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Activation of serotonin-3 receptors increases dopamine release within the ventral tegmental area of Wistar and alcohol-preferring (P) rats

Wen Liu¹, Richard J. Thielen^{1,2}, Zachary A. Rodd¹, and William J. McBride^{1,2}

1 Institute of Psychiatric Research, Department of Psychiatry, Indiana University School of Medicine, Indianapolis, IN 46202

2 Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN 46202

Abstract

The objectives of the present study were to examine (a) the effects of activating serotonin-3 (5-HT₃) receptors on dopamine (DA) release in the anterior and posterior ventral tegmental area (VTA) of Wistar rats; and (b) determine if there are differences in 5-HT₃-stimulated DA release in the VTA between alcohol-preferring (P) and Wistar rats. Local perfusion with the 5-HT₃ agonist 1-(m-chlorophenyl)-biguanide (CPBG) in the anterior and posterior VTA stimulated DA release in the both regions. The CPBG-stimulated increase in extracellular DA levels was significantly higher in the posterior than anterior VTA of Wistar rats. The basal extracellular DA levels were not significantly different between the anterior and posterior VTA of Wistar rats. However, the basal extracellular DA levels were significantly higher in the posterior VTA of Wistar rats than P rats. Local perfusion of CPBG into posterior VTA stimulated somatodendritic DA release significantly more in the P than Wistar rat. Overall, the results indicate that there may be a heterogeneous distribution of functional 5-HT₃ receptors within the VTA with higher numbers in the posterior than anterior VTA, and that, compared to 5-HT₃ receptors in Wistar rats, 5-HT₃ receptors in the posterior VTA of P rats may be more responsive to stimulation.

Keywords

serotonin-3 receptor; dopamine; ventral tegmental area; CPBG; alcohol-preferring P rat

INTRODUCTION

Alcohol-preferring P rats were originally derived, along with the alcohol-nonpreferring NP rats, from an outbred Wistar rat strain to develop a genetic model of alcohol-seeking behavior (Lumeng et al., 1977). To this end, the P rats have been extensively characterized over the last two decades (Murphy et al., 2002) and have been shown to meet most of the requirements for an animal model of alcoholism (Cicero, 1979)

Corresponding author: Dr. R.J. Thielen, Institute of Psychiatric Research, 791 Union Dr., Indianapolis, IN 46202-4887. Phone: 317-278-4809; Fax: 317-274-1365; e-mail: rthielen@iupui.edu

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P rats show a number of differences compared to NP and Wistar rats in measure of the mesolimbic dopamine (DA) system. These include reduced tissue levels of DA in the nucleus accumbens compared to NP rats (Murphy et al., 1987) and reduced numbers of tyrosine hydroxylase stained fibers in the nucleus accumbens compared to NP rats (Zhou et al., 1995). Additionally, P rats have been shown to have reduced numbers of D2 DA receptors in the ventral tegmental area (VTA) compared to NP rats (McBride et al., 1993). P rats also show a higher percentage of burst firing activity of DA VTA neurons compared to Wistar rats (Morzorati et al., 1998).

Electrophysiological and microdialysis studies indicate that serotonin-3 (5-HT₃) receptors play a role in regulating the activity of the ventral tegmental area (VTA) DA neurons. Acute systemic administration of 5-HT₃ receptor antagonists decreased the number of spontaneously active VTA DA neurons (Minabe et al., 1991; Rasmussen et al., 1991), suggesting that the activity of these neurons is regulated in part by tonic activation of 5-HT₃ receptors. Local administration of a 5-HT₃ receptor agonist increased the extracellular levels of DA in the VTA (Campbell et al., 1996), supporting the idea that 5-HT₃ receptors regulate the activity of VTA DA neurons. A recent microdialysis study from our laboratory demonstrated that local perfusion with a 5-HT₃ receptor agonist stimulated DA release in the posterior VTA of Wistar rats (Liu et al., 2006). However, neither this latter study nor the study of Campbell et al (1996) attempted to determine whether there were sub-regional differences within the VTA for 5-HT₃ stimulated DA release.

Studies on the cytoarchitecture, anatomical connectivity and behavioral function of the VTA indicate that this region is not a homogeneous structure. Within the VTA, the nucleus parabrachialis, nucleus parabrachialis pigmentosus and nucleus linearis raphe caudalis are the main DA containing nuclei (Phillipson, 1979a). Moreover, the neurons within each nucleus of VTA have distinct characteristics (Phillipson, 1979b). The studies of Oades and Halliday (1987) reported that the VTA could be divided into 5 sub-nuclei, and the study of Herve, et al. (1987) indicated that there is an uneven distribution of serotonin inputs within the VTA. Behavioral functional studies indicated that Wistar rats self-administered a GABA_A receptor antagonist directly into the anterior, but not posterior VTA (Ikemoto et al., 1997), whereas a GABA_A receptor agonist was self-administered into the posterior but not anterior VTA (Ikemoto, et al., 1998), suggesting that there were differences between the anterior and posterior VTA with regard to the distribution and function of GABA_A receptors.

The study of Rodd-Henricks et al. (2000) indicated that Wistar rats initiated and maintained the self-infusion of ethanol into the posterior but not anterior VTA, and that the reinforcing effects of ethanol required activation of VTA DA neurons and local 5-HT₃ receptors (Rodd-Henricks, et al., 2003). Another study indicated a similar finding for alcohol-preferring (P) rats, namely, ethanol was self-infused into the posterior but not anterior VTA, and that the reinforcing effects of ethanol required activation of VTA DA neurons and local 5-HT₃ receptors (Rodd et al., 2005b). Moreover, a higher dose of a 5-HT₃ antagonist was needed to inhibit self-infusion of ethanol into the posterior VTA of P than Wistar rats, suggesting that there might be differences between the rat lines in 5-HT₃ receptor function in the posterior VTA.

A recent study indicated that the 5-HT₃ agonist 1-(m-chlorophenyl)-biguanide (CPBG) was self-infused into the posterior but not anterior VTA of Wistar and P rats (Rodd et al., 2006), suggesting that there might be differences in the distribution of 5-HT₃ receptors between the anterior and posterior VTA. Moreover, the posterior VTA of P rats was more sensitive to the reinforcing effects of CPBG than was the posterior VTA of Wistar rats (Rodd et al., 2006).

The present study was undertaken to examine (a) whether there were differences between the posterior and anterior the VTA on the effects of local perfusion with the 5-HT₃ agonist CPBG on DA release; and (b) whether there were differences in the dose-response effects of CPBG on somatodendritic DA release in the posterior VTA between P and Wistar rats. The hypotheses to be tested were (a) CPBG would have a greater effect on DA release in the posterior than anterior VTA; and (b) the posterior VTA of P rats is more sensitive than is the posterior VTA of Wistar rats to the stimulating effects of CPBG on DA release.

MATERIALS AND METHODS

Animals

Adult male and female Wistar (Harlan, Indianapolis, IN) rats and female P rats (from the 56th generation), weighing 250–300 g at the time of surgery were used. All animals were housed in a constant temperature and relative humidity colony room under a regular 12-hr light/dark cycle (lights on at 0700 hr) with food and water available ad libitum. The animals used in this study 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).

Male and female Wistar rats were used in the present study. Female Wistar rats were used so that a comparison with female P rats could be made. Female P rats were used in the present study because previous studies with P rats, requiring critical stereotaxic placements, had been conducted with females (Gatto et al., 1994; Rodd-Henricks et al., 2002; Rodd et al., 2003, 2004, 2005b). Stereotaxic coordinates (Paxinos and Watson 1998) were determined for a rat weighing around 275–325 g. Because male P rats are larger than male Wistar rats or female P rats, they have larger heads and thicker skulls making it more difficult to reliably insert cannulas that delineate the anterior and posterior VTA. Previous studies (Ikemoto et al., 1997, 1998; Gatto et al., 1994; Rodd-Henricks et al., 2000, 2002, 2003; Rodd et al., 2004, 2005b) did not indicate any significant effect of the estrous cycle, as suggested by the self-infusion of the same concentration of the infusate across sessions over a 2–3 week period.

Stereotaxic surgery

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 an 18-gauge stainless steel guide cannula (Plastics One). The guide cannula was aimed at the anterior or 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 –4.6 mm, L +2.0 mm, D/V –5.9 mm for the anterior VTA; and (b) AP –5.8 to –6.0 mm, L +2.0 mm, D/V –5.9 mm for posterior VTA with a 10° angle to the vertical. Two stainless steel screws were placed in the skull to secure the guide cannula with cranioplastic cement (Plastics One). The rats were allowed at least 5–6 days to recover from surgery 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 hr each day for 2 days.

The day before microdialysis, rats were briefly anesthetized with isoflurane (less than 15 min), and the microdialysis probe was inserted through 18-gauge guide cannula and cemented into place. The probe was made as previously described (Kohl et al., 1998; Rahman and McBride 2002); the length of active dialysis probe was 1.5 mm and the probe was oriented in a rostral-caudal direction.

Local perfusion of VTA with CPBG

On the day of dialysis, six animals were placed in the microdialysis chambers; the probes were connected to the microinfusion pumps and perfused at a flow rate of 0.5 μ l/min with artificial CSF (145 mM NaCl, 2.7 mM KCl, 1.0 mM MgCl₂, 1.2 mM CaCl₂, 2.0 mM Na₂HPO₄, 0.2 mM ascorbate; pH 7.2~7.4) for 120 min prior to collecting the baseline samples. Three 20-min baseline samples were then collected. Animals were then perfused with artificial CSF containing CPBG (17.5, 35 or 70 μ M; Sigma-Aldrich) by disconnecting/reconnecting the lines at the top of the collection assembly. After 60 min perfusion with artificial CSF containing CPBG, the rats were switched back to artificial CSF 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 et al., 1996; Liu et al., 2005). Samples were collected in 0.5 ml polyethylene tubes containing 2 μ l 0.1 M perchloric acid to prevent DA oxidation during storage. Samples were immediately frozen on dry ice and stored at -70°C until analyzed.

Probe placements

Probe placements were verified by perfusion of a 1% bromphenol blue solution through the probe at the end of the experiment. Rats were overdosed with carbon dioxide, and decapitated; the brains were removed. Frozen 40 μ m 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 anterior or posterior VTA were used.

DA analysis

DA levels were determined using microbore HPLC with electrochemical detection. The samples were injected onto a reverse-phase microbore column (1.0 $\times$ 100 mm BDS Hypersil C18 column, 3 μ m particle size; Thermo Hypersil-Keystone, Bellefonte, PA) connected to a Rheodyne injector (5 μ l loop, Cotati, CA, USA). Mobile phase consisted of 77 mM sodium acetate, 0.5 mM EDTA, 3.5 mM sodium octanesulfonic acid, 9.9 mM NaCl and 6% acetonitrile, the pH was adjusted to 4.0 with acetic acid; the flow rate was 0.07 ml/min (Modal 582 pump, ESA, Chelmsford, MA, USA). The concentration of DA was determined with electrochemical detection using a Unijet radial-flow detector cell with 6 mm glassy carbon electrode using a model LC-4C amperometric detector (Bioanalytical System, West Lafayette, IN, USA) set at a potential of 450 mV and a sensitivity of 0.5 μ A).

Data analysis

DA baseline data in the anterior and posterior VTA are expressed as nM DA after correction for dilution by the perchloric acid. DA baseline between the anterior and posterior VTA of Wistar rats or between the posterior VTA of Wistar and P rats was separately analyzed using 2- way ANOVAs followed by Student's t test where appropriate. For determining the effect of CPBG in the anterior and posterior VTA of Wistar rats, DA levels were normalized and expressed as percent of baseline. Time-course data for the two CPBG concentrations in the VTA subregions were analyzed by a mixed ANOVA (region $\times$ concentration $\times$ time) with time as the repeated measure. For the effect of CPBG in the posterior VTA of Wistar and P rats, time-course data for the three CPBG concentrations were analyzed by a mixed ANOVA (line $\times$ concentration $\times$ time) with time as the repeated measure. Significant interactions were further analyzed by orthogonal contrasts. Where appropriate, Student's t-test was used to compare individual means. In addition, area under the curve was calculated using Prism (ver. 4, GraphPad Software, Inc. San Diego, CA, USA). Area under the curve was analyzed by Student's t-test.

RESULTS

Effects of CPBG on DA release in the anterior and posterior VTA of male Wistar rats

Only data from animals with probe correctly implanted in the anterior or posterior VTA were included in the analysis (Fig. 1). Within the anterior VTA, the main part of the probe was located at -4.6 to -5.2 mm relative to bregma, according to the atlas of Paxinos and Watson (1998). Within the posterior VTA, the main part of the probe was located at -5.8 to -6.3 mm relative to bregma. Approximately 85% of the animals that had undergone surgery had probes correctly implanted within the anterior or posterior VTA. However, because of the diameter of the probes, there was generally some overlap between the anterior and posterior VTA placements. Therefore, it was not always possible to clearly delineate the anterior and posterior VTA.

Examination of basal DA levels (average of the three 20-min baseline samples) in the anterior and posterior VTA of each of the doses by 2-way ANOVA (region $\times$ CPBG concentration) did not reveal any significant differences. The pooled basal extracellular levels of DA were 1.05 ± 0.19 and 0.97 ± 0.20 nM ($p = 0.77$, $n = 11$) in the anterior and posterior VTA of male Wistar rats, respectively. Local perfusion of 35 and 70 μ M CPBG into the posterior VTA significantly increased the extracellular levels of DA in the posterior VTA to approximately 370% for 35 μ M CPBG, and 800% for 70 μ M CPBG (Fig. 2). Within the anterior VTA, local perfusion with 35 and 70 μ M CPBG resulted in increases in extracellular DA levels of approximately 225% for 35 μ M CPBG, and 410% for 70 μ M CPBG (Fig. 2). The time course of both doses of CPBG on stimulating DA release indicated that the maximal response occurred 40–60 min after adding the CPBG in both the anterior and posterior VTA.

A mixed AVOVA revealed that there was a significant region $\times$ concentration $\times$ time interaction [$F(7,126) = 57.6$, $p < 0.001$]. Two-way ANOVAs were conducted on each CPBG concentration separately with the significant overall multivariate F obtained. With both 35 μ M and 70 μ M CPBG, there was a significant effect of time [$F(7, 70) = 22.8$, $p < 0.001$ for 35 μ M; $F(7, 56) = 33.4$, $p < 0.001$ for 70 μ M], and a region $\times$ time interaction [$F(7, 70) = 5.3$, $p < 0.001$ for 35 μ M; $F(7, 56) = 6.2$, $p < 0.001$ for 70 μ M]. The extracellular levels of DA in the posterior VTA were significantly ($p < 0.01$) higher than the values in the anterior VTA at 40, 60 and 80 min after initiating perfusion with 35 μ M CPBG, and at 20, 40 and 60 min after perfusion with 70 μ M CPBG (Fig. 2). The area under the curve values (insets Fig. 2) indicated that the overall CPBG-stimulated increase in the extracellular levels of DA in the posterior VTA was 2- to 3-fold higher than levels in the anterior VTA.

Comparison of the effects of CPBG on extracellular levels of DA in the posterior VTA of female P and Wistar rats

For this study, the main part of the probe was located -5.80 to -6.30 mm relative to bregma, according to the atlas of Paxinos and Watson (1998). Approximately 90% of the probes were correctly implanted within the posterior VTA. Examination of basal DA levels (average of the three 20-min baseline samples) in the posterior VTA of P and Wistar rats for each of the doses by 2-way ANOVA (region $\times$ CPBG concentration) did not reveal any significant dose effect or dose $\times$ strain interaction; however, there was a significant main effect of strain [$F(1, 28) = 7.67$, $p < 0.05$]. The pooled basal extracellular DA levels, were 0.95 ± 0.12 nM for Wistar rats ($n = 17$) and 0.59 ± 0.07 nM for P rats ($n = 17$). Local perfusion with all three doses of CPBG (17.5, 35, 70 μ M) into the posterior VTA significantly increased the extracellular levels of DA of both Wistar and P rats (Fig. 4). A three-way ANOVA indicated that there was a main effect of dose [$F(2, 28) = 63.08$; $p < 0.0001$], time [$F(7, 70) = 16.91$; $p < 0.0001$] and strain [$F(1, 28) = 43.63$; $p < 0.0001$], and a significant strain $\times$ concentration $\times$ time interaction [$F(14,196)$

= 1.84; $p < 0.05$]. The time course of the 3 concentrations of CPBG indicated that the maximal response occurred 40–60 min after CPBG for both Wistar and P rats.

With 17.5 μM CPBG (Fig. 4A), local perfusion resulted in a significant increase in the extracellular levels of DA in both P and Wistar rats [$F(7, 70) = 16.9$, $p < 0.001$], but there was no significant difference in the percent increase in DA relative to baseline between the two strains [$F(1, 10) = 2.53$; $p > 0.05$] nor a significant time $\times$ strain interaction [$F(7, 70) = 1.87$, $p > 0.05$]. With 35 μM CPBG (Fig. 4B), local perfusion resulted in a significant increase in the extracellular levels of DA in both P and Wistar rats [$F(7, 70) = 24.4$, $p < 0.001$], but there was no significant main effect of strain [$F(1, 10) = 1.77$, $p > 0.05$]. There was, however, a significant time $\times$ strain interaction [$F(7, 70) = 2.42$, $p < 0.05$]. The percent increase, relative to baseline, in the extracellular levels of DA was significantly higher in the posterior VTA of P than Wistar rats at 60 min ($p < 0.05$) after initiating perfusion with the agonist. With 70 μM CPBG (Fig. 4C), local perfusion resulted in a significant increase in the extracellular levels of DA in both P and Wistar rats [$F(7, 56) = 24.5$, $p < 0.001$], as well as a significant main effect of strain [$F(1, 8) = 56.2$, $p < 0.001$] and a significant time $\times$ strain interaction [$F(7, 56) = 3.01$, $p < 0.01$]. The peak percentage increase in the extracellular levels of DA was significantly higher in the posterior VTA of P rats (1,200% of baseline) than Wistar rats (600% of baseline). There were significant differences between the P and Wistar rats at 20, 40 and 60 min ($p < 0.05$) after perfusion with 70 μM CPBG. Analysis of the area under the curve of the normalized data indicated a significantly greater overall effect of CPBG on increasing the extracellular levels of DA in the posterior VTA of P than Wistar rats with both 35 μM CPBG (inset, Fig. 4B) and 70 μM CPBG (inset, Fig. 4C).

Since basal DA levels were significantly different in the posterior VTA of P and Wistar rats, the maximal effect of CPBG administration on the DA concentrations (not normalized to baseline) within the ACB was examined to determine if the increased response of 5-HT₃ receptors observed resulted in higher DA concentrations in the posterior VTA of P compared to Wistar rats or if it corrected the apparent deficit in VTA DA levels of P rats. Two-way ANOVA revealed a significant effect of dose of CPBG on maximal DA concentrations within the ACB [$F(2, 29) = 12.7$, $p < 0.001$]. In Wistar rats, 17.5 μM CPBG increases extracellular DA levels to 2.1 ± 0.8 nM ($n = 6$), while 35 μM CPBG increased it to 4.4 ± 0.5 nM ($n = 6$) and 70 μM CPBG increased DA levels to 6.3 ± 0.6 nM ($n = 5$). In P rats, 17.5 μM CPBG increases extracellular DA levels to 1.8 ± 0.3 nM ($n = 6$), while 35 μM CPBG increased it to 4.6 ± 0.6 nM ($n = 6$) and 70 μM CPBG increased DA levels to 7.1 ± 1.7 nM ($n = 5$). There was no significant effect of line [$F(1, 28) = 0.10$, $p = 0.75$] or a CPBG concentration $\times$ line interaction [$F(2, 28) = 0.21$, $p = 0.81$].

DISCUSSION

The major findings of this study are that local perfusion with the 5-HT₃ receptor agonist CPBG activated 5-HT₃ receptor–stimulated DA release significantly more (a) in the posterior than anterior VTA of Wistar rats (Fig. 2), and (b) in the posterior VTA of P than Wistar rats (Fig. 4). These results suggest that there may be more functional 5-HT₃ receptors in the posterior than anterior VTA of Wistar rats, and higher numbers of functional 5-HT₃ receptors within the posterior VTA of P than Wistar rats.

The present results are in agreement with a previous study that reported local perfusion with CPBG increased the extracellular levels of DA in the VTA of Wistar rats, and these effects of CPBG could be blocked with co-perfusion of a 5-HT₃ antagonist (Campbell et al., 1996). However, in this latter study, there was no attempt to differentiate the anterior and posterior VTA. In the present study, the anterior VTA is defined as -4.6 to -5.2 mm relative to bregma, and the posterior VTA is defined as -5.6 to -6.3 mm relative to bregma (Figs. 1 and 3). These

boundaries have been defined by several studies that indicated differences along the anterior-posterior VTA axis for the intracranial self-infusion of GABA_A agonists and antagonists (Ikemoto et al., 1997,1998), ethanol (Rodd-Henricks et al., 2000), acetaldehyde (Rodd et al., 2005b), and cocaine (Rodd et al., 2005a). In these cases, the posterior VTA supported the self-infusion of ethanol (Rodd-Henricks et al., 2000), acetaldehyde (Rodd et al., 2005b), cocaine (Rodd et al., 2005a) and muscimol (Ikemoto et al., 1998), whereas the anterior VTA did not. On the other hand, the anterior VTA supported the self-infusion of GABA_A antagonists, whereas the posterior VTA did not (Ikemoto et al., 1997). Furthermore, the self-infusion of ethanol and cocaine into the posterior VTA was inhibited by co-infusion with 5-HT₃ antagonists (Rodd et al., 2005 a,b), suggesting that local activation of 5-HT₃ receptors is involved in mediating their self-infusions.

The elevated extracellular levels of DA in the VTA following local perfusion with CPBG (Fig. 2) suggest that activation of 5-HT₃ receptors is stimulating somatodendritic DA release. However, it is not known if 5-HT₃ receptors are located on DA cell bodies and/or on terminals regulating the activity of DA neurons. The greater stimulating effect of CPBG on DA release in the posterior VTA compared to the anterior VTA may be a result of higher numbers of functional 5-HT₃ receptors in the posterior than anterior VTA, and/or a difference in the cellular/terminal distribution within the VTA, such that stimulation of DA neurons takes place in the posterior but not in the anterior VTA when local 5-HT₃ receptors are activated.

Because of the diameter of the microdialysis probe (approximately 0.5 mm), it is unlikely that the anterior and posterior VTA were exclusively delineated. Therefore, it is possible that the relatively low response to CPBG observed in the anterior VTA may mainly arise from the contribution of the posterior VTA as a result of some probe overlap into that region. An intracranial self-infusion study, which allows a better delineation of the anterior and posterior VTA, indicated that CPBG was self-infused into the posterior but not anterior VTA (Rodd et al., 2006), supporting the idea that there are higher numbers of functional 5-HT₃ receptors regulating DA neuronal activity in the posterior than anterior VTA.

The apparent slow onset of the effects of CPBG (Figs. 2 and 4) are likely due to initial dilution of the CPBG with the perfusion media in combination with diffusion time constants for CPBG and DA. However, it is also possible that CPBG may be having an effect that reflects a more distal or indirect action.

A previous study showed that the effect of CPBG on DA overflow in the VTA of Wistar rats could be completely blocked by the selective 5-HT₃ receptor antagonist ICS 205–930 (Campbell et al., 1996). This, taken with the data showing that CPBG is a highly selective agonist at 5-HT₃ receptors compared to other serotonin receptors (Kilpatrick et al., 1990), supports the contention that the effects of CPBG on DA overflow seen in this study are the result of activation of 5-HT₃ receptors by CPBG. Additionally, Rodd and coworkers (2006) showed that the self-administration of CPBG directly into the posterior VTA can be blocked by ICS 205–930, further supporting the role of 5-HT₃ receptors in the actions of CPBG within this region in both Wistar and P rats. This, however, does not rule out the possibility that other serotonin receptors may be involved in mediating the effects of the natural agonist serotonin on VTA DA neurons.

The basal level of extracellular DA was approximately 40% lower in the posterior VTA of the P than Wistar rat. This suggests that, in the posterior VTA of the P compared to the Wistar rat, there may be (a) fewer DA neurons, (b) the same numbers of DA neurons but the activities of the DA neurons are lower, and/or (c) the clearance (uptake, recovery) of DA is greater. Neurochemical and neuroanatomical data suggest that there might be deficiencies in the mesolimbic DA system of the P rat compared to the alcohol-non-preferring NP rats (Murphy

et al., 1987; McBride et al., 1993; Zhou et al., 1995). Murphy et al. (1987) reported lower tissue levels of DA in the nucleus accumbens of the P compared to the NP rat, and Zhou et al. (1995) reported that there were fewer tyrosine hydroxylase immunostained fibers projecting to the nucleus accumbens from the VTA of the P compared to the NP rat. The study of McBride et al. (1993) found fewer D2 receptors in the VTA and nucleus accumbens of P compared to NP rats, which could be interpreted to reflect fewer DA neurons in the P than NP rat. If the low alcohol-drinking NP and Wistar rats have similar mesolimbic DA systems, then the present finding would be consistent with a reduced number of DA neurons in the posterior VTA of P rats compared to Wistar rats. Smith and Weiss (1999), using no-net-flux quantitative microdialysis, examined the extracellular levels and clearance of DA in the nucleus accumbens of ethanol-naïve P, NP and Wistar rats and reported no significant difference in either measurement between any of the strains. However, in this study, the probes were primarily located in the core region of the nucleus accumbens, which might not receive significant DA projections from the posterior VTA.

Morzorati (1998), using extracellular recording techniques, examined basal DA neuronal activity in the VTA of P and Wistar rats, and reported a higher percentage of DA neurons demonstrating burst firing activity in the P than Wistar rat; no differences in other burst firing parameters or firing frequencies were detected. These electrophysiological results suggest that the lower basal extracellular DA levels, observed in the present study, may not be due to lower DA neuronal activity in the VTA of the P than Wistar rat. However, the recordings in the study of Morzorati (1998) were taken between -5.3 to -5.6 mm bregma, which is anterior to the microdialysis probe placements (Fig. 3). Additional studies in the posterior VTA (e.g., no-net-flux quantitative microdialysis, extracellular recordings) will need to be conducted to better understand the mechanisms underlying the difference in the basal extracellular levels of DA observed in the present study.

The dose-response effects of CPBG on the extracellular levels of DA in the posterior VTA of P and Wistar rats (Fig. 4) indicated that CPBG had a greater effect in the P than Wistar rat, and this effect was most pronounced at the highest concentration. Because the results are presented as percent of baseline, the higher increases in the extracellular levels of DA in the posterior VTA of the P rat is partly due to the lower basal extracellular levels of DA in the P than Wistar rat. This would suggest that the increased response of 5-HT₃ receptors to CPBG in the posterior VTA of P rats restores DA neurotransmission to the level of Wistar rats when these receptors are activated. That appears to be the case as there were no significant differences between Wistar and P rats in the maximal DA level achieved at any of the CPBG concentrations tested, although the net increase was approximately 20 % greater in the P than Wistar rat (6.5 nM versus 5.3 nM) at the highest CPBG concentration. The results suggest that there may be higher numbers of functional 5-HT₃ receptors in the posterior VTA of the P than Wistar rat. Alternatively, it is also possible that there are no differences in the number of functional 5-HT₃ receptors but there might be differences in the distribution of 5-HT₃ among the cellular elements within the posterior VTA between P and Wistar rats. Either way, this may be an attempt by the system to restore proper function in response to a reduced basal DA tone observed in P rats.

The present finding of a higher response with CPBG on the extracellular levels of DA in the posterior VTA of the P relative to the Wistar rat is also in agreement with results for intracranial self-infusion studies. A 4-fold higher concentration of a 5-HT₃ receptor antagonist was required to inhibit ethanol self-infusion into the posterior VTA of P than Wistar rats (Rodd-Henricks et al., 2003; Rodd et al., 2005b), suggesting differences in 5-HT₃ receptor function between P and Wistar rats. Another study (Rodd et al., 2006) indicated that P rats, compared to Wistar rats, self-administered lower concentrations and received more infusions of CPBG into the posterior VTA. These latter findings also suggest that there are functional differences in 5-HT₃ receptors

within the posterior VTA between P and Wistar rats, with the 5-HT₃ receptors of the P rat being more sensitive and responsive to CPBG. Thus, all of the findings are consistent with increased response of 5-HT₃ receptors to agonist activation in the posterior VTA of P compared to Wistar rats. However, it is not clear if greater response to CPBG is due to more 5-HT₃ receptors, and/or changes in the functional properties of the receptor.

The current results indicated that the basal extracellular levels of DA in the posterior VTA of adult male and female Wistar rats were similar (1.05 ± 0.20 and 0.95 ± 0.12 nM). Although the response with 35 μ M CPBG was similar between the male and female Wistar rats, the increase in the extracellular levels of DA with 70 μ M CPBG was approximately 40% higher ($p < 0.05$) in the male than the female Wistar rat. These results suggest that there may be differences in the number and/or functional activity of 5-HT₃ receptors in the posterior VTA between male and female rats; however, because the studies with the male and female Wistar rats were conducted at different times (separated by weeks), additional studies will need to be undertaken to confirm and extend a comparison between male and female Wistar rats.

In conclusion, the results of the present study suggest that there is 5-HT₃ receptor functional heterogeneity within the VTA of Wistar rats. In addition, the results of the present study suggest that 5-HT₃ receptors in the posterior VTA of P rats may be more responsive to stimulation than those in the posterior VTA of Wistar rats.

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Fig 1. Microdialysis probe placements in the anterior (left side) and posterior ventral tegmental area (VTA, right side). Each line indicates the active dialysis membrane of probes used in the study. Overlapping placements are not shown. The numbers in the right of each slice indicate distance (mm) from bregma (adapted from Paxinos and Watson 1998).

Fig 2.

Effects of local perfusion with 35 μ M and 70 μ M CPBG (A and B) on extracellular DA levels in the anterior and posterior VTA of male Wistar rats. The data were expressed as percentage of baseline, and each point is means $\pm$ S.E.M. (n = 5–6/group). A mixed ANOVA with repeated measure revealed significant time $\times$ concentration $\times$ region interaction [F (7,126) = 57.6, p < 0.001]. * p < 0.05 compared with the anterior VTA group.

Fig 3.

Microdialysis probe placements in the posterior VTA of female Wistar (left side) and P (right side) rats. Each line indicates the active dialysis membrane of probes used in the study. Overlapping placements are not shown. The numbers in the right of each section indicate distance (mm) from bregma (adapted from Paxinos and Watson 1998).

Fig 4.

Effects of local perfusion with different concentrations of CPBG (A: 17.5; B: 35 and C: 70 μ M) on extracellular DA levels in the posterior VTA of female P and Wistar rats. The data were expressed as percentage of baseline, and each point is means $\pm$ S.E.M. ($n=6$ /group). A mixed ANOVA with repeated measure revealed a significant line $\times$ time $\times$ concentration interaction [$F(14,196) = 1.84$; $p < 0.05$]. * $p < 0.05$ compared with Wistar rats.