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Effects of repeated daily treatments with a 5-HT_3 receptor antagonist on dopamine neurotransmission and functional activity of 5-HT_3 receptors within the nucleus accumbens of Wistar rats

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Abstract

A previous study indicated that pretreatment with repeated daily injections of serotonin-3 (5-HT_3) receptor antagonists subsequently reduced the effectiveness of the 5-HT_3 antagonists to attenuate ethanol intake under 24-h free-choice conditions; one possibility to account for this is that the functional activity of the 5-HT_3 receptor may have been altered by prior treatment with the antagonists. The present experiments were conducted to examine the effects of local perfusion of the 5-HT_3 agonist 1-(*m*-chlorophenyl)-biguanide (CPBG) on the extracellular levels of dopamine (DA) in the nucleus accumbens (ACB) and ventral tegmental area (VTA) of adult male Wistar rats that had received repeated daily injections of the 5-HT_3 antagonist, MDL 72222 (MDL). In vivo microdialysis was used to test the hypothesis that alterations in 5-HT_3 receptor function have occurred with repeated antagonist injections. One group was given daily injections of MDL (1 mg/kg, s.c.) for 10 consecutive days (MDL group), and the other group was administered saline for 10 days (saline group). On the day after the last treatment, rats were implanted with a unilateral guide cannula aimed at either the ACB or VTA. Two days later, the microdialysis probe was inserted into the guide cannula; on the next day, microdialysis experiments were conducted to determine the extracellular levels of DA in the ACB or VTA. Local perfusion of CPBG (17.5, 35, 70 μM) in the ACB significantly stimulated DA release in the saline- and MDL-treated animals. In terms of percent baseline, the CPBG-stimulated DA release was higher in the MDL-treated group than in the saline-treated group in both the ACB and VTA; however, on the basis of the extracellular concentration, there were no significant differences in the ACB between the two groups. Using the no-net-flux microdialysis, it was determine that the basal extracellular concentration of DA in the ACB was approximately 60% lower in the MDL group than saline group; there was no difference between the groups in the extraction fraction (clearance). Overall, the results suggest that repeated daily treatments with MDL decreased basal DA neurotransmission in the ACB and did not have a clear effect on functional activity of 5-HT_3 receptors in the ACB.

Keywords: Serotonin-3 receptor; Dopamine; Nucleus accumbens; Ventral tegmental area; MDL 72222; 1-(*m*-Chlorophenyl)-biguanide; No-net-flux microdialysis

1. Introduction

Several studies demonstrated that serotonin-3 (5-HT_3) receptors in the nucleus accumbens (ACB) and the ventral tegmental area (VTA) are involved in regulating this dopamine (DA) system, as well as the nigro-striatal DA pathway. Data indicate that 5-HT_3 receptor agonists increased the extracellular concentrations of DA both in vivo in the ACB and striatum (Chen et al., 1991; Benloucif et al., 1993), and in vitro in the striatum (Blandina et al., 1988, 1989). Furthermore, acute systemic administration of 5-HT_3 receptor antagonists decreased the number of spontaneously active VTA DA neurons (Minabe et al., 1991; Rasmussen et al., 1991). In addition, 5-HT_3 receptor antagonists have been shown to attenuate the increase of extracellular DA levels in the ACB induced by several drugs of abuse (Carboni et al., 1989; Imperato and Angelucci, 1989; Wozniak et al., 1990; Pei et al., 1993; Yoshimoto et al., 1992; McNeish et al., 1993; Kankaanpää et al., 1996). Previous studies

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from our laboratory demonstrated that local administration of a 5-HT_3 receptor agonist into the ACB or VTA increased the extracellular levels of DA in both regions (Campbell and McBride, 1995; Campbell et al., 1996), supporting the idea that 5-HT_3 receptors are involved in regulating terminal and somatodendritic release of DA.

It has been previously reported that local inhibition of 5-HT_3 receptors blocked ethanol-induced DA release in the ACB (Carboni et al., 1989; Wozniak et al., 1990; Yoshimoto et al., 1992; Campbell and McBride, 1995) and VTA (Campbell et al., 1996). Systemic administration of 5-HT_3 antagonists suppressed voluntary ethanol consumption of alcohol-preferring rats under 24-h free-choice conditions (Fadda et al., 1991; McKinzie et al., 1998; Rodd-Henricks et al., 2000). However, the study of Rodd-Henricks et al. (2000) indicated that pretreatment with repeated daily injections of ICS205-930 or MDL72222 (MDL), both 5-HT_3 receptor antagonists, subsequently reduced the effectiveness of ICS205-930 and MDL to attenuate ethanol intake of P rats under 24-h free-choice drinking conditions. One possibility to account for the reduced effectiveness of ICS205-930 and MDL to attenuate ethanol drinking is that repeated daily treatment with the 5-HT_3 antagonists altered the function of 5-HT_3 receptors. As a consequence, higher doses of 5-HT_3 antagonists were required to reduce alcohol intake.

The present study was undertaken to test the hypothesis that repeated daily treatments with a 5-HT_3 receptor antagonist alter the function of 5-HT_3 receptors in the ACB and VTA and changes mesolimbic DA neurotransmission. This hypothesis was examined by determining the effects of local perfusion with the 5-HT_3 agonist 1-(*m*-chlorophenyl)-biguanide (CPBG) into the ACB and VTA on DA release in Wistar rats after daily treatments with MDL. In addition, because alterations in 5-HT_3 receptor function could also alter DA neurotransmission, the no-net-flux microdialysis technique was used to determine basal extracellular levels of DA in the ACB following MDL pretreatment.

2. Methods

2.1. Animals

Adult male Wistar rats weighing 280–340 g (Harlan, Indianapolis, IN) at the time of surgery were used. All animals were housed at constant temperature and relative humidity under a regular 12-h light/dark cycle (lights on at 0700 h) with food and water ad libitum. Prior to the injection period, all rats were handled daily for at least 7 days. During handling, the napes of the rats' necks were gently pinched to habituate the animal to the injection procedure. The animals used in this experiment were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). All research protocols were approved by the institutional animal care and use committee and are in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute on Drug Abuse, NIH, and the Guide for the Care and Use of Laboratory Animal (National Research Council, 1996).

2.2. Pretreatment with MDL

Rats were assigned to one of two groups: one group was given 1 mg/kg MDL subcutaneously (s.c.) once daily for 10 consecutive days with a volume of 1.0 ml/kg body weight (MDL group), as previously used (Rodd-Henricks et al., 2000). Rats in the other group received an equal volume of saline (saline group). MDL (Sigma, St. Louis, MO) was first dissolved in a drop of glacial acetic acid, sterile saline was then added, and pH of the solution was adjusted to 6.0 with 1 N NaOH.

2.3. Stereotaxic surgery

On the day after the last pretreatment, rats were anaesthetized with 2% isoflurane and placed in the stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA); the skull was exposed and a small hole was drilled to insert unilaterally an 18-gauge stainless steel guide cannula (Plastics One). The guide cannula was aimed at the ACB-shell or the posterior VTA, according to the atlas of Paxinos and Watson (1998). The following coordinates relative to bregma were used with the incisor bar set at −3.3 mm: (a) AP +1.9 mm, L +2.2 mm, D/V −4.2 mm for the ACB; and (b) AP −5.8 to −6.0 mm, L +2.0 mm, D/V −5.9 mm for the VTA with a 10° angle to the vertical. Two stainless steel screws were placed in the skull to secure the guides with cranioplastic cement (Plastics One). The rats were allowed 2 days to recover before microdialysis probes were inserted. During the recovery period, animals were habituated to the clear Plexiglas microdialysis chambers (22.5 × 44.5 × 38.0 cm, W × L × H) for approximately 2–3 h.

On the day before microdialysis, rats were briefly anesthetized with isoflurane, and the loop style microdialysis probe was inserted through the 18-gauge guide cannula and cemented into place. The loop style probe was made as previously described (Kohl et al., 1998; Rahman and McBride, 2002). The loop length of active dialysis surface was 2 mm for ACB and 1.5 mm for VTA, and the total length of the dialysis membrane was 4.0 and 3.0 mm, respectively. The loop was oriented in a rostral–caudal direction. After probe insertion, rats were again placed in the Plexiglas microdialysis chambers for approximately 2–3 h to habituate. The weights of the rats prior to surgery averaged 309 ± 3 g and on the day of dialysis they averaged 335 ± 4 g.

2.4. Local perfusion of ACB with CPBG

Dialysis was performed 4 days after the last treatment with MDL. Although pharmacokinetic data for MDL in rats is unavailable, data in other mammals show that by this time MDL should be fully eliminated (Cheng et al., 1992). On the day of dialysis, six animals were placed in the microdialysis chambers and the microdialysis probes were perfused at a flow rate of 0.5 μl/min with artificial cerebrospinal fluid (aCSF; 145 mM NaCl, 2.7 mM KCl, 1.0 mM $mgCl_2$, 1.2 mM $CaCl_2$, 2.0 mM Na_2HPO_4, 0.2 mM ascorbate; pH 7.2–7.4) for 120 min prior to collecting the baseline samples. The time of exposure of the tissue to changes in the perfusion media took into account the length of the inlet and exit lines, which were similar from animal to animal. Therefore,

all animals had similar durations of drug exposure. Three 20-min baseline samples were then collected. Animals were then switched to lines with aCSF containing CPBG (17.5, 35 or 70 μM; Sigma) by disconnecting/reconnecting the lines at the top of the collection assembly. After 60-min perfusion with aCSF containing CPBG, the rats were switched back to aCSF for an additional 80 min. Each rat was perfused with only one concentration of CPBG. These concentrations were chosen based upon previous studies from our laboratory (Campbell and McBride, 1995; Campbell et al., 1996). Samples were collected in 0.5 ml polyethylene tubes containing 2 μl 0.1 N perchloric acid. Samples were immediately frozen on dry ice and stored at −70 °C until analyzed.

2.5. Local perfusion of VTA with CPBG

The perfusion method is similar to the above. After the 2-h equilibration period, three 20-min baseline samples were collected. Animals were then perfused with aCSF containing 35 μM CPBG for 60 min and aCSF was returned for an additional 80 min. The single concentration of 35 μM CPBG was chosen because this concentration produced a submaximal but reliable increase in extracellular DA levels in the ACB (Campbell and McBride, 1995) and VTA (Campbell et al., 1996), and this concentration has been used to detect changes in the functional activity of 5-HT_3 receptors in the ACB of P rats following chronic alcohol drinking (Thielen et al., 2004). Samples were immediately frozen on dry ice and stored at −70 °C until analysis.

2.6. Dopamine no-net-flux

The DA no-net-flux experiment and data analysis were conducted as described previously (Parsons and Justice, 1992; Justice, 1993; Thielen et al., 2004). In brief, animals were placed in the microdialysis chambers and the microdialysis probes were perfused at a flow rate of 0.5 μl/min with aCSF. After a 2-h equilibration period, three baseline samples were collected every 20 min in microfuge tubes containing 2 μl 0.1 N perchloric acid. As indicated above, changes in perfusion media and sample collection took into account the length of the inlet and outlet lines. The ACB was then perfused, in random order, with aCSF containing 5, 10 or 20 nM DA for five 20-min periods at each concentration; aCSF was returned for an additional 60 min (three samples) after perfusion of all three concentration of DA. Samples were immediately frozen on dry ice and stored at −70 °C until analyzed.

2.7. Probe placements

Probe placements were verified by perfusion of 1% bromphenol blue solution through the probe at the end of the experiment. Rats were overdosed with CO_2 and the brains removed and stored at −70 °C. Frozen 40 μM coronal sections were prepared and used to evaluate probe placements according to the atlas of Paxinos and Watson (1998). Only data from animals in which placements were clearly in the ACB or VTA were used.

2.8. Dialysis sample analysis for DA concentrations

DA levels were analyzed by using microbore high-performance liquid chromatography (HPLC) with electrochemical detection. The samples were injected onto a reverse-phase microbore column (1.0×100 mm BDS Hypersil C18 column, 3 μM particle size; Thermo Hypersil-Keystone, Bellefonte, PA, USA) connected to a BAS custom injection valve with a Rheodyne injector (5 μl loop, Cotati, CA, USA). Mobile phase consisted of 77 mM sodium acetate, 0.5 mM EDTA, 3.5 mM sodium octanesulfonic acid, 10 mM NaCl and 6% acetonitrile,; the pH was adjusted to 4.0 with acetic acid; the flow rate was 0.07 ml/min (Model 582 pump, ESA, Chelmsford, MA, USA). The concentration of DA was assayed by electrochemical detection using a Unijet radial-flow detector cell with 6 mm glassy carbon electrode with a LC-4C amperometric detector (Bioanalytical Systems, Lafayette, IN, USA) at a potential of 450 mV, and a sensitivity setting of 0.5 to 5 nA depending upon the expected concentrations of DA in the no-net-flux experiment and at the highest concentration of CPBG.

2.9. Data analysis

ACB and VTA baseline data are expressed as nM DA after correction for dilution by the perchloric acid and were analyzed by Student's *t*-test. For the CPBG experiments, DA levels were normalized and expressed as percent of baseline. For the ACB, time-course data for the three CPBG concentrations were analyzed by a mixed ANOVA (pretreatment × concentration × time) with time as the repeated measure. For the VTA, a mixed ANOVA (pretreatment × time) with time as the repeated measure was conducted. Significant interactions were further analyzed by orthogonal contrasts. Where appropriate, Student's *t*-tests were used to compare individual means.

For the no-net-flux experiment, extracellular DA concentrations and extraction fraction (E_d) values were obtained from multiple linear regression modeling using the SAS System for Windows, version 8.02 (SAS Institute, Cary, NC). Regression lines were constructed by plotting the difference between inflow and outflow concentrations of DA. $[DA]_{in}$ was the concentration of DA added in the perfusate and $[DA]_{out}$ was the dialysate DA concentration in the collected samples. The amount of DA gained or lost from the brain was expressed as $[DA]_{in} - [DA]_{out}$. The zero point on the *y*-axis represents no net gain or loss of DA across the dialysis membrane. ANOVA was used to compare the basal extracellular DA concentration (*x*-intercept) and the E_d value (slope) in the ACB between saline- and MDL-treated rats.

The presentations of time-course data are corrected for the delay of the CPBG or DA in reaching the tissue and the delay in collecting the sample because of the length of the inlet and outlet lines and the slow perfusion rate.

3. Results

Fig. 1 shows representative probe placements in the ACB and VTA. Only data from animals with probes correctly implanted in the ACB or VTA were included in the analysis.

Within the ACB, almost all probe placements were in the shell alone or in the shell plus core. Probes were mainly located between 1.0 and 1.6 mm rostral of bregma, according to the atlas of Paxinos and Watson (1998). Within the VTA, placements were mainly in the posterior VTA, coronal sections at −5.8 to −6.0 mm bregma. No probes were located in the substantia nigra. Approximately 85% of the animals that had undergone surgery had probes correctly implanted within the ACB (shell or shell plus core) or posterior VTA.

Local perfusion of all three doses of CPBG (17.5, 35 and 70 μM) into the ACB significantly increased the extracellular levels of DA in the ACB in both the saline and MDL group (Fig. 2). A three-way ANOVA revealed that there was a significant group × concentration × time interaction [$F(14,203)=3.57$, $p<0.001$]. Two-way ANOVAs were conducted on each CPBG concentration separately with the significant overall multivariate F-value obtained.

Fig. 2A shows the time-course of the effects of local perfusion with 17.5 μM CPBG on the extracellular levels of DA in the ACB. The basal extracellular levels of DA in the ACB of these groups, obtained by averaging the three 20-min baseline samples, were 4.2±1.2 nM for the saline group ($n=6$) and 3.1±0.7 nM for the MDL group ($n=7$); these values were not statistically different. The results indicated that, with 17.5 μM CPBG (Fig. 2A), the effect of time was significant [$F(7,77)=22.1$, $p<0.001$]; although there was a trend, the group effect was not significant [$F(1,11)=3.41$, $p=0.09$] and the group × time interaction was also not significant [$F(7,77)=1.17$, $p=0.33$]. The significant effect of time was a result of 17.5 μM CPBG increasing the extracellular levels of DA within 40 min after adding CPBG.

The basal extracellular levels of DA in the ACB of rats perfused with 35 μM CPBG were 6.2±1.0 nM for the saline group ($n=6$) and 3.9±0.5 nM for the MDL group ($n=6$); these values were not statistically different. With 35 μM CPBG (Fig. 2B), there was a significant effect of time [$F(7,70)=40.1$, $p<0.001$], group [$F(1,10)=9.49$, $p<0.05$] and group × time interaction [$F(7,70)=2.32$, $p<0.05$]. The time-course of 35 μM CPBG-enhanced extracellular levels of DA indicated that the maximal response occurred approximately 40 to 60 min after adding the CPBG in both the saline and MDL groups; the increase in the extracellular levels of DA for the MDL group was significantly ($p<0.05$) higher than the saline group 60 and 80 min after initiating perfusion with CPBG. DA levels returned to basal levels within 60–80 min after ending the perfusion with 35 μM CPBG.

The basal extracellular levels of DA in the ACB of rats perfused with 70 μM CPBG were 3.1±0.6 nM for the saline group ($n=5$) and 2.1±0.6 nM for the MDL group ($n=5$); these values were not statistically different. With perfusion of 70 μM

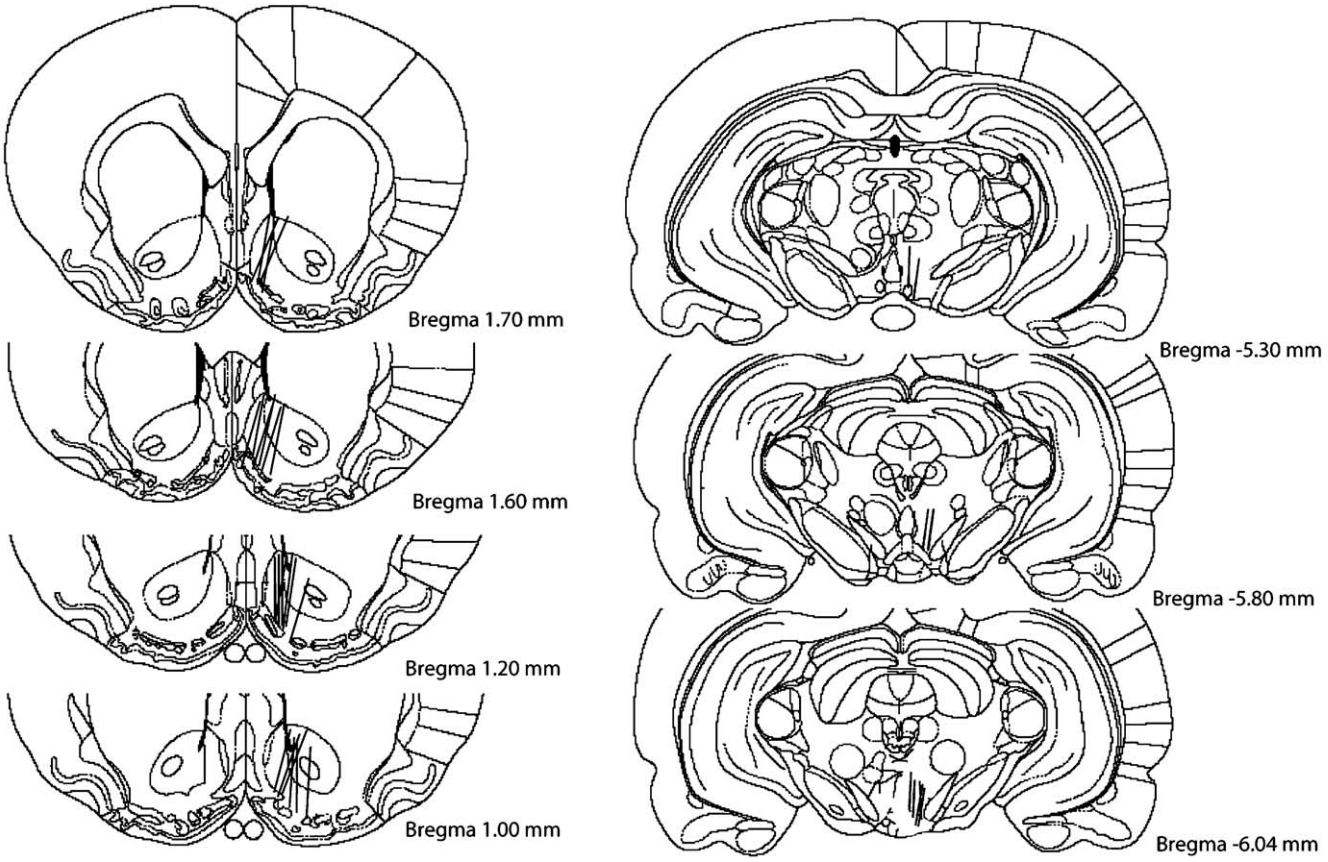

Fig. 1. Microdialysis probe placements in the nucleus accumbens (ACB, left side) and ventral tegmental area (VTA, right side). Each line represents the active dialysis loop of probes used in the study. The length of active dialysis loop was 2 mm for ACB and 1.5 mm for VTA. Overlapping placements are not shown. The numbers to the right of each section indicate distance (mm) from bregma (adapted from Paxinos and Watson, 1998).

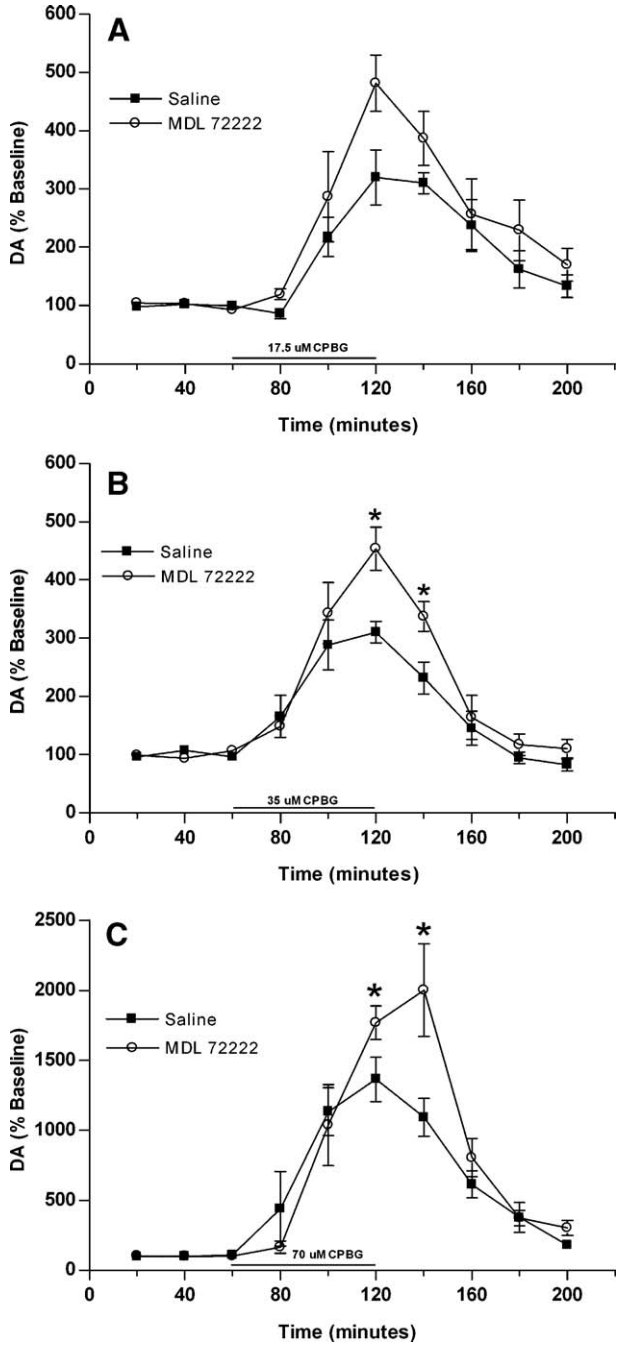

Fig. 2. Effects of local perfusion with three doses of CPBG (A: 17.5, B: 35, C: 70 μM) on extracellular DA levels in the ACB of Wistar rats. Animals in the MDL group were injected (s.c.) with 1 mg/kg MDL once daily for 10 consecutive days. Animals in the saline group were administrated an equivalent volume of saline. The data are expressed as percentage of baseline, and each point is the group mean±S.E.M. (n=5–7/group). A three-way ANOVA with repeated measure revealed significant group×concentration×time interaction [$F(14,203)=3.57$, $p<0.0001$]. $*p<0.05$ compared with the saline group.

CPBG (Fig. 2C), there was a significant effect of time [$F(7,56)=39.0$, $p<0.001$], a non-significant effect of group [$F(1,8)=2.75$, $p=0.14$] and a significant group×time interaction [$F(7,56)=3.74$, $p<0.005$]. The time-course of 70 μM CPBG-induced changes in the extracellular levels of DA indicated that the maximal response occurred approximately 60 min after starting local perfusion with CPBG. The groups significantly ($p<0.05$) differed at the 60- and 80-min time points after starting perfusion with CPBG. DA levels had nearly returned to basal levels within 80 min of ending the perfusion with 70 μM CPBG.

Although there were no statistically significant differences in the baseline values between the MDL- and saline-treated groups for the individual dialysis experiments (Fig. 2A–C), when values from the individual experiments were pooled, there was a statistically significant difference ($p<0.05$) in basal extracellular levels of DA within the ACB between saline (4.7±0.7 nM, $n=17$) and MDL (3.1±0.4 nM, $n=18$) pretreated rats. The overall baseline values were approximately 35% lower in the MDL group than saline group.

To determine if the percentage increase in the extracellular levels of DA produced by perfusion with CPBG resulted in higher DA concentrations in the ACB of the MDL group, the maximal effect of CPBG administration on the extracellular concentrations of DA within the ACB was examined. Two-way ANOVA revealed a significant effect of dose of CPBG on maximal DA concentrations within the ACB [$F(2,29)=12.7$, $p<0.001$]. Perfusion with 17.5 μM CPBG increased extracellular DA levels to 15.2±4.2 nM ($n=6$) for saline rats and 15.2±3.4 nM ($n=6$) for MDL rats; perfusion with 35 μM CPBG increased DA levels to 20.6±3.6 nM ($n=6$) for the saline group and 18.0±3.0 nM ($n=6$) for the MDL group; and perfusion with 70 μM CPBG increased DA levels to 41.9±8.0 nM ($n=5$) for saline rats and 40.4±9.5 nM ($n=5$) for MDL rats. There was no significant effect of treatment or a dose×treatment interaction.

Within the VTA (Fig. 3), the basal extracellular levels of DA were 2.0±0.8 nM ($n=5$) for the saline group and 1.8±0.8 nM ($n=5$) for the MDL group; these values were not significantly different. In both the saline- and MDL-treated groups, perfusion with 35 μM CPBG resulted in a significant increase in extracellular DA levels as indicated by a main effect of time within the VTA [$F(7,56)=14.9$, $p<0.001$]. The increase in

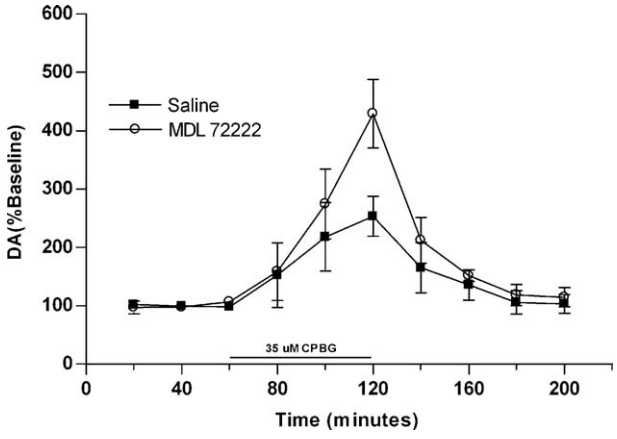

Fig. 3. Effects of local perfusion with 35 μM CPBG on extracellular DA levels in the VTA of Wistar rats. Animals in the MDL group were injected (s.c.) with 1 mg/kg MDL once daily for 10 consecutive days. Animals in the saline group were administrated with an equivalent volume of saline. The data are expressed as percentage of baseline and each point is the group mean±S.E.M (n=5/group).

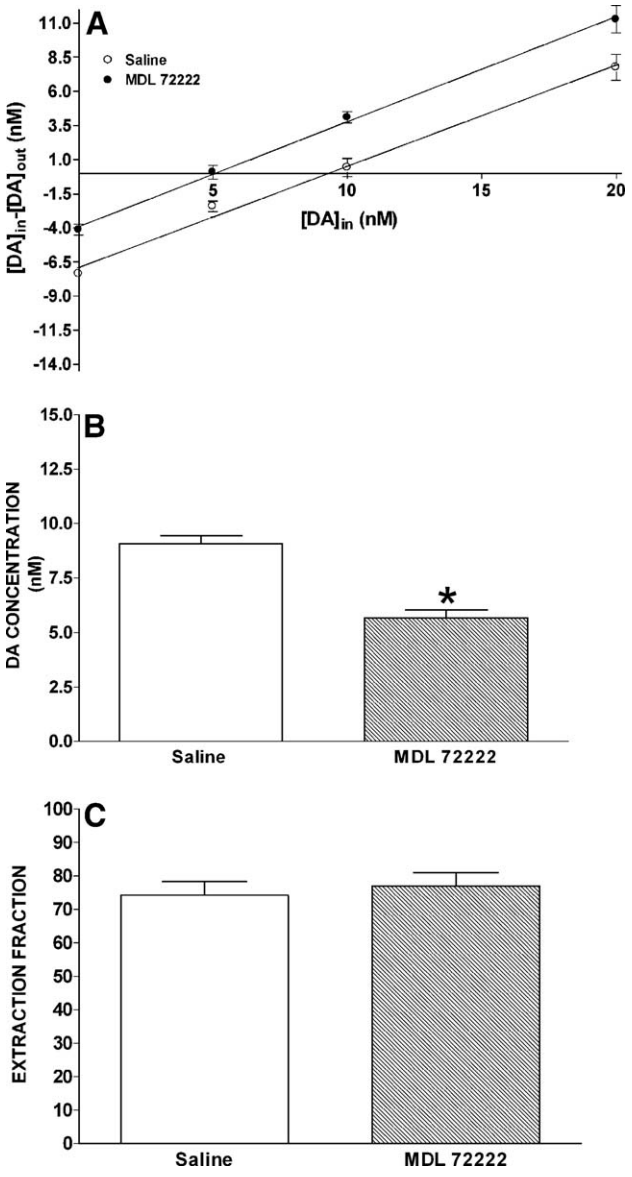

Fig. 4. Extracellular concentrations and extraction fractions (E_d) for DA in the ACB of Wistar rats treated with saline ($n=6$) or MDL ($n=5$) determined by no-net-flux microdialysis. (A) Net gain or loss of DA across the dialysis membrane as a function of the concentration of DA in the perfusate for the saline group (open circles) and the MDL group (closed circles). The basal extracellular concentration of DA (zero point intercept) and clearance (E_d value) were determined from this analysis. (B) Extracellular concentrations of DA for the both groups. The DA concentration was significantly lower in the MDL group compared with the saline treated group ($*p<0.05$). (C) In vivo DA extraction fractions (E_d values, clearance) for both groups. The E_d values were not statistically different. Data are the means±S.E.M.

extracellular DA levels in response to 35 μM CPBG was greater in the MDL group compared to the saline group as indicated by a significant main effect of group [$F(1,8)=7.78$, $p<0.05$]. There was not a significant time×treatment interaction [$F(7,56)=1.93$, $p>0.05$]. The significant group effect was likely due to higher extracellular DA levels in the MDL group compared to the saline group 60 min after starting the perfusion with CPBG.

The no-net-flux technique was used to verify that there were differences in the extracellular baseline concentrations between the MDL and saline groups in the ACB. With this quantitative microdialysis approach (Fig. 4A–C), the basal extracellular concentrations of DA and DA clearance (E_d values) were, respectively, 9.3±0.4 nM and 74±4% for the saline group ($r^2=0.78$, $p<0.05$) and 5.0±0.4 nM and 77±4% for the MDL group ($r^2=0.82$, $p<0.05$). There was a significant effect of repeated daily treatment with MDL on the basal extracellular concentration of DA (intercept) [$F(1,184)=43.6$, $p<0.0001$] with the basal extracellular concentration of DA in the MDL group being significantly lower than the saline group (Fig. 4A and B, $p<0.05$). The E_d values (slope) were not significantly different between the two pretreatment groups (Fig. 4A and C).

4. Discussion

The major findings of this study suggested that repeated daily treatments of male Wistar rats with a 5-HT_3 receptor antagonist reduced basal DA neurotransmission in the ACB (Fig. 4) and that this effect did not appear to be a result of alterations in the functional activity of local 5-HT_3 receptors. Probe placements indicated these effects occurred in the shell and shell-core of the ACB (Fig. 1).

Evidence for reduced DA neurotransmission in the ACB of the MDL group compared to the saline group is indicated by the results of the no-net-flux experiment, which indicated lower extracellular concentrations of DA in the MDL group, compared to control values, without a change in the extraction fraction (Fig. 4). However, it is not clear if the effects of the MDL injections on DA neurotransmission occurred directly or indirectly on the mesolimbic DA system. Because basal DA neurotransmission decreased, MDL treatments may have up-regulated 5-HT_3 receptors at sites sending inhibitory inputs to the VTA and/or ACB.

The present results are in agreement with the findings of several studies indicating that the effects of chronic administration of 5-HT_3 receptor antagonists reduces VTA DA neuronal activity. Chronic treatment with the 5-HT_3 receptor antagonist DAU 6215 reduced basal extracellular levels of DA in the rat ACB (Invernizzi et al., 1995). Additionally, electrophysiological studies showed that chronic treatment with 5-HT_3 receptor antagonists reduced the number of spontaneously active DA cells in the VTA (Minabe et al., 1992; Rasmussen et al., 1991; Prisco et al., 1992). Therefore, the overall results suggest that chronic treatment with 5-HT_3 receptor antagonists increases the net inhibitory tone regulating VTA DA neuronal activity.

In the present study, only the MDL compound was tested. Because MDL can act at $GABA_A$ receptors (Klein et al., 1994) and possesses affinity for the 5-HT_4 receptor (Gebauer et al., 1993), it is possible that the effects of chronic MDL treatment on reducing DA neurotransmission may be related to its effects at these receptors. This is not a likely explanation because chronic treatment with several different 5-HT_3 receptor antagonists appears to have similar effects on reducing VTA DA neuronal activity (Invernizzi et al., 1995; Minabe et al., 1992; Prisco et al., 1992; Rasmussen et al., 1991).

It might be expected that a reduction in basal extracellular DA levels should also have been observed in the VTA as was observed in the ACB of the MDL group, if chronic MDL

treatment reduced spontaneously active DA neurons in the VTA. However, in the ACB, a difference in basal DA levels was only observed when the baseline values for several CPBG experiments were pooled or when extracellular concentrations were determined with the quantitative no-net flux microdialysis procedure. Future studies would need to be undertaken to assess the effects of repeated MDL injections on the basal extracellular concentrations of DA in the VTA using the no-net flux procedure.

The current study indicated that local perfusion of the ACB and VTA with CPBG increased the extracellular levels of DA in both regions (Figs. 2 and 3). These results suggest that local activation of 5-HT_3 receptors increases terminal DA release in the ACB and somatodendritic DA release in the VTA of Wistar rats, and are in general agreement with previous studies from our laboratory (Campbell and McBride, 1995; Campbell et al., 1996). However, the study of Campbell and McBride (1995) reported dose–response increases in DA overflow occurring following perfusion with 5 to 100 μM CPBG. In the present study, there was little difference between the effects of 17.5 and 35 μM CPBG on DA overflow in the ACB of the saline-treated rats. It is not clear why a dose–response effect was not observed in the present study between these two concentrations of CPBG. With the exception of a lower perfusion rate in the present study (0.5 μl/min) compared to the previous study (1.2 μl/min), the microdialysis parameters were similar.

Although the slower perfusion rate used in the present study may not have resulted in CPBG reaching steady state levels within the 60-min time period, comparison of the present data with that previously published with a higher flow rate indicate similar findings. Comparison of the time-course plots for 35 μM CPBG between the present and the previous study indicated that the shape of the plot between the two studies was similar and that the peak effects in both studies occurred between 40 and 60 min. However, the peak response was higher in the previous study (~440%) than in the present study (~300%) for 35 μM CPBG. This was not the case for 17.5 and 70 μM CPBG. Comparable percent increases were observed for the lowest CPBG concentration in both studies (~300%), and higher DA extracellular concentrations were observed in the present study (~1300%) than previously reported (~900%) at the highest CPBG concentration. Therefore, it does not appear that a difference in the perfusion flow rate between the two studies is a factor contributing to the response of CPBG in the ACB. In addition, the lower flow rate used in the present study did not apparently influence CPBG effects in the VTA because the shape of the plot and peak response (250% vs. 300%) were similar between the present study and that previously published (Campbell et al., 1996).

The results of the present study indicate that perfusion with CPBG increases the extracellular concentrations of DA more in the ACB and VTA of the MDL-treated rats than saline injected rats when determined on the basis of percent of baseline (Figs. 2 and 3). However, if the baseline difference in DA concentration is taken into account, there are no differences between the MDL-treated group and the control group with regard to the effects of CPBG on DA release in the ACB. Additional data will need to be obtained for the VTA to assess whether any changes in the 5-HT_3 receptor occurred in this region.

Within the ACB or VTA, it is not known if CPBG is acting at 5-HT_3 receptors located on DA neurons (terminals and/or cell bodies), or indirectly on other inputs that regulate terminal or somatodendritic DA release. However, within the VTA, there is evidence that DA neurons receive direct 5-HT innervation (Van Bockstaele et al., 1994), and the posterior VTA receives a high density of 5-HT fibers from the raphe nuclei (Halliday and Tork, 1989; Herve et al., 1987; Parent et al., 1981).

The effective tissue concentration of CPBG during perfusion is difficult to determine. Although the diffusion radius for this compound has not been determined, based on previous studies with ethanol, dopamine and mannitol (Gonzales et al., 1998; Höistad et al., 2000), the concentration of CPBG is highest immediately around the probe and decreases to near zero at distances of 1 mm from the probe. CPBG can act at the DA transporter to block reuptake (Campbell et al., 1995). However, at the lowest concentration of CPBG used in the ACB and the 35 μM concentration used in the VTA, CPBG does not appear to block DA uptake as suggested by the lack of effect of CPBG on extracellular levels of DOPAC and HVA (Campbell and McBride, 1995; Campbell et al., 1996). If CPBG was blocking DA uptake as well as activating 5-HT_3 receptors, then the extracellular levels of DOPAC and HVA would decline over time. In addition, at the lowest concentration of CPBG in the ACB and at the 35 μM CPBG concentration in the VTA, co-perfusion with a 5-HT_3 receptor antagonist blocks the CPBG-enhanced release of DA (Campbell and McBride, 1995; Campbell et al., 1996). Because 5-HT_3 receptor antagonists do not appear to bind to the DA transporter (Svingos and Hitzemann, 1992), it would appear that the antagonists are blocking CPBG effects mainly at the 5-HT_3 receptor, as opposed to the DA transporter. Also, in the present study, if the effects of CPBG at the highest concentrations were mainly due to blocking DA uptake, then the CPBG would be expected to have a more pronounced effect on the extracellular levels of DA in the saline-treated group because this group had higher basal DA release than did the MDL group.

In summary, the present research provides additional support that 5-HT_3 receptors are involved in regulating extracellular DA release in the ACB and posterior VTA. In addition, basal DA neurotransmission is reduced in the ACB after repeated daily treatment with a 5-HT_3 receptor antagonist.

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