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Blockade of accumbens 5-HT_3 receptor down-regulation by ondansetron administered during continuous cocaine administration

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Abstract

The present experiment examined whether ondansetron, co-administered with continuous cocaine, would block the down regulation of accumbens 5-HT_3 receptors. Rats were exposed to a 14-day pretreatment regimen that involved the continuous infusion of 40 mg kg^{-1} day^{-1} cocaine or 0.9% saline via a subcutaneously implanted osmotic minipump. In addition to the continuous cocaine or saline administration, all subjects received daily subcutaneous (s.c.) injections of either vehicle or 0.1 mg kg^{-1} ondansetron for the entire 14-day pretreatment regimen. The rats were then withdrawn from this pretreatment regimen for seven days, and slices from the nucleus accumbens obtained. The slices were perfused with 25 mM K^+ in the absence and presence of 0, 12.5, 25, or 50 μM *m*-Chlorophenyl-biguanide HCl (mCPBG). The efflux samples were assayed for dopamine content by high pressure liquid chromatography (HPLC) with electrochemical detection. Continuous cocaine administration significantly attenuated the ability of mCPBG to facilitate K^+-induce dopamine overflow compared to saline control rats. In addition, the rats that received ondansetron and cocaine during the 14-day pretreatment period, the ability of mCPBG to enhance K^+ stimulated dopamine release was not significantly different from the saline control subjects. For all groups except the cocaine alone group, the effects of mCPBG on K^+ stimulated dopamine release were Ca^{2+} dependent, suggesting that these effects are receptor mediated. These results suggest that continuous cocaine administration functionally down-regulates 5-HT_3 receptors in the nucleus accumbens, and that this down-regulation can be blocked by chronic ondansetron administration. Hence, a functional down regulation of accumbens 5-HT_3 receptors represents a significant contribution to the tolerance induced by continuous cocaine administration.

Keywords: Cocaine, continuous; Tolerance; 5-HT_3 receptor; Dopamine release; Nucleus accumbens; (Rat)

1. Introduction

Previous research involving chronic cocaine administration clearly indicates that the continuous administration of cocaine via osmotic minipump induces tolerance to its behavioral and neurochemical effects (Reith et al., 1987; King et al., 1992, 1993; Chen and Reith, 1993). Behavioral tolerance is determined by decreased locomotor activity (measured with activity monitors) and/or a decrease in the presence of stereotypes (as measured by the Ellinwood and Balster rating scale), following a cocaine challenge. Tolerance to the neurochemical properties of cocaine is determined by a decreased ability of cocaine to enhance synaptic dopamine levels. The contribution of different mechanisms mediating this tolerance has not been clearly established.

Although much research indicates that the effects of cocaine are dependent on dopamine neurotransmission, several additional lines of evidence indicate that serotonin (5-hydroxytryptamine, 5-HT) systems have a regulatory role in dopamine release and cocaine-induced behaviors. This literature includes the research of Carroll and colleagues who have evaluated the role of 5-HT in modulating cocaine self-administration (Carroll et al., 1990a,b), and Cunningham and colleagues who have 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.

Both in vivo and in vitro research indicates that 5-HT_3 receptor mechanisms can modulate dopaminergic systems (Blandina et al., 1988, 1989; Benuck and Reith, 1992). Several microdialysis studies have reported that 5-HT_3 receptor agonists induce dopamine release in a variety of

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mesocorticolimbic areas, including the nucleus accumbens (e.g., Chen et al., 1991; Campbell and McBride, 1995). For example, Campbell and McBride (1995) reported that perfusion of 3.3–100 μM m-Chlorophenyl-biguanide HCl (mCPBG), a selective 5-HT_3 receptor agonist, through the dialysis probe increased dialysate dopamine levels in a concentration dependent 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.

Our current research is based on the hypothesis that the tolerance induced by continuous cocaine administration is significantly mediated by a down-regulation of 5-HT_3 receptors. First, continuous cocaine administration should result in prolonged, elevated synaptic levels of 5-HT, due to the 5-HT uptake inhibiting properties of cocaine. The increased synaptic levels of 5-HT would result in prolonged occupancy of 5-HT_3 receptors over the continuous infusion period. This would have the effect of further increasing synaptic dopamine levels because of the dopamine releasing effects of 5-HT_3 receptor activation described above. Because of prolonged receptor occupancy, 5-HT_3 receptors would presumably be down regulated or desensitized as a neuroadaptation to counteract the excitatory effects of prolonged 5-HT_3 receptor occupancy. Such decreased stimulatory abilities of 5-HT_3 receptors would contribute to the behavioral and dopaminergic tolerance produced by the continuous infusion of cocaine because the 'normal' stimulatory effects of 5-HT_3 receptor activation on dopamine release would be attenuated or eliminated.

Our previous research is consistent with this hypothesis (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 receptor agonist, mCPBG, to induce dopamine 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. These results indicate that continuous cocaine administration does, indeed, functionally down regulate 5-HT_3 receptors. Furthermore, we reported that ondansetron, co-administered with continuous cocaine, blocks the development of behavioral tolerance (King et al., 1997). Steketee and Crissman (1997) have recently supported these results by reporting that the local application of ICS-205-930 and LY-278-584 into the ventral tegmental area blocked the development of sensitization to systemically administered cocaine.

These results suggest that blockade of behavioral tolerance may be associated with a blockade of the down-regulation of 5-HT_3 receptors. The present study evaluated two areas of concern. The first was to replicate our previous finding of an attenuation of the ability of 5-HT_3 receptor agonists to induce dopamine release following continuous cocaine administration (i.e., replicate the finding of 5-HT_3 receptor down-regulation). The second concern was that since ondansetron blocks the development of behavioral tolerance to cocaine (King et al., 1997), can ondansetron also block the development of functional 5-HT_3 receptor down-regulation in the accumbens? If ondansetron can block the development of 5-HT_3 receptor down-regulation, this would indicate that the previous behavioral results are due to a blockade of 5-HT_3 receptor down-regulation, and not the result of some non-specific change induced by ondansetron. Thus, the current experiment evaluated the ability of either 0.0 or 0.1 mg kg^{-1} ondansetron, co-administered with chronic cocaine, to block the development of 5-HT_3 receptor down-regulation. The 0.1 mg kg^{-1} dose of ondansetron was chosen because this was the middle dose used in our previous research (King et al., 1997), and as stated above, the effects of ondansetron on tolerance were not dose-dependent. The lack of a dose–response effect in the behavioral experiment is not uncommon, as the literature regarding the effects of 5-HT_3 receptor antagonists indicates that they generally exhibit an inverted U-shaped dose–response curve (see, e.g., Grant, 1995 for a discussion of this issue).

2. Materials and methods

2.1. 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 0700 h) for 1 week. They were maintained on free food and water, and were housed in pairs. Terminal weights ranged from 275–325 g.

2.2. Drugs

Cocaine HCl (received from NIDA) was dissolved in 0.9% saline. 1-*m*-Chlorophenyl-biguanide HCl, was dissolved in distilled water. Ondansetron hydrochloride dihydrate, (±) 1,2,3,9-tetrahydro-9-methyl-3[(2-methyl-1*H*-imidazol-1-yl)methyl]-4*H*-carbazol-4-one, monohydrochloride dihydrate, (generously supplied by Glaxo Wellcome, Middlesex, UK) was also dissolved in distilled water. All doses were calculated as the salt.

2.3. Minipump preparation

Alzet Osmotic pumps (model 2ML2 Alza) were filled with 2.5 ml of either 100 mg ml^{-1} 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^{-1} resulting in an overall average dose of 40 mg kg^{-1} day^{-1} cocaine. The pumps were primed by warming in a warm water bath (37°C) for 4 h before pump implantation.

2.4. 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. This occurred in approximately 2% of the subjects.

2.5. Pretreatment regimen

The cocaine–ondansetron 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 an average rate of 40 mg kg^{-1} day^{-1} (continuous administration group), or (2) implanted with 2ML2 Alzet minipumps continuously infusing saline (saline control group). All subjects also received a daily s.c. injection of ondansetron. The ondansetron doses were 0.0 or 0.1 mg kg^{-1}.

2.6. Slice preparation

Seven days after the removal of the minipump (and cessation of the ondansetron injections), the rats were decapitated and their brains removed. 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^{-1}. Slices were maintained at 35°C, and the buffer continually aerated with a mixture of 95% O_2/5% CO_2; pH 7.4.

2.7. 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 MgSO4, 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 dopamine 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 (three baseline, three K^+-stimulated, three post-K^+ samples) constituted the S1 period. At this point, Krebs buffer containing either 0, 12.5, 25, or 50 μM mCPBG was substituted for the 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 a single concentration of mCPBG constituted the S2 period. For the experiments evaluating the Ca^{2+} dependency of mCPBG enhancement of K^+ stimulated dopamine release, the perfusion buffer contained 0.6 mM, instead of 2.4 mM, Ca^{2+} throughout both the S1 and S2 periods.

2.8. 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.

2.9. Chromatography

Perfusate concentrations of dopamine were determined by HPLC using electrochemical detection. The chromatographic system consisted of a ThermoSeparations P1000 solvent delivery system, a Keystone Octyl/B 5-μm particle (150 × 4.6 mm) column and a ThermoSeparations AS 100 refrigerated autosampler with a 200-μl sample loop. Electrochemical detection of dopamine was 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.

2.10. Data analysis

There were five subjects per pretreatment group, per withdrawal day, per calcium condition, where each subject is the average of two slices: Slices from the left and right accumbens were run in separate perfusion chambers and data separately collected for each chamber. These two data sets, for a single subject, were then averaged to form the data for a single subject for subsequent analysis. This averaging method was used, instead of simply treating each slice as an independent observation so that within- and between-subjects factors were not conflated. The design of the experiment is a three-way between subjects

design, with the factors Pretreatment drug (i.e., saline vs. cocaine), Ondansetron dose (vehicle vs. 0.1 mg kg^{-1}), and Ca^{2+} level (normal vs. low). The main dependent measure was the amount of dopamine present for each sample, separately for pretreatment group, mCPBG concentration, and Ca^{2+} concentration. Dopamine levels are expressed as either pg/200 μl sample, or as an area under the curve (AUC) determined by PeakFit 4.0 (Jandel). The AUCs are calculated on the K^+ and post-K^+ (separately during S1 and S2 periods) samples only; in other words, the AUCs do not include the baseline samples. The significance level was set at $P < 0.05$ for all comparisons.

3. Results

3.1. Basal dopamine levels

Table 1 presents the mean amount of baseline dopamine present (average of the first three, baseline collection periods) for the high and low Ca^{2+} conditions. A two-way ANOVA on the baseline dopamine levels for the high Ca^{2+} conditions indicated that no effect was significant [Pretreatment Group: $F(3,64) = 0.85$; mCPBG Concentration: $F(3,64) = 0.82$; Pretreatment Group × mCPBG Concentration: $F(9,64) = 1.29$]. A two-way ANOVA for the low Ca^{2+} conditions indicated that no effect was significant [Pretreatment Group: $F(3,64) = 2.33$; mCPBG Concentration: $F(3,64) = 0.53$; Pretreatment Group × mCPBG Concentration: $F(9,64) = 2.02$]. Thus, there are no differences in basal dopamine levels across the different pretreatment groups, for either the normal and low Ca^{2+} conditions.

3.2. K^+ Stimulated dopamine release

Fig. 1 presents mean dopamine levels during the S1 and S2 periods, for each mCPBG concentration, separately for each pretreatment group. 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. The data suggest that there are differences between the pretreatment groups in terms of the effects of mCPBG on K^+ stimulated dopamine release, and that these effects are Ca^{2+} dependent. To determine whether there were any differences in K^+ stimulated dopamine release in the absence of any mCPBG (i.e., during the S1 period), two-way ANOVAs were conducted on the AUCs. The AUCs were determined over the K^+ and post-K^+ samples only; in other words, the baselines were not included in the determination of the AUC. The factors were Pretreatment Group, and mCPBG concentration.

3.2.1. Normal Ca^{2+} conditions

The results of the ANOVA indicated that no effect was significant (Pretreatment Group: $F(3,64) = 0.68$; mCPBG Concentration: $F(3,64) = 2.17$]; Pretreatment Group × mCPBG concentration interaction: $F(9,64) = 0.63$).

3.2.2. Low Ca^{2+} condition

The results of the ANOVA indicated that no effect was significant (Pretreatment Group: $F(3,64) = 0.89$; mCPBG Concentration: $F(3,64) = 0.35$]; Pretreatment Group × mCPBG concentration interaction: $F(9,64) = 1.53$).

3.3. Effects of mCPBG on basal dopamine levels

Fig. 2 presents the differences between the mean baseline dopamine levels in the S1 and S2 periods. Averaging the three baseline dopamine levels in the S1 and S2 periods, separately for each mCPBG concentration, and then taking the difference between the two averages determined the differences (i.e., S2–S1). To evaluate whether mCPBG increased basal dopamine levels during the S2 period, two-way ANOVAs were conducted on the differences, separately for the normal and low Ca^{2+} conditions. In these ANOVAs Pretreatment Group was one factor, and mCPBG concentration was a second factor.

3.3.1. Normal Ca^{2+} condition

The results of the ANOVA indicated that neither the main effect of Pretreatment Group [$F(3,64) = 2.02$], nor the Pretreatment Group × mCPBG concentration interaction [$F(9,64) = 1.77$] were significant. However, the main effect of mCPBG Concentration was significant [$F(3,64) = 31.23$]. The results of post-hoc Bonferroni comparisons indicate that the 50 μM mCPBG condition is significantly different than all of the other mCPBG concentrations. In addition, the 25 μM condition is significantly different than the 0 μM condition.

Table 1
Basal dopamine levels for each pretreatment group, mCPBG concentration, and Ca^{2+} level

Pretreatment group	0 μM mCPBG		12.5 μM mCPBG		25 μM mCPBG		50 μM mCPBG	
	High Ca^{2+}	Low Ca^{2+}	High Ca^{2+}	Low Ca^{2+}	High Ca^{2+}	Low Ca^{2+}	High Ca^{2+}	Low Ca^{2+}
Continuous saline	1.88 (0.62)	0.64 (0.32)	3.55 (1.64)	2.11 (1.12)	1.75 (0.66)	3.64 (1.73)	1.28 (0.67)	0.87 (0.55)
Continuous saline + ondansetron	1.02 (0.36)	1.44 (0.6)	2.42 (1.36)	0.03 (0.02)	3.58 (1.45)	0.46 (0.39)	1.37 (0.60)	2.07 (0.56)
Continuous cocaine	4.66 (2.66)	0.78 (0.41)	2.63 (1.16)	1.99 (1.13)	0.88 (0.41)	0.73 (0.61)	1.78 (0.94)	0.91 (0.54)
Continuous cocaine + ondansetron	1.51 (0.77)	0.31 (0.13)	1.41 (0.30)	0.15 (0.10)	1.00 (0.44)	0.81 (0.39)	1.46 (0.70)	0.81 (0.32)

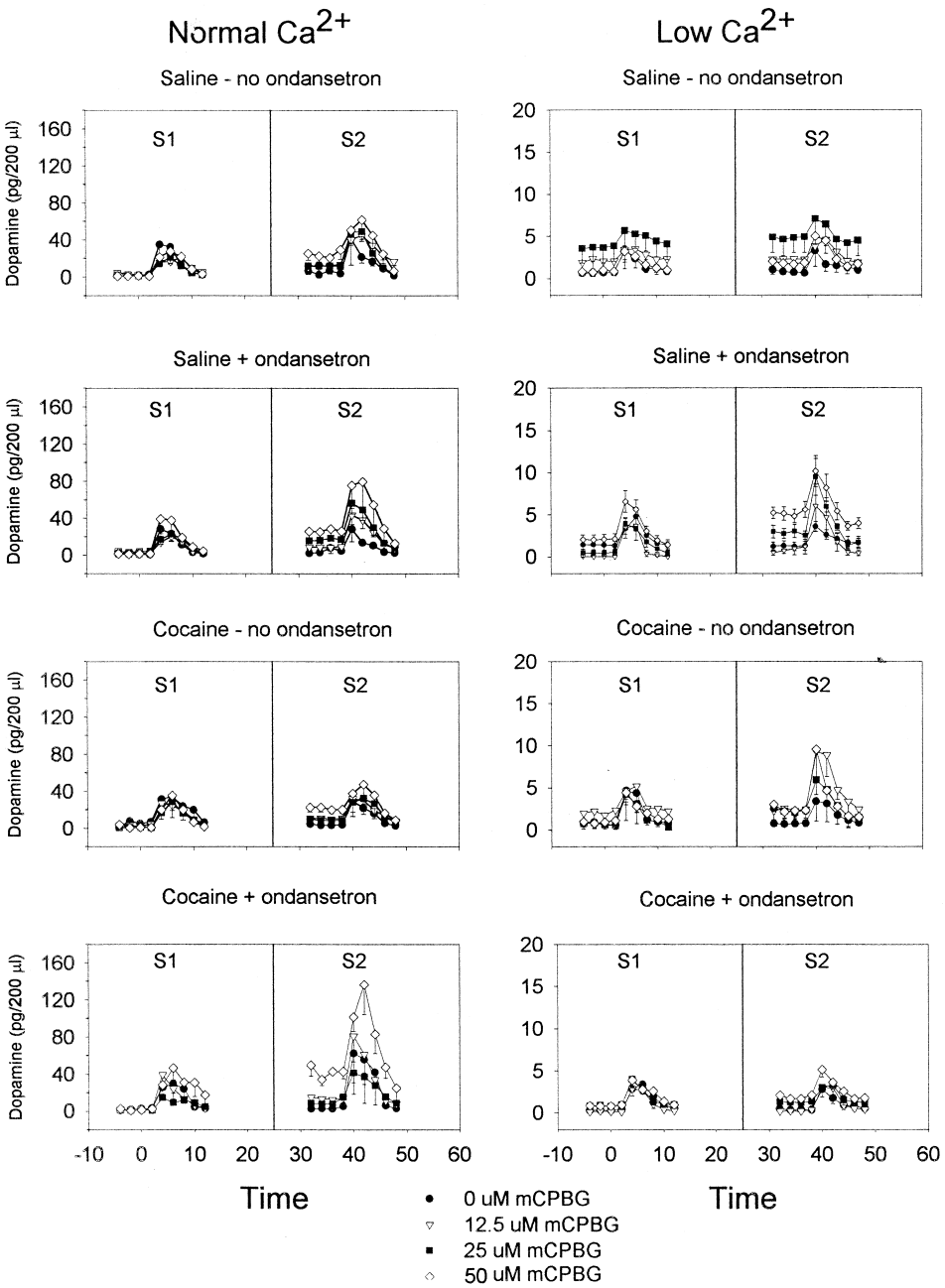

Fig. 1. The first figure shows the mean dopamine levels during the S1 and S2 periods, for each mCPBG concentration, separately for each pretreatment group. The left-hand panels present the data from the normal Ca^{2+} conditions, while the right-hand panels present the data from the low Ca^{2+} conditions. The circles (●) represent the 0 μM conditions, the open-inverted triangles (▽) represent the 12.5 μM conditions, the filled squares (■) represents the 25 μM conditions, and the open diamonds (□) represents the 50 μM conditions. The bars represent one standard error of the mean.

3.3.2. Low Ca^{2+} condition

The results of the ANOVA indicated that the main effects of Pretreatment Group [$F(3,64) = 6.56$] and mCPBG concentration [$F(3,64) = 18.46$] were significant. However, the Pretreatment Group × mCPBG concentration was not significant [$F(9,64) = 1.99$]. The results of post-hoc Bonferroni's tests indicated that the saline alone and saline plus ondansetron groups were significantly different ($t = 3.38$) as were the saline plus ondansetron and cocaine plus ondansetron groups ($t = 4.18$). In addition, the 12.5, 25 and 50 μM conditions were all significantly different from the 0 μM, and the 12.5 μM condition was significantly different than both the 25 and 50 μM conditions.

3.4. mCPBG and K^+ stimulated dopamine release

Because the results presented in Fig. 2 indicated that mCPBG increased basal dopamine levels in a Ca^{2+} dependent manner, the raw data was normalized. The data for each subject, in the K^+ and post-K^+ samples of the S1

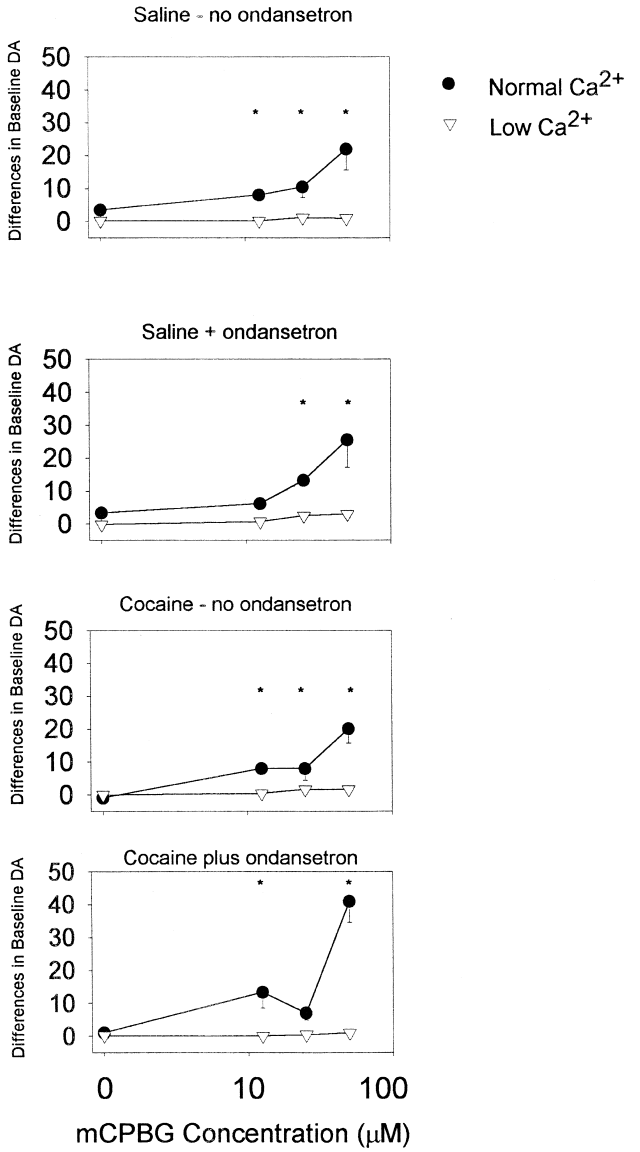

Fig. 2. Effects of mCPBG concentration on basal dopamine levels. The first figure presents the differences between the mean baseline dopamine levels in the S1 and S2 periods. Averaging the three baseline dopamine levels in the S1 and S2 periods, separately for each mCPBG concentration, and then taking the difference between the two averages determined the differences (i.e., S2–S1). The panels present the concentration effect data for the normal and low Ca^{2+} conditions, separately for each pretreatment group. The circles (●) represent the normal Ca^{2+} conditions, while the open-inverted triangles (▽) represent the low Ca^{2+} conditions. For all figures the bars represent one standard error. The asterisks indicate a significant difference as determined by post-hoc Bonferroni's *t*-tests.

and S2 period, was normalized relative to the minimum and maximum amount of dopamine (DA) in a sample during K^+ and post-K^+ period of the S1 period according to the following formula:

$$(DA_{sample} - DA_{min\,S1})/(DA_{max\,S1} - DA_{min\,S1})$$

Because the normalization occurs only during the K^+ and post-K^+ samples only, this allows one to assess the effects of mCPBG on K^+ stimulated dopamine release while factoring out the effects of mCPBG on basal dopamine levels.

Fig. 3 presents normalized S2:S1 ratios as a function of mCPBG concentration, for each pretreatment group. The top panel presents the data for the normal Ca^{2+} conditions, while the bottom panel presents the data from the low Ca^{2+} conditions.

3.4.1. Normal Ca^{2+} condition

The results of the ANOVA indicated that the main effects of Pretreatment Group [$F(3,64) = 7.95$] and mCPBG Concentration [$F(3,64) = 17.16$] were significant.

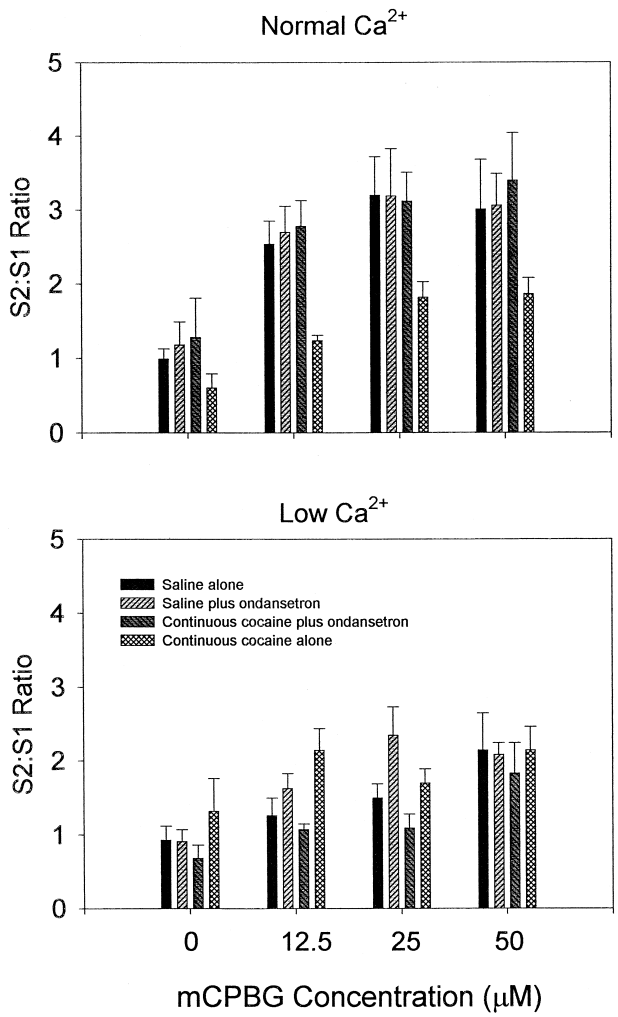

Fig. 3. Mean S2:S1 ratios for each pretreatment group as a function of mCPBG concentration. The ratios are based on the normalized dopamine levels during the S1 and S2 periods, which were determined using only the K^+ and post-K^+ samples only. The top panel presents the data from the normal Ca^{2+} conditions, while the bottom panel presents the data from the low Ca^{2+} conditions. The black bars represent the saline–no ondansetron subjects, the gray hatched bars represent the saline plus ondansetron subjects, the gray cross-hatched bars represent the continuous cocaine–no ondansetron subjects, and the dark gray hatched bars represent the continuous cocaine plus ondansetron subjects. The bars represent one standard error of the mean.

However, the Pretreatment Group × mCPBG concentration interaction [$F(9,64) = 0.29$] was not significant. The results of post-hoc Bonferroni comparisons indicate that the cocaine–no ondansetron group is significantly different than all of the other pretreatment groups. In addition, the 12.5, 25, and 50 μM mCPBG conditions are significantly different than the 0 μM condition.

3.4.2. Low Ca^{2+} condition

The results of the ANOVA indicated that the main effects of Pretreatment Group [$F(3,64) = 4.65$] and mCPBG Concentration [$F(3,64) = 10.65$] were significant. However, the Pretreatment Group × mCPBG concentration interaction [$F(9,64) = 1.02$] was not significant. The results of post-hoc Bonferroni comparisons indicate that the saline–ondansetron and cocaine ondansetron groups are significantly different as are the cocaine–no ondansetron and cocaine–ondansetron groups. In addition, the 12.5, 25, and 50 μM mCPBG conditions are significantly different than the 0 μM condition.

4. Discussion

The results of the present study show that perfusion of nucleus accumbens slices with mCPBG, a 5-HT_3 receptor agonist, enhances K^+ stimulated dopamine release. This result replicates previous findings that 5-HT_3 receptors can induce dopamine release in the nucleus accumbens (Chen et al., 1991; Campbell and McBride, 1995). The results are also consistent with our previous report demonstrating a functional down-regulation of accumbens 5-HT_3 receptors following continuous cocaine administration (Matell and King, 1997). Additionally, we have shown that chronic intermittent ondansetron, co-administered with continuous cocaine, blocks the development of this functional down-regulation of accumbens 5-HT_3 receptors.

4.1. Cocaine tolerance and 5-HT_3 receptors

The overall pattern of results from the current, and our previous, experiments strongly suggest that a functional down-regulation of 5-HT_3 receptors is a mechanism that has a significant contribution to cocaine tolerance. Continuous cocaine administration induces behavioral tolerance to its behavioral effects (Reith et al., 1987; King et al., 1992), as well a functional down-regulation of accumbens 5-HT_3 receptors (current study; Matell and King, 1997). In addition, the co-administration of a single daily injection of ondansetron and continuous cocaine blocks the development of 5-HT_3 receptor down-regulation (current results) as well as behavioral tolerance (King et al., 1997). Thus, blockade of accumbens 5-HT_3 receptor down-regulation is associated with normal behavioral responsivity to cocaine.

The present results indicate that chronic intermittent ondansetron had no consistent, systematic effect on the subsequent ability of mCPBG to enhance K^+ stimulated dopamine release in the nucleus accumbens in the saline control subjects. In other words, there were no differences between the saline alone and saline plus ondansetron groups on any of the current measures under the normal Ca^{2+} conditions. This result is consistent with previous research indicating that 5-HT_3 receptor antagonists have no effect on locomotor behavior or neurotransmitter levels when administered alone in drug naive rats (e.g., Costall and Naylor, 1974; Costall et al., 1987, 1989, 1990a,b; King et al., 1994).

4.2. Ondansetron and blockade of 5-HT_3 receptor down-regulation

In the current experiment continuous cocaine administration functionally down-regulated accumbens 5-HT_3 receptors, consistent with our previous results (Matell and King, 1997). However, the current results clearly indicate that ondansetron, co-administered with continuous cocaine, blocked the development of the functional down-regulation of accumbens 5-HT_3 receptors. This result is consistent with our behavioral study (King et al., 1997) which demonstrated that ondansetron–continuous cocaine treatments blocked the development of behavioral tolerance to a cocaine challenge on day 7 of withdrawal from continuous cocaine administration. The current results therefore clearly indicate that the previous behavioral results are due to a blockade of the down-regulation of accumbens 5-HT_3 receptors, and are not due to some non-specific change induced by chronic ondansetron administration.

It is somewhat surprising that a single daily injection of ondansetron, during continuous cocaine administration, would block the development of tolerance and 5-HT_3 receptor down-regulation, to the extent that cocaine is always present during the pretreatment regimen, but ondansetron is only present for a portion of the day. Indeed, in humans, following an oral or IV dose, the T_{max} for ondansetron is approximately 2 h, while the half-life is approximately 3.5 h (Blake et al., 1993; Roila and Delfavero, 1995); in older humans, the half-life is approximately 6–8 h (Blake et al., 1993; Roila and Delfavero, 1995). It should be noted that the metabolism of ondansetron following oral and IV dosing is extremely rapid, and that the half-life of ondansetron following a subcutaneous injection is liable to be longer. Results from an ongoing study in our laboratory indicate that the continuous administration of cocaine for 16 h per day, for 14 days, does not induce behavioral tolerance to the effects of a cocaine challenge on day 7 of withdrawal. Thus, the daily injection could block the induction of tolerance and receptor down-regulation. This analysis does imply that the functional down-regulation of accumbens 5-HT_3 receptors represents a significant mechanism in cocaine tolerance.

4.3. Continuous cocaine and dopamine release

We had previously reported that continuous cocaine administration produces decreased cocaine-induced dopamine release (King et al., 1993), and decreased 5-HT_3 receptor agonist induced dopamine release (King et al., 1995) in the striatum. This pattern of previous results might suggest that continuous cocaine administration induces some generalized impairment of the release process. However, the present results indicate that this is not the case. There were no differences in the amount of K^+ stimulated dopamine release between the saline and continuous cocaine groups during the S1 period. This result indicates that, under the appropriate conditions, dopamine release in the nucleus accumbens is normal (unchanged) in slices from rats continuously administered cocaine. This result is consistent with our previous report which also did not find any differences in K^+ stimulated dopamine release in the nucleus accumbens during withdrawal from continuous cocaine administration (Matell and King, 1997).

4.4. Mechanisms of 5-HT_3 receptor action

Our current results are 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 in a concentration dependent manner. Second, this enhancement of release was Ca^{2+} dependent: Reducing the Ca^{2+} levels in the perfusion buffer to 25% of normal levels eliminated the ability of mCPBG to enhance K^+ stimulated dopamine release in all groups. 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 dopamine release, in saline control subjects.

A second proposed mechanism for mCPBG's mode of action is its ability to block the dopamine transporter. 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]dopamine reuptake into synaptosomes in both the striatum ($IC_{50} = 5.1 \pm 0.3$ μM) and nucleus accumbens ($IC_{50} = 6.5 \pm 0.4$ μM). These results therefore suggest that part of the mechanism of mCPBG's action is blockade of the dopamine transporter.

Although blockade of the dopamine transporter by mCPBG may have contributed to increased dopamine levels, it is unlikely that this mechanism accounts for the differences between the pretreatment groups in mCPBG's ability to enhance K^+ stimulated dopamine release. The effects of mCPBG on baseline dopamine levels in the pretreatment groups were not different. However, there were significant differences in the ability of mCPBG to enhance K^+ stimulated dopamine release. Thus, if the effects of mCPBG were largely mediated by mCPBG's action at the dopamine transporter, then there should not have been any differences between the pretreatment groups. Furthermore, the effects of mCPBG on basal dopamine levels were concentration and Ca^{2+} dependent: Reduction of Ca^{2+} in the perfusion medium reduced the ability of mCPBG to enhance basal dopamine levels. This result suggests that the effects of mCPBG on basal dopamine levels are receptor mediated, and not due to blockade of the dopamine transporter.

In summary, the current results indicate that continuous cocaine administration induces a functional down-regulation of accumbens 5-HT_3 receptors. Furthermore, this development of this down-regulation can be blocked by the co-administration of ondansetron during the continuous cocaine administration period. Therefore, the down-regulation of accumbens 5-HT_3 receptors is a mechanism that contributes significantly to the tolerance induced by continuous cocaine administration.

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