylaminotetralin (8-OH DPAT (RBI/Sigma, Natick, MA) was dissolved in sterile distilled H_20 to concentration of 0.2 mg/ml. Haloperidol (RBI/Sigma, Natick, MA) was initially dissolved in glacial acetic acid and 0.1 M NaOH was used to achieve a pH of 4.0. Sterile distilled H_2O was used to dilute the solution to a concentration of 0.1 mg/ml. All injections were IP.

Apparatus. All of the behavioral tests were conducted in square open field compartments that were 60 × 60 × 45 cm. Closed-circuit video cameras (RCA TC7011U) were mounted 50 cm above the open-field enclosures. All signals were analyzed by a video tracking system, the Videomex-V from Columbus Instruments (Columbus, OH), and the data imported into a PC-compatible computer. The walls of the chamber were white, and the floor of the open- field was covered by plain white paper that was changed after each animal. Ambient white noise (80 dB) was provided by a white-noise generator (San Diego Instruments, San Diego, CA) and was turned on immediately prior to placement of the animal in the test chamber and turned off upon removal from the test chamber. Testing was conducted under conditions of red light illumination to avoid the aversive quality of white light and to enhance the contrast between the subject and background as well as to reduce the animal's shadow. The animal's head was blackened with a nontoxic marker, and the camera only tracked this feature of the rat's body. During each session, data was collected every 2.5 min by the computer. Dot matrix printers (Epson FX-286e) were placed outside the test rooms, and were connected to the image analyzers by a parallel cable, and the computer screen tracings of the animal's movement were printed out every 2.5 min. The complete test procedure was conducted automatically without the presence of the experimenter in the test room. In addition, a VHS VCR was also connected to each camera providing the ability for one to review and reinput the video tape signal to the image analyzer in case of a malfunction of either the analyzer or the printer during the experiments.

Behavioral testing. With repeated treatments, cocaine typically induces sensitization and conditioned drug effects (2,4,14,32,37,38,40). As a result of these factors, the effects on locomotor behavior of a fixed dose level of cocaine is not constant, but may change with repeated treatments. This change in the efficacy of a cocaine treatment makes it appropriate to assess initial as well as repeated cocaine treatment effects.

Initially, all animals underwent 10 days of daily handling, including 3 days of saline injections, to acclimate the animals to manipulation and injection procedures. Next, all animals were given two 10-min tests in the test environment to form groups that were statistically equivalent with respect to the dependent variable of locomotion. Four days after the completion of the matching protocol, the four matched groups (n = 7) received five successive 20-min tests in which spontaneous locomotor behavior was recorded. The four treatment groups were saline-saline, saline-cocaine (10 mg/kg), haloperidol (0.1 mg/kg)-saline, and haloperidol (0.1 mg/kg)-cocaine 10 mg/kg). The treatments were administered as two separate injections. The haloperidol and saline injections were given 40 min before testing in the home cage, and the second series of injections of either saline or cocaine were given immediately before testing. Four days after the completion of this testing the animals were given a final treatment, and sacrificed immediately after the 20 min behavioral test. In this final test all groups received the same treatment they had previously received.

Statistical analyses. Two-way analysis of variance (ANOVA) was used to analyze the behavioral data to determine the group effects, repeated-treatment effects, as well as the interaction between variables. Subsequently, to make more specific comparisons one-way ANOVAs were used. For the biochemical data, one-way ANOVAs or independent t-tests were performed. To make specific group comparisons, post hoc Duncan's multiple range tests were performed. $p < 0.05$ was used as the criterion for statistical significance.

Biochemical procedures. Ex vivo measurements were made on dopamine (DA) and 5-hydroxytryptamine (5-HT) and metabolites 3,5-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindole-3-acetic acid (5-HIAA). In ex vivo measurements, DA and 5-HT concentrations were primarily determined by intracellular concentrations which, on the basis of our previous findings (5), were not affected by the drug treatments used in the present experiments. The drug treatments, however, were expected to impact upon 5-HT and DA metabolites. In that transmitter/metabolite correlations were generally high, variability in tissue sample concentrations of 5-HT and DA could contribute substantially to the variability in metabolite concentrations. Accordingly, we express the changes on metabolites induced by pharmacological treatments used in this study in terms of metabolite/transmitter ratios. In this way, the possible contribution of variability in transmitter concentration among tissue samples obtained from a particular brain area to the observed metabolite concentrations is diminished. In addition to measurements of tissue concentrations of DA, 5-HT, DOPAC, and 5-HIAA, we also measured cocaine concentrations in plasma and in brain. In this way, we were able to validate that animals receive the appropriate cocaine treatment and whether or not a coadministered drug may have altered the availability of cocaine to brain tissue. Tissue samples were obtained immediately following completion of the behavioral testing. Animals were placed in a plastic restraining cone (Braintree Scientific, Braintree, MA) and sacrificed by decapitation. Trunk blood was collected in tubes containing 200 μl of 0.5% sodium fluoride and centrifuged for 15 min at 2,500 rpm. The plasma was

frozen at −70°C and subsequently assayed for cocaine. The brain was rapidly removed and dissected on a chilled plate. Under magnification, bilateral samples of limbic brain were dissected, which included nucleus accumbens, olfactory tubercle, and overlying pyriform cortex. Following dissection, the samples of brain tissue are weighed, placed in tubes containing 0.5 ml of 0.1 M perchloric acid and 4.5 μl of 10 μg/ml dihydroxybenzylamine (DHBA) as an internal standard, and then homogenized and centrifuged. The resulting supernatant was filtered through 0.2 μm pore filters and the extracts were stored at −70°C until the HPLC-EC analysis, which was completed within 24–48 h. The tissue samples were analyzed for dopamine, DA (3-hydroxytyramine), the dopamine metabolite, DOPAC (3,4-dihydroxyphenyl-acetic acid), 5-HT (5-hydroxytryptamine), the 5-HT metabolite, 5-HIAA (5-hydroxyindole-3-acetic acid), and cocaine. For the catecholamine and indoleamine analyses of brain tissue, a BAS biophase column [C18 reverse phase (4.6 × 250 mm 5 μm)] was used. The buffer used was 0.15 M moncholoroacetic acid, pH 3.1, 2 mM EDTA 0.86 mM SOS (sodium octyl sulfate). This was added to 35 ml acetonitrile (3.5%) to make 1 liter. This solution was then filtered and degassed, and 18 ml (1.8%) tetrahydrofuran (THF) was added. The mobile phase flow rate was 1.2 ml/min, and a BAS 4B EC detector was set at 0.8 V. For cocaine (brain samples), the mobile phase was 24% acetonitrile and 76% 0.02 M potassium phosphate buffer (pH 3.0). A Nucleosil C18 column (100 × 4 mm, 3 μm) was used, with a flow rate of 0.5 ml/min. Cocaine was detected using the BAS variable wavelength UV detector at a setting of 235 nm. For plasma cocaine (6), the sample (0.5 ml plasma) was prepared by precipitating out the protein with 1.5 ml 100% acetonitrile. Sodium phosphate buffer (0.3 ml of 0.1 M) (pH 6.0) was added to the supernate, and the pH was between 4 and 6. A Bond Elut Certify (Varian, Harbor City, CA) solid-phase column (125 mg and 3 ml) was conditioned with methanol and 0.1 M sodium phosphate buffer. Before the column could run dry, the prepared sample was passed through the column. The column was then washed with 3 ml HPLC-grade water, 3 ml 0.1 M HC1, and 10 ml methanol. The cocaine was eluted with 2 ml methylene chloride:isopropanol:ammonium hydroxide (77:19:4). The sample was then evaporated under a stream of nitrogen and reconstituted in 0.2 ml of buffer. For cocaine (plasma and brain samples), the mobile phase was 24% acetonitrile and 76% 0.02 M potassium phosphate buffer (pH 3.0). A Nucleosil C18 column (100 × 4 mm, 3 μm) was used, with a flow rate of 0.5 ml/min. Cocaine was detected using the BAS variable wavelength UV detector at a setting of 235 nm.

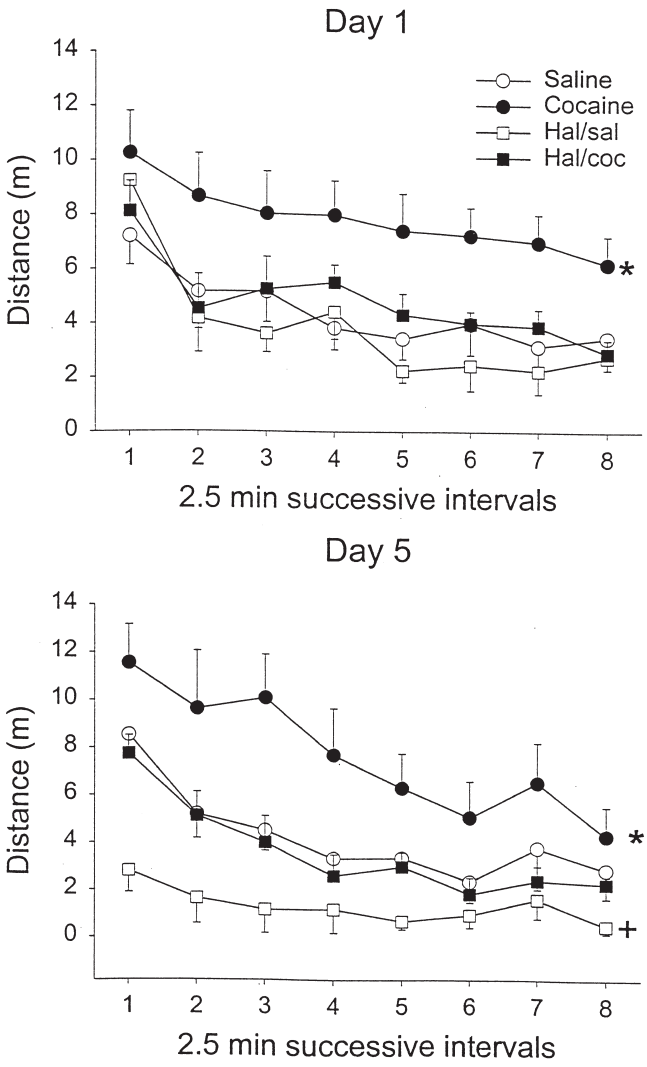

FIG. 1. Means and SEMs of within session locomotion distance scores of groups ($n = 7$) treated with either IP injections of saline, cocaine (10 mg/kg), haloperidol (0.1 mg/kg), or haloperidol (0.1 mg/kg) + cocaine (10 mg/kg). The upper half of the figure presents the first day of treatment, and the lower half of the figure presents the results from the fifth day of treatment. Asterisk (*) denotes $p < 0.01$ higher than all other groups, and + denotes $p < 0.01$ lower than all other groups.

Results

The drug treatments had substantial effects upon locomotion over the course of the 5 treatment days. Statistical analysis of the locomotion data indicated that there was a statistically significant treatment effect, $F(3, 24) = 12.1$, 0.001, and statistically significant interaction, $F(12, 96) = 2.6$, $p < 0.01$. The primary contributor to the interaction was the marked decline in locomotion in the haloperidol-saline group over the 5 days of treatment. Figure 1 represents the within-session locomotion scores for the four groups on day 1 and day 5 of testing to display the initial treatment effects as well as the repeated-treatment effects. It is apparent in Fig. 1 that the haloperidol treatment on day 1 (upper) had little effect upon locomotion, but by the fifth treatment (lower) this resulted in a marked retardation in locomotion. On day 1 the group × interval ANOVA indicated that there were statistically significant group differences, $F(3, 24) = 5.2$, $p < 0.01$, and post hoc comparisons indicated that the cocaine group had higher locomotion scores than all other groups that did not differ from each other. On day 5 there also were statistically significant group differences, $F(3, 24) = 8.1$, $p < 0.01$. On this test day, the cocaine group had higher locomotion scores than all other groups, and the haloperidol group had lower scores than all other groups. The saline and haloperidol plus cocaine groups were statistically equivalent, $p > 0.05$. Although these results suggest that haloperidol blocked the stimulant effect of cocaine, it is also evident that the haloperidol treatment came to exert a substantial suppressive effect upon locomotion. Figure 2 presents a comparison of the haloperidol-sal vs. the haloperidol-cocaine groups (upper) and the sal-sal vs. sal-cocaine

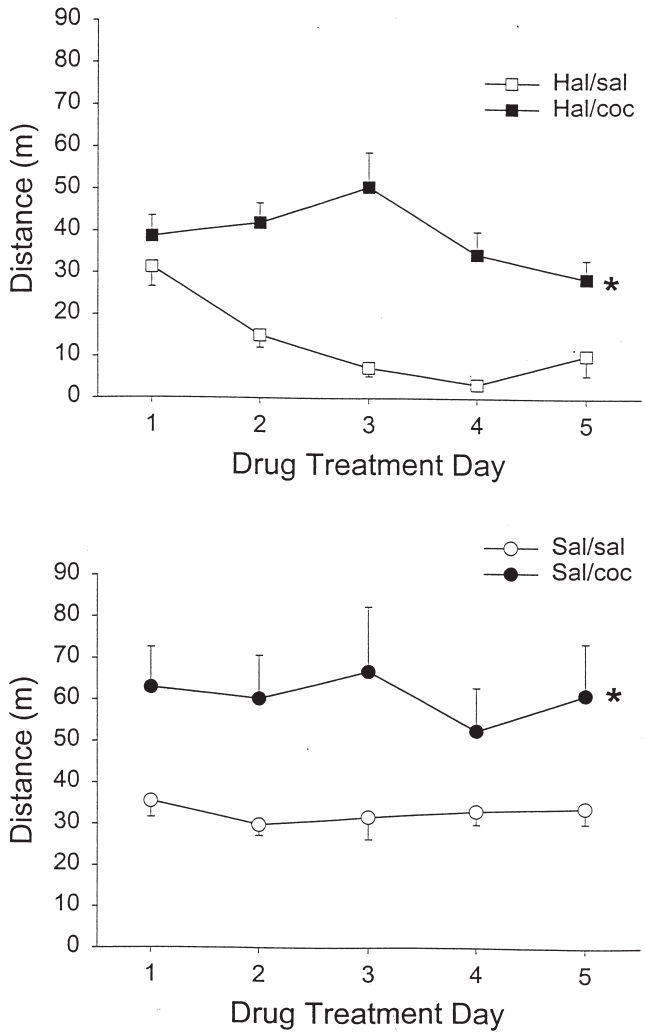

FIG. 2. Means and SEMs of total distance locomotion scores over the five treatment sessions. The upper half of the figure compares the haloperidol (0.1 mg/kg) vs. the haloperidol (0.1 mg/kg) + cocaine (10 mg/kg) treatment groups and the lower half compares the saline vs. cocaine (10 mg/kg) treatment groups. Asterisk (*) denotes $p < 0.01$.

groups (lower) over the course of the 5 test days. Presented in this way, it is apparent that the cocaine treatment had a substantial locomotor stimulant effect in both the saline and haloperidol treated animals, $F(1, 13) = 26.7$, $p < 0.01$, and $F(1, 13) = 7.8$, $p < 0.01$, respectively. The biochemical measurements of dopamine and the dopamine metabolite DOPAC indicated that there were no statistically significant treatment effects upon DA concentration, $p > 0.05$, but that there were substantial effects upon dopamine metabolism. Figure 3 represents the biochemical findings. As can be seen in Fig. 3 (upper), haloperidol and cocaine had the expected opposite effects upon DOPAC/DA ratios. Furthermore, the cocaine and haloperidol effects upon DOPAC/DA ratios appeared to interact in a straightforward additive manner. As was in the case for the behavioral findings, cocaine had effects when compared with its respective control group. That is, the sal-cocaine group had a lower DOPAC/DA ratio than the saline group, and the haloperidol-cocaine group had a lower DOPAC/DA ration than the haloperidol-saline group. In the lower half of Fig. 3, it can be seen that the haloperidol treatment did not alter the availability of cocaine in the brain tissue samples. Plasma cocaine concentrations also were unaffected by haloperidol, $p > 0.05$.

Discussion

The results of this experiment indicate that haloperidol can blunt the locomotor stimulant effects of cocaine. Haloperidol given alone, however, induced a substantial reduction in locomotor behavior with repeated treatments. Although the motoric suppressive effects of haloperidol occurred with repeated treatments, it is relevant that haloperidol attenuated the effects of cocaine on even the first test when the effects of haloperidol upon locomotion were slight. Overall, the results of this study can be looked at in two ways, depending upon which noncocaine group is used as the reference point. If the locomotion level in the saline group is used as the baseline response, then one could argue that the dopamine antagonist efficacy of haloperidol was sufficient to balance the increase in dopamine availability induced by cocaine. Indeed, the saline and haloperidol-cocaine groups had statistically equivalent levels of locomotion. Thus, there appears to be no net behavioral effect in terms of dopaminergic stimulation with the cocaine-haloperidol combination such that locomotor activity occurred at nondrug levels. Alternatively, if the haloperidol

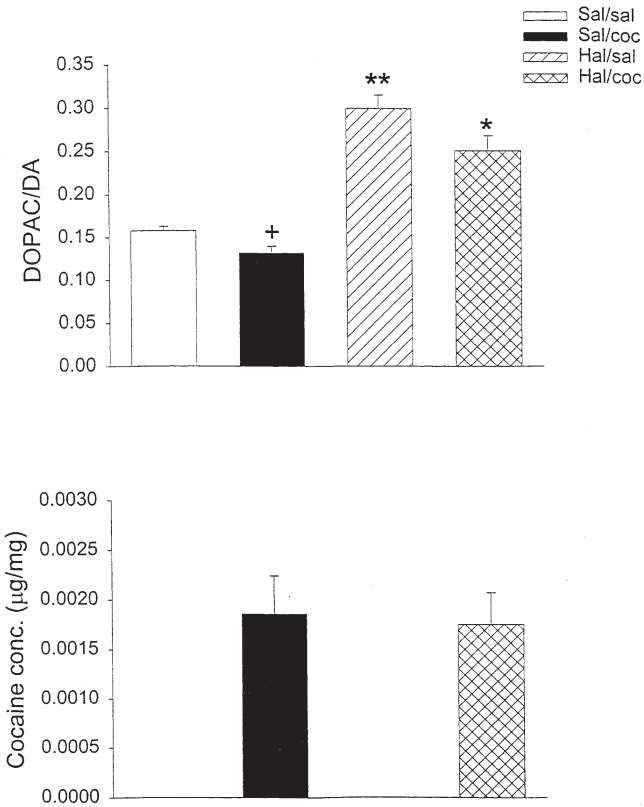

FIG. 3. Means and SEMs for DOPAC/DA (upper) and cocaine concentrations (lower) obtained from limbic brain samples for the four treatment groups. Asterisks (**) denote $p < 0.01$ higher than all other groups, * denotes $p < 0.01$ higher than saline and cocaine groups, and + denotes $p < 0.01$ lower than all other groups.

treatment is used as the baseline to assess cocaine effects in the haloperidol-cocaine treatment group, then it is apparent that cocaine induced a locomotor stimulant effect in the haloperidol-cocaine group. From this latter perspective, the effects of haloperidol upon locomotor behavior demonstrate that dopamine receptor antagonism can profoundly affect locomotion behavior, and that this effect can be reversed by cocaine.

EXPERIMENT 2

A difficulty with conducting behavioral studies in which dopamine function is attenuated throughout the brain (e.g., peripheral injection of DA antagonists) is that a diminution of DA function can substantially modify the baseline upon which this behavior is measured. In the first experiment, haloperidol blocked the stimulant effect of cocaine as compared to saline-treated animals, but not compared to haloperidol-treated animals. Interestingly, there have been several reports in which motoric effects of haloperidol (i.e., catalepsy) have been reversed by the 5-HT_{1A} agonists such as buspirone and (±)-8-hydroxy-dipropylaminotetralin (8-OH DPAT) (1,10,15,16,23,29,33). Given alone, the buspirone or 8-OH DPAT treatments did not affect catalepsy. In the second experiment, we administered 8-OH DPAT in combination with haloperidol to determine if this combined treatment would reverse the locomotion reduction induced by haloperidol and, in addition, restore the stimulant efficacy of cocaine. To determine a relevant dose level of 8-OH DPAT, we measured its impact upon 5-HT activity as manifested in 5-HIAA/5-HT

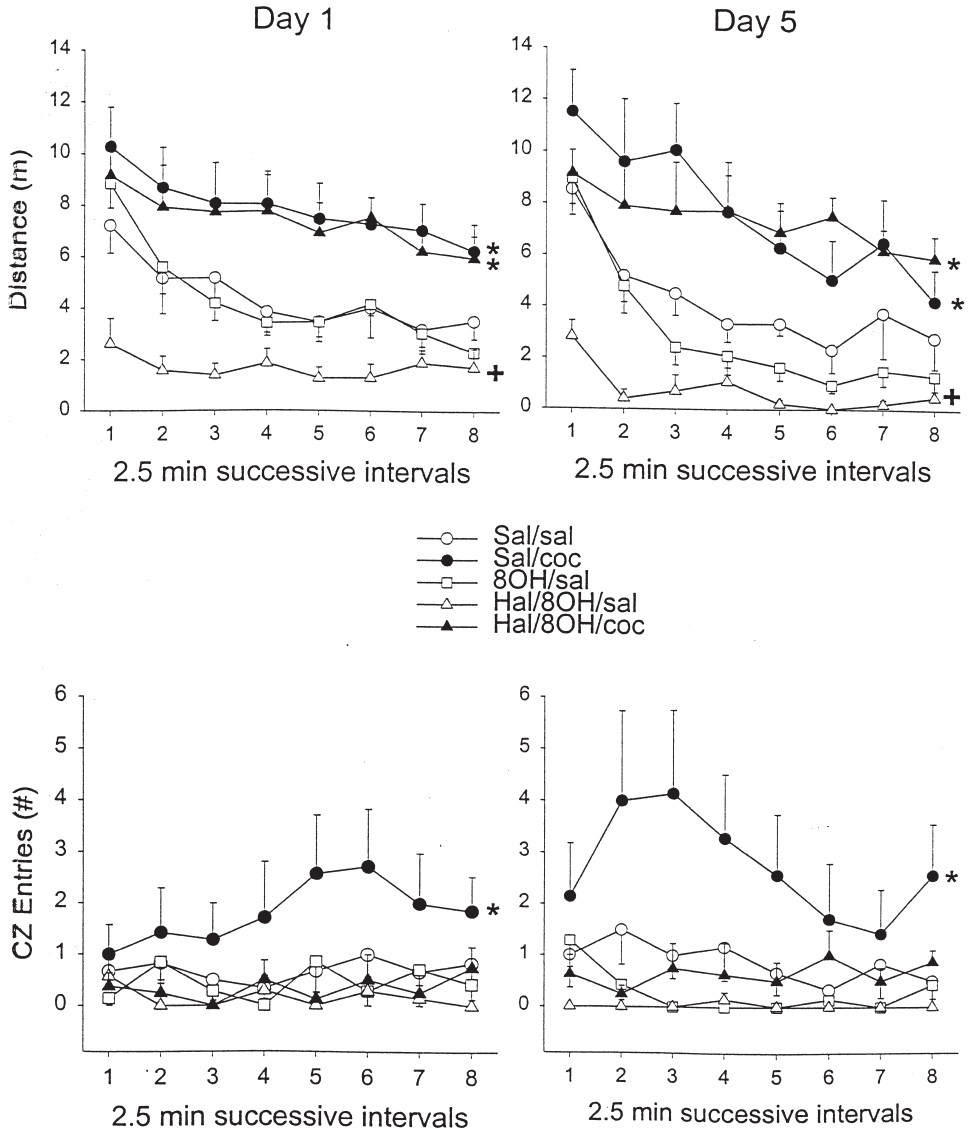

FIG. 4. Means and SEMs for within session locomotion distance scores (upper) and central zone entries (lower) on the first (left) and fifth (right) days of IP injection treatments (saline, cocaine (10 mg/kg), 8-OH DPAT (0.2 mg/kg), haloperidol (0.1 mg/kg) + 8-OH DPAT (0.2 mg/kg), or haloperidol (0.1 mg/kg) + 8-OH DPAT (0.2 mg/kg) + cocaine (10 mg/kg). Asterisk (*) denotes $p < 0.01$ scores higher than all other groups and + denotes $p < 0.01$ scores lower than all other groups.

ratios. On the basis of a series of dose level treatments (0.1–0.8 mg/kg), we employed a dose level of 8-OH DPAT (0.2 mg/kg), which decreased 5-HIAA/5-HT ratios to an extent that was equivalent to that induced by 10 mg/kg cocaine.

Method

The methods and materials and biochemical procedures were the same as in the first experiment. The only substantive change was that the software program for the video testing was altered so the entries into the central zone of the open field could be counted. This field was defined as the central area of the open field and comprised 1/9 of the total area of the arena.

Behavioral testing. Initially, all animals underwent 10 days of daily handling, including 3 days of saline injections to acclimate the animals to manipulation and injection procedures. Next, all animals were given two 10-min tests in the test environment to form groups that were statistically homogenous with respect to the dependent variable of locomotion. Four days after the completion of the matching protocol, the five matched groups ($n = 7$) received five daily 20-min tests in which spontaneous locomotor behavior was recorded. The five treatment groups were saline + saline, saline + cocaine (10 mg/kg), 8-OH DPAT (0.2 mg/kg) + saline, haloperidol (0.1 mg/kg) + 8-OH DPAT (0.2 mg/kg) + saline, and haloperidol (0.1 mg/kg) + 8-OH DPAT (0.2 mg/kg) + cocaine (10 mg/kg). The treatments were administered as two sets of IP injections. The first set of injections, haloperidol, 8-OH DPAT, haloperidol + 8-OH DPAT, or saline were given 40 min before testing in the home cage, and the second set of injections of either saline or cocaine were given immediately before testing. Four days after the completion of this testing the animals were given a final treatment and sacrificed immediately after the 20-min behavioral test. In this final test all groups received the same treatment they had previously received except for the 8-OH DPAT + haloperidol + saline group. On the final test, this group received 8-OH DPAT + haloperidol + cocaine (10 mg/kg). This modification in the treatment protocol was made to directly ascertain if the cocaine stimulant effect could be generated in animals with an established decreased behavioral baseline.

Results

There were statistically significant treatment effects over the 5-day testing period on both measures of distance and central zone entries (CZ), $F(4, 30) = 13.2$, $p < 0.001$, and $F(4, 30) = 4.7$, $p < 0.01$, for distance and entries, respectively. The day by treatment interactions for both measures were not statistically significant. Figure 4 presents the results for distance (upper) and CZ entries (lower) and day 1 (left) and day 5 (right). The 8-OH DPAT treatment by itself had no overall statistically significant effect upon locomotion distance compared to the saline treatment, although mean locomotion in the latter part of session 5 was decreased. The 8-OH DPAT treatment, however, not only did not prevent the response suppression effect of haloperidol, but appeared to enhance the response suppression particularly on day 1. Interestingly, the 8-OH DPAT treatment did permit the cocaine locomotor stimulant effect in animals treated with haloperidol. Another facet of the cocaine locomotor stimulation measured in this study was central zone entries that increase with cocaine treatment (7). For this behavioral measure, cocaine given by itself increased central zone entries. The group given cocaine plus haloperidol and 8-OH DPAT, however, had CZ entries at or below the level of the saline treatment group.

In the final drug treatment prior to sacrifice, the group which had received haloperidol and 8-OH DPAT in combination with saline over the first 5 treatment days was again given haloperidol + 8-OH DPAT, but on this test these animals also received cocaine (10 mg/kg). On this test day this group exhibited a statistically significant locomotor stimulant effect compared to the saline group, $p < 0.01$. This finding is consistent with the results obtained with the group treated with haloperidol + 8-OH DPAT + cocaine throughout testing.

In agreement with the first experiment, the biochemical measurements indicated that there were no statistically significant drug treatment effects upon DA and 5-HT concentrations, $p > 0.05$, but that there were reliable effects upon DA and 5-HT metabolism. Figure 5 presents the effects of the drug treatments upon DOPAC/DA (upper) and 5-HIAA/5-HT (lower) ratios obtained from limbic tissue samples. As can be seen in Fig. 5 (upper), the 8-OH DPAT treatment did not affect DOPAC/DA ratios. On the other hand, cocaine lowered and haloperidol increased DA/DOPAC ratios, as was observed in Experiment 1. 5-HIAA/5-HT ratios (lower),

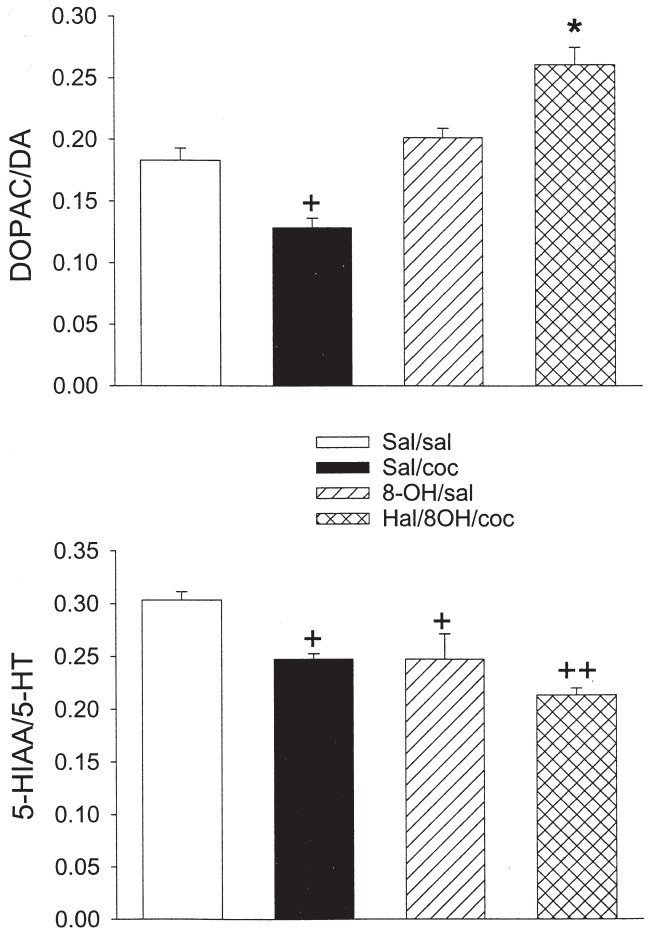

FIG. 5. Means and SEMs for DOPAC/DA (upper) and 5-HIAA/5-HT (lower) ratios obtained from limbic brain samples. Asterisk (*) denotes $p < 0.01$ ratios higher than all other groups; + denotes $p < 0.01$ ratios lower than the saline group; ++ denotes $p < 0.01$ ratios lower that all other groups.

however, were affected by 8-OH DPAT. As expected, the 8-OH DPAT reduced 5-HIAA/5-HT ratios similar to cocaine, and in the group given 8-OH DPAT + haloperidol + cocaine, the reduction was even greater, indicating an additive effect of the 8-OH DPAT and cocaine treatments upon 5-HIAA/5-HT ratios. Measurement of cocaine concentrations in the same brain tissue samples revealed that there were no statistically significant differences in cocaine concentrations among different cocaine treatment groups $p > 0.05$. In addition, there were no statistically significant differences among groups in plasma cocaine concentrations, $p > 0.05$.

EXPERIMENT 3

In the previous experiment, the 8-OH DPAT treatment was able to reverse the suppressive effects of haloperidol upon the locomotor stimulant effects of cocaine. Although the 8-OH DPAT treatment by itself did not have an effect upon locomotor activity that was different from saline treatment, it is possible the 8-OH DPAT may have directly enhanced the locomotor stimulant effect of cocaine. To assess this possibility, another experiment was conducted in which animals were treated with cocaine and 8-OH DPAT together to determine if the 8-OH DPAT treatment enhanced the locomotor stimulant effect of cocaine. All experimental procedures were the same in this experiment as they were in the previous experiments except for the drug treatment groups. In this experiment, there were four treatment groups: saline-saline, saline-cocaine (10 mg/kg), 8-OH DPAT (0.2 mg/kg)-saline, and 8-OH DPAT (0.2 mg/kg)-cocaine (10 mg/kg).

Results

Overall, the results of this study showed that the 8-OH DPAT treatment did not affect the overall locomotor activity of the saline- or cocaine-treated animals. The 8-OH DPAT treatment, however, did decrease entries in to the central zone. Figure 6 presents the locomotion distance scores (upper) and central zone entries (middle) for the four treatment groups on the fifth treatment session. As can be seen in Fig. 6, the 8-OH DPAT treatment did not modify the locomotor stimulant effect of cocaine (upper panel), but did reduce the tendency of cocaine-treated animals to enter the central zone (middle panel). A two-way ANOVA indicated that cocaine induced statistically significant effects upon locomotion, $F(3, 24) = 6.3$, $p < 0.01$, and central zone entries $F(3, 24) = 9.0$, $p < 0.01$. None of the treatment group $\times$ interval interactions were statistically significant, $p > 0.05$. The finding that the 8-OH DPAT treatment decreased entries into the central zone is similar to the observations in Experiment 2 in which the combined 8-OH DPAT, haloperidol, and cocaine treatment decreased central zone entries compared to cocaine treated animals. The bottom graph in Fig. 6 presents the central zone entries adjusted for distance traversed (central zone entries/meter). As can be seen in Fig. 6, cocaine increased the propensity to enter the central zone even when entries were adjusted for locomotor distance traveled. The 8-OH DPAT treatment effectively blocked this effect of cocaine. Although the 8-OH DPAT treatment by itself reduced the level of central zone entry rate, the effects was not statistically significant. It needs to be noted that saline-treated animals have a fairly low central zone entry rate so that it may be difficult to demonstrate a decrease in this tendency because of floor effects. Figure 7 presents the DOPAC/DA (upper) and 5-HIAA/5-HT (lower) ratios obtained from limbic brain tissue samples. As can be seen in Figure 7, cocaine reduced both

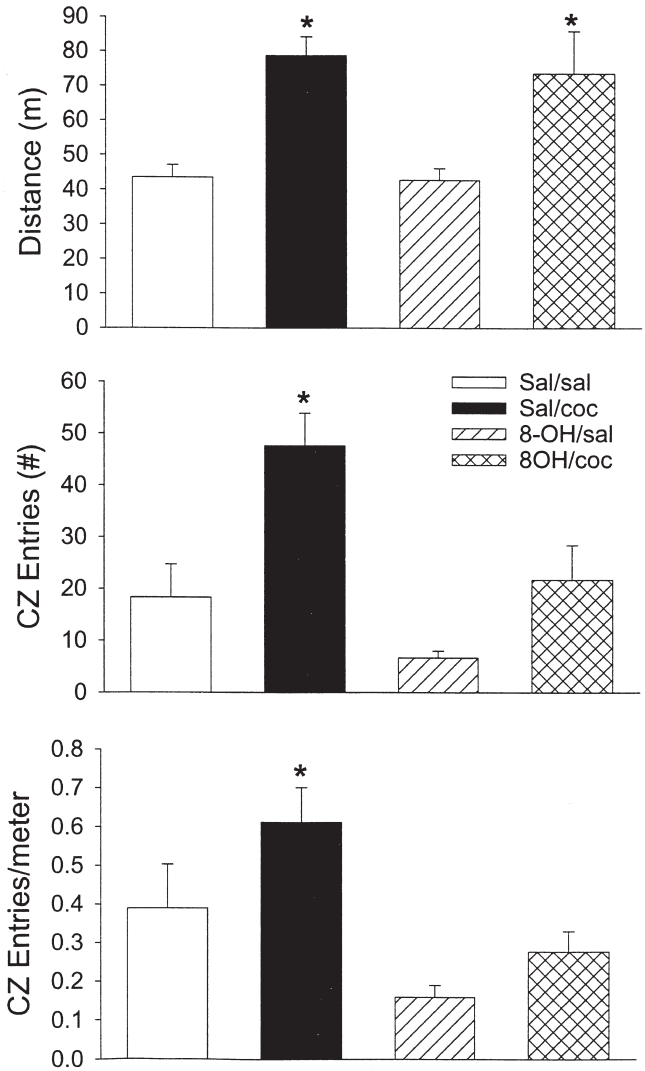

FIG. 6. Means and SEMs for session totals of locomotion distance scores (upper), central zone entries (middle), and frequency of central zone entries/meter (bottom) on the fifth day of IP injection treatments [saline, cocaine (10 mg/kg), 8-OH DPAT (0.2 mg/kg), or 8-OH DPAT (0.2 mg/kg) + cocaine (10 mg/kg)]. Asterisk (*) denotes $p <$ 0.01 scores higher than the other groups.

DOPAC/DA and 5-HIAA/5-HT ratios. This finding is consistent with the established effects of cocaine in attenuating the reuptake of DA and 5-HT, thereby increasing stimulation of DA and 5-HT autoreceptors. As expected, the effects of 8-OH DPAT were selective to 5-HT, and are consistent with the established 8-OH DPAT agonist activity at the 5-HT_{1A} autoreceptor site. As we observed in Experiment 2, the 8-OH DPAT (0.2 mg/kg) treatment exerted an autoreceptor effect equivalent to the 10 mg/kg cocaine treatment and the combined 8-OH DPAT and cocaine treatment effects appeared to be additive. As in the two previous experiments, the cocaine concentrations in the brain tissue samples were statistically equivalent for the 8-OH DPAT + cocaine and the cocaine groups, $p > 0.05$.

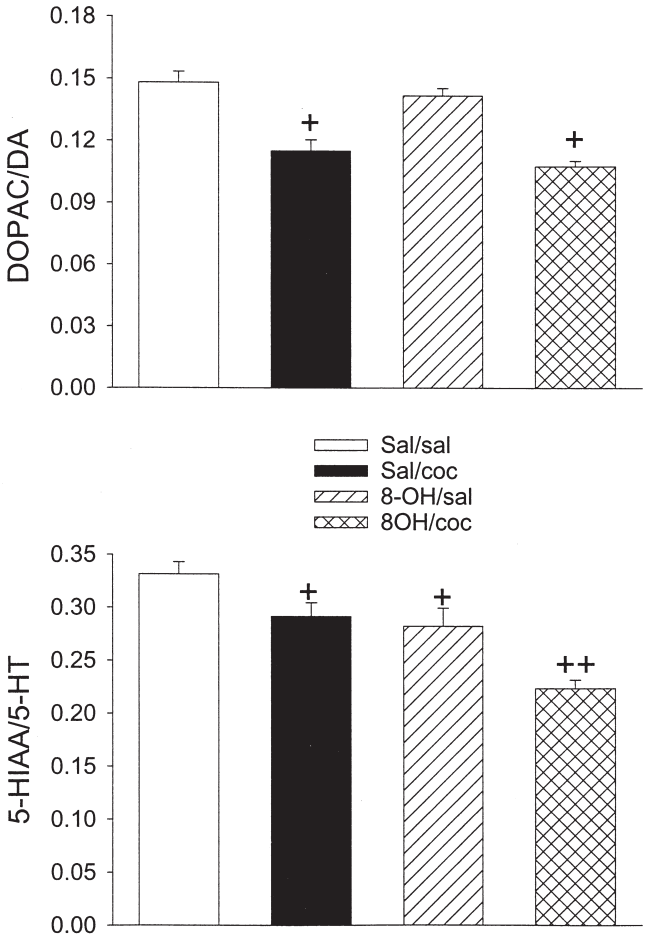

FIG. 7. Means and SEMs for DOPAC/DA (upper) and 5-HIAA/5-HT (lower) ratios obtained from limbic brain samples. + Denotes $p < 0.01$ ratios lower than the sal-sal-group. ++ denotes $p < 0.01$ ratio lower compared to all other groups.

GENERAL DISCUSSION

Unlike previous reports in which 5-HT_{1A} agonists have been found to reverse the cataleptic effects of haloperidol (1, 23), we did not find that the 5-HT_{1A} agonist, 8-OH DPAT reversed the locomotor suppressant effects of haloperidol. While the dose level of 8-OH DPAT used in the present study (0.2 mg/kg) was comparable to the doses used in previous studies, the major difference was in the dose of haloperidol used and the behavioral measure employed. In the present study, we used a 0.1 mg/kg haloperidol treatment and the behavioral measurement was spontaneous locomotor behavior. This contrasts with the use of a 1.0 mg/kg haloperidol dose and catalepsy as the behavioral measurement of the haloperidol effect. The finding that 8-OH DPAT did not reverse the locomotor retardation effect of haloperidol indicates that the efficacy of 8-OH DPAT in countering the cataleptic effects of dopamine receptor antagonism of high-dose haloperidol effects does not extend to locomotor behavior and to low-dose haloperidol effects. Thus, the idea that 5-HT_{1A} agonists might be useful in the attenuation of extrapyramidal side effects of haloperidol used in clinical situations may be questionable (28,44). The difficulty with using an anticataleptic effect as a therepeutic indicator is that it does not necessarily indicate a restoration of normative motoric function. The fact that spontaneous motor behavior is severely reduced by a dose of haloperidol, which is one-tenth of the dose used to induce catalepsy coupled with the fact that an 8-OH DPAT treatment, which is generally effective in reversing catalepsy, does not diminish the locomotion retardation of a low dose of haloperidol makes it likely that animals made cataleptic by a high dose of haloperidol still have severe motor retardation when the catalepsy is reversed by the 8-OH DPAT. Thus, a reversal of catalepsy does not imply a restoration of normative motoric function. Indeed, motoric impairments in rats can be detected with a dose of haloperidol lower than 0.1 mg/kg when operant response performance is measured (13,22).

In the present study, the 8-OH DPAT treatment itself had no effect upon locomotor behavior in terms of a distance measure, and did not prevent the locomotion retardation induced by haloperidol. Although the haloperidol plus 8-OH DPAT group had baseline locomotion levels well below saline-treated animals, cocaine-restored locomotor stimulant effects equivalent to those observed when cocaine was given by itself. This finding indicates that the cocaine interaction with haloperidol in Experiment 1 is not explicable simply in terms of haloperidol effects upon baseline locomotion. Although the overall locomotion level of the cocaine and the cocaine plus haloperidol + 8-OH DPAT groups were equivalent in terms of the distance measure, the cocaine group exhibited a substantially greater number of penetrations of the central zone. Recently (12), it has been shown that cocaine also increases the time spent in the central zone. The increased propensity of cocaine-treated animals to enter the central zone was also observed in Experiment 3 and, in addition, giving animals a combined 8-OH DPAT + cocaine treatment eliminated this effect of cocaine upon central zone penetrations. In this latter experiment, the 8-OH DPAT treatment did not modify the locomotor stimulant effect of cocaine. The behavioral significance of increased entries into the central zone of an open field remains somewhat uncertain, although there have been attempts to relate this behavior to increased stress as well as decreased anxiety (9,20,21,31,39). The fact that an increase in pharmacological agonism of the 5-HT_{1A} receptor blocks the facilitory effect of cocaine upon entry into the central zone without altering overall locomotor stimulation suggests that this added 5-HT_{1A} stimulation induced by 8-OH DPAT exerts an important effect upon the hedonic/emotive properties of cocaine. Thus, it will be important to ascertain if 8-OH DPAT would alter the capacity of cocaine to induce place preference or self-administration.

The biochemical findings were generally in line with the expectations for the drug treatments used. Studies using in vivo microdialysis have shown that cocaine and haloperidol increase extracellular dopamine, and that 8-OH DPAT has no effect upon extracellular dopamine (8,17). In ex vivo measurements, however, the predominant source of transmitter and transmitter metabolite measured is intracellular, so the impact of the drug treatments upon neurochemical measurements is different. With the drug doses and time intervals of measurement used in the present study, intracellular stores of transmitter are unaffected. Primarily, the intracellular effects of the drug treatments are detected as alterations in metabolism mediated by autoreceptor activity. Cocaine, by increasing extracellular DA and 5-HT, increases autoreceptor stimulation and thereby decreases DA and 5-HT metabolism. 8-OH DPAT is an agonist at 5-HT_{1A} autoreceptor sites as

well as postsynaptic sites, and thereby deceases 5-HT metabolism (11). Haloperidol, on the other hand, is an autoreceptor and postsynaptic receptor antagonist and, therefore, can increase intracellular dopamine metabolism (8,17,30,34). The ex vivo results obtained in the present study are consistent with these drug effects upon DA and 5-HT neurons. In attempting to account for the differences in levels of locomotor behavior in animals that received haloperidol and cocaine vs. haloperidol + 8-OH DPAT and cocaine, the biochemical findings indicate that there was an increase in 5-HT stimulation in this latter treatment group. Perhaps this increased intensity of 5-HT stimulation was sufficient to override the behavioral suppression effects of haloperidol. The biochemical findings, however, indicate that haloperidol had a similar impact upon dopamine metabolism in animals given haloperidol and cocaine vs. haloperidol + 8-OH DPAT + cocaine. This observation supports the suggestion (23) that 8-OH DPAT interacts with haloperidol at a still-to-be-determined location, but apparently not directly at the dopamine neuron.

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