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ORIGINAL INVESTIGATION

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Withdrawal from continuous cocaine administration: time dependent changes in accumbens 5-HT_3 receptor function and behavioral tolerance

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Abstract We have previously reported that continuous cocaine administration functionally down regulates 5-HT_3 receptors in the nucleus accumbens. The current experiments evaluated the duration of behavioral tolerance to cocaine and whether the duration of behavioral tolerance and 5-HT_3 receptor down-regulation co-varied. Rats were withdrawn from a pretreatment regimen (40 mg/kg/per day cocaine or 0.9% saline for 14 days) for 1, 7 or 14 days. The rats were either sacrificed, and slices from the nucleus accumbens obtained, or were exposed to behavioral rating procedures. The results indicated that continuous cocaine administration significantly attenuated the ability of mCPBG to facilitate K^+-stimulated DA release on days 1 and 7, but not day 14, of withdrawal. Furthermore, continuous cocaine administration induced behavioral tolerance to a cocaine challenge on days 1 and 7, but not day 14, of withdrawal. These results suggest that continuous cocaine administration functionally down-regulates 5-HT_3 receptors in the nucleus accumbens, and this functional down-regulation co-varies with the behavioral tolerance induced by continuous cocaine administration. Hence, a functional down-regulation of accumbens 5-HT_3 receptors may represent a partial mechanism for the tolerance following continuous cocaine administration.

Key words Continuous cocaine · Tolerance · 5-HT_3 receptor · DA release · Nucleus accumbens · Rat

Introduction

Previous research involving chronic cocaine administration clearly indicates that the continuous administration of cocaine via osmotic minipump results in tolerance to its behavioral and neurochemical effects (Reith et al. 1987; King et al. 1992, 1993, 1997; Chen and Reith 1993). The mechanisms mediating this tolerance have not been clearly established. Although much research indicates that the effects of cocaine are dependent on DA neurotransmission, several additional lines of evidence indicate that serotonin (5–HT) systems have a regulatory role in DA release and cocaine-induced behaviors. This literature includes, but is not limited to, the research of Carroll and colleagues who have evaluated the role of 5-HT in modulating cocaine self-administration (Carroll et al. 1990a, b). Cunningham and colleagues have also explored the effects of acute and chronic cocaine administration on dorsal raphe 5-HT_{1A} receptor electrophysiology (Cunningham et al. 1987, 1992; Cunningham and Lakoski 1988, 1990). Overall, this research indicates that cocaine has substantial effects on central 5-HT systems, and that 5-HT systems can modulate the effects of cocaine.

Specifically, research, both in vivo and in vitro, indicates that 5-HT_3receptors can modulate dopaminergic systems. For example, several microdialysis studies have reported 5-HT_3 agonists induced dopamine release in a variety of mesocorticolimbic areas, including the accumbens (Chen et al. 1991; Campbell and McBride 1995; Campbell et al. 1996). For example, Campbell and McBride (1995) reported that perfusion of 3.3–100 μM mCPBG, a selective 5-HT_3 receptor agonist, through the dialysis probe increased dialysate DA levels in a concentration manner. Furthermore, this release was Ca^{2+} dependent and blocked by local perfusion of 100 μM ICS 205–930, a 5-HT_3 receptor antagonist. The results of in vitro experiments are generally consistent with the microdialysis experiments (e.g., Blandina et al. 1988, 1989; Benuck and Reith 1992; Schmidt and Black 1989). Lastly, two reports indicate that the 5-HT_3 receptor antagonists MDL 72222 (Kankaanpaa et al. 1996) and zacopride (McNeish et al. 1993) can attenuate

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cocaine-induced increases in accumbens DA levels, as assessed by in vivo microdialysis. These results suggest that 5-HT_3 receptors may be important in the chronic effects of cocaine.

We have previously reported that continuous cocaine administration alters the ability of 5-HT_3 receptors to regulate behavior and DA release (e.g., King et al. 1994, 1995, 1997; Matell and King 1997). For example, we reported that continuous cocaine administration attenuates the ability of the 5-HT_3 agonist, mCPBG, to induce DA release in the caudate-putamen (King et al. 1995), and the nucleus accumbens (Matell and King 1997) on day 7 of withdrawal from continuous cocaine administration (40 mg/kg per day). Furthermore, we reported that ondansetron co-administered with continuous cocaine inhibits the development of behavioral tolerance produced by continuous cocaine administration (King et al. 1997). The overall pattern of results strongly suggests a role for 5-HT_3 receptors in the effects of withdrawal from continuous cocaine and the tolerance associated with this dosing regimen.

The current program of research is based on the general hypothesis that continuous cocaine administration functionally down-regulates accumbens 5-HT_3 receptors, and that such down-regulation represents a (partial) mechanism of cocaine tolerance. Continuous cocaine administration is postulated to induce a functional down-regulation of 5-HT_3 receptors in the following manner. The continuous infusion of cocaine results in prolonged, elevated synaptic levels of 5-HT due to the 5-HT uptake inhibiting properties of cocaine. These increased synaptic levels of 5-HT result in sustained occupancy of 5-HT_3 receptors over the continuous infusion period. Because of sustained receptor occupancy, 5-HT_3 receptors would presumably be down regulated or desensitized as a neuroadaptation to counteract the excitatory effects of 5-HT_3 receptor activation. Such decreased stimulatory abilities of 5-HT_3 receptors would contribute to the behavioral and neurochemical tolerance produced by the continuous infusion of cocaine because the "normal" stimulatory effects of 5-HT_3 receptor activation on DA release would be attenuated or eliminated. To the extent that this hypothesis is correct, then the presence of behavioral tolerance and 5-HT_3 receptor down-regulation should wax and wane together.

The present study evaluated several issues. The first issue was to replicate our finding of 5-HT_3 receptor down-regulation in the accumbens following continuous cocaine administration. The second issue is the duration of the functional down-regulation of accumbens 5-HT_3 receptors. For example, does the down-regulation persist for an extended period of time, or does it dissipate after a short withdrawal period? Third, what is the duration of behavioral tolerance induced by continuous cocaine administration? Lastly, do the functional down-regulation of accumbens 5-HT_3 receptors and behavioral tolerance wax and wane together?

Materials and methods

General methods

Subjects

Male Sprague-Dawley rats weighing 125–150 g (Charles River Laboratories), were acclimated to the vivarium (12-h light/dark cycle, light on at 7 a.m.) for 1 week. They were maintained on free-food and water, and were housed in pairs. Terminal weights ranged from 275–325 g.

Drugs

Cocaine HCL (received from NIDA) was dissolved in 0.9% saline. 1-(m-Chlorophenyl-biguanide HCL (RBI Inc.) was dissolved in distilled water. All doses were calculated as the salt.

Minipump preparation

Alzet Osmotic pumps (model 2ML2 Alza Corporation) were filled with 2.5 ml of either 100 mg/ml cocaine HCL or isotonic (0.9%) saline. The pumps were slightly modified by adding a microdialysis fiber to the output portal to eliminate tissue necrosis from the cocaine (Joyner et al. 1993). The infusion rate for the cocaine was 5 µl/h resulting in an overall dose of 40 mg/kg per day cocaine. The pumps were primed by warming in a warm water bath (37°C) for 4 h before pump implantation.

Surgery

Rats were anesthetized briefly by inhalation with methoxyflurane (Metofane). They were then shaved along the dorsal midline and injected with 0.1 cc lidocaine (Abbot) proximal to the incision site. A 2-cm incision was made with scissors and a large subcutaneous pocket was made with the scissors. The minipumps were inserted into the pocket with the delivery portal towards the head and the incision closed with surgical autoclips. Removal of the minipumps entailed the identical procedure. The amount of residual cocaine solution was measured. Subjects that had more than 10% of the drug remaining in the pump were discarded from the study.

Pretreatment regimen

The cocaine pretreatment was for a 14-day period. On day 1 of treatment animals were either: (1) implanted with 2ML2 Alzet minipumps continuously infusing cocaine at a rate of 40 mg/kg per day (continuous administration group), or (2) implanted with 2ML2 Alzet minipumps continuously infusing saline (saline control group).

Neurotransmitter release methods

Slice preparation

One, 7, or 14 days after the removal of the minipump, the rats were decapitated and the brains removed over ice. Brain slices 1 mm thick containing only the nucleus accumbens were obtained over ice using a brain block. The slices were then placed in a superfusion chamber and perfused with Krebs buffer (124 mM NaCl, 1 mM KCl, 1.24 mM KH_2PO_4, 1.3 mM $MgSO_4$, 26 mM $NaHCO_3$, 2.4 mM

$CaCl_2$, 10 mM glucose, saturated with 95% O_2/5% CO_2: pH 7.4) at a flow rate of 1 ml/min. Slices were maintained at 35°C, and the buffer continually aerated with a mixture of 95% O_2/5% CO_2; pH 7.4.

Sample collection

After a 40-min incubation period, three 2-min samples were collected. The perfusion medium was then changed to a high K^+ Krebs buffer (124 mM NaCl, 25 mM KCl, 1.24 mM KH_2PO_4, 1.3 mM $MgSO_4$, 26 mM $NaHCO_3$, 2.4 mM $CaCl_2$, 10 mM glucose, saturated with 95% O_2/5% CO_2: pH 7.4) in order to stimulate DA release, and another three 2-min samples collected. The perfusion medium was then changed back to the original Krebs buffer and three more samples collected. This sequence of events (3 baseline, three K^+-stimulated, three post-K^+ samples) constituted the S1 period. At this point, Krebs buffer containing 25 μM mCPBG was substituted for the agonist-free perfusion medium. After a 20-min incubation period, the collection procedures were repeated (three baseline, three K^+-stimulated, three post-K^+). This sequence of events, in the presence of 25 μM mCPBG, constituted the S2 period. For the experiments evaluating the Ca^{2+} dependency of mCPBG enhancement of K^+ stimulated DA release, the Krebs buffer contained 25% of the normal Ca^{2+} levels (i.e., 0.6 mM) throughout the entire experiment, and the sequence of events was identical to that for the high Ca^{2+} experiments. Because the design of the experiment uses two stimulation periods (S1 and S2), and one dependent measure is the S2:S1 ratio, Ca^{2+} was not completely eliminated from the perfusion buffer, so that there was some DA released during both stimulation periods.

Sample preparation

As each sample was collected 400 μl of Krebs buffer containing 2.5 mM EDTA, 0.5% sodium azide, and the internal standard epinine (final concentration 50 pg per 200 μl sample), were added to each sample collection tube. The samples were then transferred to autosampler vials and placed in a ThermoSeparations AS 100 refrigerated autosampler for analysis.

Chromatography

Perfusate concentrations of DA and DOPAC were determined by HPLC using electrochemical detection. The chromatographic system consisted of a ThermoSeparations P1000 solvent delivery system, a Keystone Octyl/B 5 micron particle (150 × 4.6 mm) column and a ThermoSeparations AS 100 refrigerated autosampler with a 200 μl sample loop. Electrochemical detection of DA and DOPAC were achieved with an ESA Coulocomb II detector, with a 5021 conditioning cell and a model 5011 analytic cell. The chromatography method consisted of setting the potentials of the conditioning cell at +450 mV, the analytical cell at detector 1 (E1) at + 100 mV, and detector 2 (E2) at −340 mV. Chromatograms were recorded from E2, whose sensitivity was set at 10 nA full scale detection, with a time constant of 5 s. The mobile phase consisted of 0.1 M KOAc, 0.05 M citric acid, 0.0005 M EDTA, 0.0008 M C_8SO_4, 3% glacial acetic acid, and 4% acetonitrile. Chromatograms were recorded using a IBM compatible computer and ThermoSeparations LCTalk chromatography software.

Data analysis

There were six subjects, per pretreatment group, per withdrawal day, per Ca^{2+} level, where each subject is the average of two slices: slices from the left and right accumbens were run in separate chambers and data collected for each chamber. These two data sets, for a single subject were then averaged to form the data for a single subject for subject analysis. This method was used, instead of simply treating each slice as an independent observation, so that within and between subjects factors were not intermixed. The main dependent measure was the amount of DA present for each sample, separately for pretreatment group and mCPBG concentration. The data were analyzed by standard ANOVAs, with specific differences analyzed by post-hoc *t*-tests. Dependent measures included baseline DA levels, the ratio of DA release in the presence of 25 μM mCPBG to DA release in the absence of any mCPBG [the S2:S1, where DA levels were calculated as the area under the curve (AUC) using PeakFit 4.1 (Jandel Scientific)]. The significance level was set at $P \leq 0.05$ for all comparisons.

Table 1 Modified Ellinwood and Balster (1974) rating scale

Rating	Category	Definition	Ancillary scale
1	Asleep	Lying down, eyes closed	
2	Inactive	Lying down, eyes partially open	Note head position Note eye position Note low-frequency behaviors
3	In place activities	Normal grooming or sniffing	Note body part being groomed
4	Normal alert activities	Investigatory movement, sniffing, or rearing	Note number of rears
5	Hyperactive	Rapid jerky movements or running	
6	Slow patterned movements	Repeated exploration of cage at normal activity levels	
7	Fast patterned movements	Repeated exploration of cage with rapid intense movements	
8	Restricted movements	Gnawing and chewing stereotypies	
9	Intermittent licking stereotypy		
10	Constant licking stereotypy		
11	Hyper-reactive	The following behaviors are noted/counted: JE– intense jerky hyperactive movement JU – jumping (popcorn like movements) DI – disjunctive behaviors OR – obstinate regression (backing up)	

Behavioral methods

Behavioral testing

On days 1, 7, or 14 following pre-treatment, the animals were acclimated to the test room in their home cage for 30 min under normal light conditions. The animals were then transferred to test cages, and allowed to acclimate to the test cages for an additional 30 min. The test cages were standard, clear plastic laboratory animal housing cages, 28 × 18 × 12 cm, with another cage taped, upside down, in place on top. The top cage had five air holes drilled uniformly on either side. Six of these test cages were placed in a row 12 inches apart. A modified version of the Ellinwood and Balster Rating Scale (1974) was used (Table 1). All subjects received a 15.0 mg/kg IP cocaine injection. A single experienced rater, performed all behavior ratings. The rater was blind to the subject types for each experimental session. A rating was given to each of the animals at 5 min preinjection and at 5-min intervals thereafter for 60 min. The observation period was for 20 s with 10 s between cages.

Data analysis

Different rats were treated and used for the behavioral experiments than those used in the slice experiments. The current experiment is a mixed model design. Specifically, there were two group factors (Cocaine pretreatment condition and withdrawal period) that produces six separate groups (two pretreatment groups × three withdrawal periods), and one repeated measures factor (Time). There were ten rats per group. In the current experiment the subject types (continuous administration and saline control) were randomized according to a Latin Square design. Because the behavior ratings are ordinal data, the group differences were analyzed by Kruskall-Wallis analyses of variance on ranks. Differences between the two groups are determined by non-parametric Mann-Whitney tests. The significance level is set at $P \leq 0.05$ for all comparisons.

Results

Neurotransmitter release experiment

Potassium-stimulated DA release

Figure 1 presents mean DA levels during the S1 and S2 periods, separately for each withdrawal day. The left-hand panels present the data from the normal Ca^{2+} conditions, while the right-hand panels present the data from the low Ca^{2+} conditions. A three-way ANOVA on the AUCs from the S1 period indicated that the main effects of Pretreatment group and Withdrawal day were not significant (Pretreatment group: $F1,60 = 0.02$; Withdrawal day: $F2,60 = 1.83$). However, the main effect of Ca^{2+} was significant (Ca^{2+} level: $F1,30 = 49.09$). No interaction was significant (Pretreatment group × Ca^{2+} level: $F2,60 = 0.31$; Pretreatment group × Withdrawal day: $F2,60 = 0.34$; Pretreatment group × Withdrawal day × Ca^{2+} Level: $F2,60 = 0.24$). This pattern of results indicates that there were no significant differences between the two pretreatment groups in the amount of K^+ stimulated DA release during the S1 period. Furthermore, the K^+ stimulated DA release was Ca^{2+} dependent.

Effects of mCPBG on basal DA levels

The results presented in Fig. 1 suggest that mCPBG increased basal DA levels during the S2 period. Figure 2 presents the differences between the mean baseline DA levels in the S1 and S2 periods. Averaging the three baseline DA levels in the S1 and S2 periods, separately for each withdrawal day, and then taking the difference between the two averages determined the differences (i.e., mean S2 minus mean S1). To evaluate whether mCPBG increased basal DA levels during the S2 period, a three-way ANOVA was conducted on the differences. In this ANOVA, Pretreatment group was one factor, Withdrawal day was a second factor, and Ca^{2+} level was the third factor. The results of the ANOVA indicated that the main effects of Pretreatment group and Withdrawal day were not significant (Pretreatment group: $F1,60 = 2.44$; Withdrawal day: $F2,60 = 0.1$). However, the main effect of Ca^{2+} was significant (Ca^{2+} level: $F1,30 = 54.59$). No interaction was significant (Pretreatment group × Ca^{2+} level:

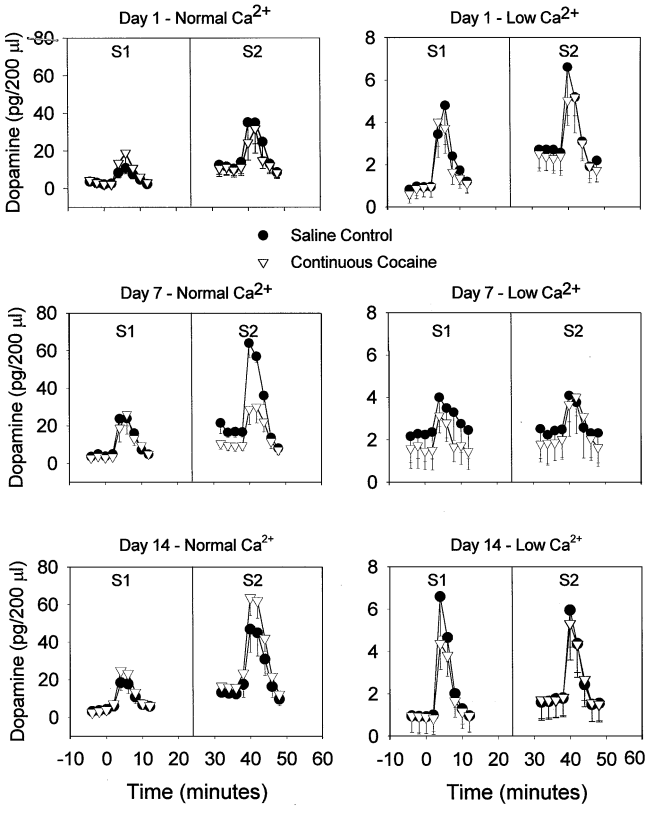

Fig. 1 K^+ stimulated DA release in the absence (*left portion of each panel*) and presence (*right portion of each panel*) of 25 µM mCPBG, separately for each withdrawal day, and Ca^{2+} level (2.4 or 0.6 mM). The *filled circles* (●) represent the saline control subjects. The *unfilled inverted triangles* (▽) represent the continuous cocaine subjects. The data are the means ± SEM. The *bars* represent 1 SEM. For all conditions, there are six subjects per pretreatment group per withdrawal day. Each subject is the mean of two slices (left and right nucleus accumbens) which were run independently, in separate perfusion chambers

$F2,60 = 1.89$; Pretreatment group × Withdrawal day: $F2,60 = 1.05$; Pretreatment group × Withdrawal day × Ca^{2+} level: $F2,60 = 0.82$). This pattern of results indicates that there were no significant differences between the two pretreatment groups in the amount that mCPBG increased DA levels during the S2 period. Furthermore, the effect of mCPBG on basal DA levels was Ca^{2+} dependent.

mCPBG and K+ stimulated DA release

Because the results presented in Fig. 2 indicated that mCPBG increased basal DA levels in a Ca^{2+} dependent manner, the raw data were normalized. The normalization was conducted only for the high K^+ and post K^+ samples only, and does not include the data from the baseline samples. The data for each subject in the S1 and S2 period was normalized relative to the minimum and maximum amount of DA in a sample during the high K^+ and post-K^+ samples only, during the S1 period, according to the following formula:

$$(DA_{sample} - DA_{minS1})/(DA_{maxS1} - DA_{minS1}).$$

In other words, the normalization formula does not include the baseline samples from either the S1 or S2 periods, as the results of the normalization would be strongly affected by the effects of mCPBG on basal DA levels. The current normalization formula factors out such effects by only using the K+ and post-K+ samples only. Thus, one can assess the effects of mCPBG on K^+stimulated DA release while factoring out the effects of mCPBG on basal DA levels.

Figure 3 presents the S2:S1 DA ratio for each withdrawal day, where the DA levels are represented by the areas under the curve from the normalized data. Figure 3 allows one to determine whether there are differences between the pretreatment groups at the withdrawal

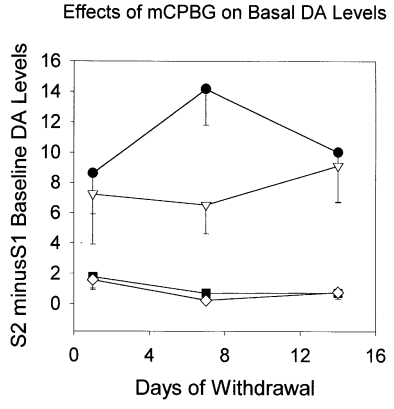

Fig. 2 Effects of 25 µM mCPBG on basal DA levels, for each pretreatment group and Ca^{2+} condition (2.4 or 0.6 mM). The data are the differences between the mean basal DA levels in the S2 and S1 periods. The differences between the mean baseline DA levels in the S1 and S2 periods were calculated in the following manner. Averaging the three baseline DA levels in the S1 and S2 periods, separately for each withdrawal day, and then taking the difference between the two averages determined the differences (i.e., mean S2 minus mean S1). The *filled circles* (●) represent the saline control subjects during the normal Ca^{2+} conditions. The *unfilled inverted triangles* (▽) represent the continuous cocaine subjects during the normal Ca^{2+} conditions. The *filled squares* (■) represent the data from the saline control subjects during the low Ca^{2+} conditions. The *unfilled diamonds* (◇) represent the data from the continuous cocaine subjects during the low Ca^{2+} conditions. The data are the means ± SEM. For all conditions, there are six subjects per pretreatment group per withdrawal day. Each subject is the mean of two slices (left and right nucleus accumbens) which were run independently, in separate perfusion chambers

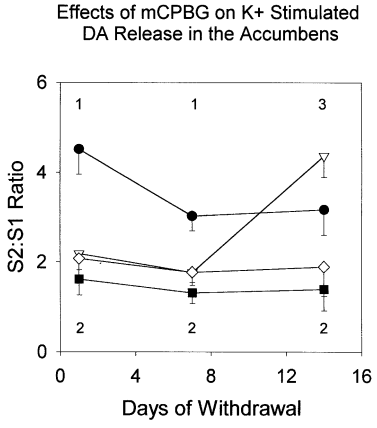

Fig. 3 Effects of 25 µM mCPBG on DA release stimulated by 25 mM K^+, for each pretreatment group and Ca^{2+} condition (2.4 or 0.6 mM). The data are presented as the S2:S1 ratios on the normalized data. The ratios were calculated in the following manner. For each subject, the AUC was determined, separately for the S1 and S2 periods. The AUC was calculated based on the three K^+ and three post-K^+ samples (i.e., the AUCs do not include the three baseline samples). The ratio for each subject was then determined. The data for each subject, in the S1 and S2 period, were normalized relative to the minimum and maximum amount of DA in a sample during the S1 period, where the minimum and maximum were selected from only the K^+ and post-K^+ samples, according to the following formula:

$$(DA_{sample} - DA_{minS1})/(DA_{maxS1} - DA_{minS1}).$$

This allows one to assess the effects of mCPBG on K^+ stimulated DA release while factoring out the effects of mCPBG on basal DA levels. The *filled circles* (●) represent the saline control subjects during the normal Ca^{2+} conditions. The *unfilled inverted triangles* (▽) represent the continuous cocaine subjects during the normal Ca^{2+} conditions. The *filled squares* (■) represent the data from the saline control subjects during the low Ca^{2+}conditions. The *unfilled diamonds* (◇) represent the data from the continuous cocaine subjects during the low Ca^{2+} conditions. The data are the means ± SEM. The *bars* represent 1 SEM. For all conditions, there are six subjects per pretreatment group per withdrawal day. Each subject is the mean of two slices (left and right nucleus accumbens) which were run independently, in separate perfusion chambers. The *number 1* indicates that, for the normal Ca^{2+} conditions, the saline control and continuous cocaine subjects are significantly different as determined by post-hoc Tukey's tests. The *number 2* indicates that, for the saline control group, the normal and low Ca^{2+} conditions are significantly different as determined by post-hoc Tukey's tests. The *number 3* indicates that, for the continuous cocaine group, the normal and low Ca^{2+} conditions are significantly different, as determined by post-hoc Tukey's tests

periods. A two-way ANOVA evaluated whether there were differences between the pretreatment groups in the ability of mCPBG to enhance K^+ stimulated DA release. Pretreatment group was one factor, and Withdrawal day was the second factor. The results of the ANOVA indicated that both main effects, as well as the interaction were significant (Pretreatment group: $F1,30 = 5.02$; Withdrawal day: $F2,30 = 5.19$; Pretreatment group × Withdrawal day: $F2,30 = 8.60$). The results of post-hoc Tukey's tests indicate that the saline control group is significantly higher than the continuous cocaine group on days 1 ($q = 5.34$) and 7 ($q = 2.90$), but not day 14 ($q = 2.75$) of withdrawal.

Behavioral experiment

Figure 4 presents median cocaine-induced behavior ratings for each pretreatment group separately for the different withdrawal days. The top panel presents the data for withdrawal day 1, the middle panel presents the data for withdrawal day 7, and the bottom panel presents the data for withdrawal day 14. The results of the Mann-Whitney tests indicated that continuous cocaine administration induces tolerance on days 1 and 7, but not day 14 of withdrawal from continuous cocaine administration.

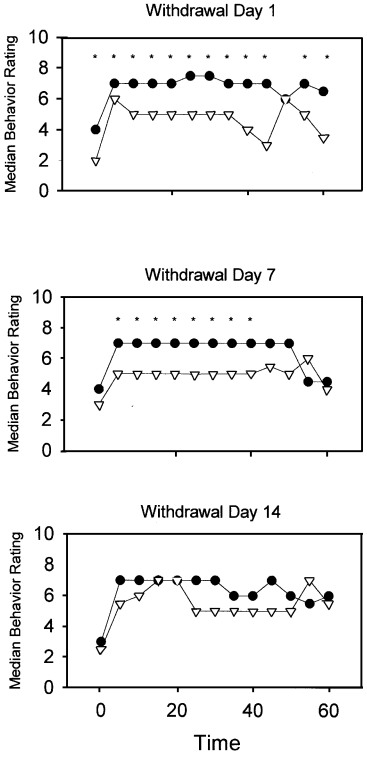

Fig. 4 Median behavior rating induced by a 15.0 mg/kg cocaine challenge. The *top panel* presents the results on day 1 of withdrawal from continuous cocaine and saline administration. The *middle panel* presents the results on day 7 of withdrawal from continuous cocaine and saline administration. The *bottom panel* presents the results on day 14 of withdrawal from continuous cocaine and saline administration. The *filled circles* (●) represent the saline control subjects. The *unfilled inverted triangles* (▽) represent the continuous cocaine subjects. The *asterisks* indicate significant differences between the two groups, at a specific time point, as determined by a non-parametric Mann-Whitney *U*-test. The significance level was set at $P \leq 0.05$. For this experiment, there are ten subjects per pretreatment group, per withdrawal day for a total of 60 subjects

Discussion

The results of the present study show that perfusion of nucleus accumbens slices with mCPBG, a 5-HT_3 agonist, enhances K^+ stimulated DA release. This result replicates previous findings that 5-HT_3 receptors can modulate DA release in nucleus accumbens (Chen et al. 1991; Campbell and McBride 1995), as well as our previous report demonstrating a functional down-regulation of accumbens 5-HT_3 receptors following continuous cocaine administration (Matell and King 1997). Furthermore, allowing for the fact that only one concentration of mCPBG was run, the data suggest this down-regulation was present on days 1 and 7, but not day 14, of withdrawal from continuous cocaine administration. Additionally, the behavioral data suggest that the behavioral tolerance induced by continuous cocaine administration is present on days 1 and 7, but not day 14, of withdrawal from continuous cocaine administration. Thus, the overall pattern of results seems to suggest that behavioral tolerance and accumbens 5-HT_3 receptor down-regulation co-exist in time.

Effects of mCPBG on basal DA release

The results presented in Fig. 2 indicate that there are no differences between the pretreatment groups in the effects of mCPBG on basal DA levels. However, if the effects of mCPBG were receptor mediated, then one would predict that there should be differences between the pretreatment groups. Thus, this result does not seem to be consistent with our hypothesis that continuous cocaine administration induces a functional down-regulation of 5-HT_3 receptors. However, we have argued that an analysis of basal conditions may not be an adequate assessment of the mechanisms mediating tolerance. The mechanisms mediating tolerance are likely to manifested when an increased demand is put on the system and the system will no longer be able to compensate for the demand. For example, our previous research has not indicated any differences in basal activity (e.g., King et al. 1992, current experiment), or basal DA levels (e.g., King et al. 1993, current experiment) between saline and cocaine treated subjects. However, when these subjects are challenged in some manner, substantial differences in both behavior and

neurotransmitter release is observed. In further support of this argument is the literature that 5-HT_3 receptors are not tonically active, and modulate dopaminergic systems only under conditions of stimulated dopaminergic activity (see Grant 1995, for a more complete discussion of this issue). Thus, the failure to find differences between the pretreatment groups in the effects of mCPBG on basal DA levels is not necessarily inconsistent with the general hypothesis.

Mechanisms of mCPBG enhancement of K^+-stimulated DA release

The mechanism(s) of mCPBG's ability to modulate DA release are unclear, although several, not necessarily exclusive possibilities exist. One mechanism that has been proposed is that 5-HT_3 receptors located on neurons regulate neurotransmitter release. Several lines of evidence support this hypothesis. First, neuroblastoma and neuroblastoma-glioma, but not C_6 or primary cultures of glial cells, can be labeled with 5-HT_3 selective ligands (see Laporte et al. 1992, for a review of these studies). Second, 5-HT_3 receptor agonists induce DA release in a variety of areas including the accumbens (Chen et al. 1991; Campbell et al. 1995), and hypothalamus (Blandina et al. 1990). For example, Campbell and McBride (1995) reported that perfusion of mCPBG through the dialysis probe increased dialysate DA levels in a concentration dependent manner. Furthermore, this release was Ca^{2+} dependent and blocked by local perfusion of ICS 205–930, a 5-HT_3 receptor antagonist.

Our current results in the saline control subjects (and day 14 of withdrawal in the continuous cocaine subjects) are generally consistent with the hypothesis that neuronally located 5-HT_3 receptors mediating neurotransmitter release. First, perfusion of accumbens slices with mCPBG enhanced K^+ stimulated release. Second, this enhancement of release was calcium dependent: reducing the Ca^{2+} levels in the perfusion buffer to 25% of normal levels eliminated the ability of mCPBG to enhance K^+ stimulated DA release. Lastly, in a previous experiment (Matell and King 1997), we reported that perfusion of accumbens slices with ICS 205–930 eliminated the ability of mCPBG to enhance K^+ stimulated DA release. These results are consistent with other research indicating that 5-HT_3 receptors exert their effects when dopaminergic systems are driven by some pharmacologic stimulus (see e.g., Costall et al. 1987; Grant 1995, for reviews of this literature).

A second proposed mechanism for mCPBG's mode of action is its ability to block the DA transporter (DAT). For example, Campbell et al. (1995) recently reported that mCPBG displaced [^{3}H]GBR 12935 in a biphasic manner in both the striatum (IC_{50}: high affinity = 0.4 ± 0.2 μM, low affinity = 34.8 ± 5.6 μM) and nucleus accumbens (IC_{50}: high affinity = 2.0 ± 0.6 μM, low affinity = 52.7 ± 15.9 μM). Furthermore, mCPBG inhibited [^{3}H]DA reuptake into synaptosomes in both the striatum (IC_{50} = 5.1 ± 0.3 μM) and nucleus accumbens (IC_{50} = 6.5 ± 0.4 μM). These results therefore suggest that part of the mechanism of mCPBG's action is blockade of the DAT.

Although blockade of the DAT by mCPBG may have contributed to increased DA levels, it is unlikely that this mechanism accounts for the differences between the two pretreatment groups in mCPBG's ability to enhance K^+ stimulated DA release. The effects of mCPBG on baseline DA levels in the saline and continuous cocaine controls were not different. However, there were significant differences in the ability of mCPBG to enhance K^+ stimulated DA release. Lastly, there were no differences between the pretreatment groups in the effects of mCPBG on K^+ stimulated DA release under the low Ca^{2+} conditions. Thus, if the effects of mCPBG were largely mediated by mCPBG's action at the DAT, then there should not have been any differences between the two pretreatment groups. Furthermore, the effects of mCPBG on basal DA levels were Ca^{2+} dependent: reduction of Ca^{2+} in the perfusion medium reduced the ability of mCPBG to enhance basal DA levels. This result suggests that the effects of mCPBG on basal DA levels are receptor mediated, and not due to blockade of the DAT.

The overall pattern of results thus suggests that continuous cocaine administration functionally down-regulates accumbens 5-HT_3 receptors for at least 7 days following cessation of cocaine administration. However, by day 14 of withdrawal, these receptors have recovered, and exert their normal effects following a cocaine challenge.

Desensitization and tolerance

The overall pattern of results from the current and our previous experiments support the hypothesis that continuous cocaine administration down-regulates 5-HT_3 receptors, and that this down-regulation represents a significant contribution to behavioral tolerance. Continuous cocaine administration induces tolerance to its behavioral effects (Reith et al. 1987; King et al. 1992), and a functional down-regulation of accumbens 5-HT_3 receptors (current study; Matell and King 1997). In spite of the limitations of the current experiment, the data suggest that the behavioral tolerance and functional down-regulation co-vary in time. When behavioral tolerance is present, 5-HT_3 receptors are down-regulated, and when behavioral tolerance is not present, 5-HT_3 receptors are not down-regulated.

It is not clear if the functional down-regulation is due to a decrease in the number or affinity of 5-HT_3 receptors (which could be determined by receptor binding experiments), or due to an increased desensitization of 5-HT_3 receptors. Evidence suggests that 5-HT_3

receptors undergo rapid desensitization. For example, Yakel et al. (1990) and Yakel and Jackson (1988) reported that the electrophysiological responses mediated by 5-HT_3 receptor activation rapidly desensitize with the continuous application of 5-HT. Hence, continuous cocaine administration may produce a more rapid rate of desensitization of 5-HT_3 receptors. Further research should examine these two possibilities.

In summary, the current results indicate that continuous cocaine administration induces: (1) behavioral tolerance on days 1 and 7, but not day 14, of withdrawal, and (2) a functional down-regulation of accumbens 5-HT_3 receptors on days 1 and 7, but not day 14, of withdrawal. Thus, behavioral tolerance and accumbens 5-HT_3 receptor down-regulation seem to co-vary in time. This pattern of results suggests that the down-regulation of accumbens 5-HT_3 receptors represents a partial mechanism for the behavioral tolerance induced by continuous cocaine administration.

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