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Activation of 5-$HT_{1B/1D}$ receptors in the mesolimbic dopamine system increases dopamine release from the nucleus accumbens: a microdialysis study

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Abstract

This study was designed to investigate the role of 5-hydroxytryptamine (5-HT)$_{1B}$ receptors located in the ventral tegmental area and nucleus accumbens in the modulation of accumbal dopaminergic transmission. The selective 5-HT_{1B} receptor agonist CP 93129 {3-(1,2,5,6-tetrahydro-4-pyridyl)pyrrolo[3,2-*b*]pyrid-5-one} was administered into the ventral tegmental area or nucleus accumbens of freely moving Sprague–Dawley rats via retrograde microdialysis. The effects of intra-accumbal and intra-tegmental CP 93129 on extracellular dopamine levels in the nucleus accumbens were measured using one- and dual-probe microdialysis, respectively. For dual-probe microdialysis, one probe was in the ventral tegmental area for drug administration and the other in the ipsilateral nucleus accumbens for dopamine measurement. The results show that infusion of CP 93129 (2, 5 and 10 μM) into the nucleus accumbens increased local dopamine levels in a concentration-related manner. Infusion of CP 93129 (10 and 20 μM) into the ventral tegmental area also increased dopamine levels in the ipsilateral nucleus accumbens. The increased dopamine release in the nucleus accumbens produced by intra-accumbal or intra-tegmental CP 93129 was antagonized by co-infusion of cyanopindolol (5 μM), a 5-$HT_{1B/1A}$ receptor antagonist, but not by WAY-100635 {*N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-2-pyridinyl-cyclohexanecarboxamide} (5 μM), a highly selective 5-HT_{1A} receptor antagonist. In addition, augmentations of dopamine release in the nucleus accumbens induced by intra-accumbal CP 93129 were sensitive to Na^+ channel blockade with tetrodotoxin. These results are not in opposition to the concept that 5-HT_{1B} receptors within the ventral tegmental area and nucleus accumbens are all involved in the modulation of dopamine release in the terminal area of the mesolimbic dopamine system.

Keywords: CP 93129; Cyanopindolol; WAY-100635; Dopamine; Ventral tegmental area; Nucleus accumbens; Microdialysis

1. Introduction

Anatomical studies of the afferent pathways to the ventral tegmental area have indicated that this region receives serotonergic innervations from the dorsal and medial raphe nuclei (Moore et al., 1978; Herve et al., 1987). The nucleus accumbens also receives a relative dense input of serotonergic fibers from raphe nuclei (Moore et al., 1978; Vertes and Martin, 1988). Consistent with anatomical evidence, behavioral, biochemical and electrophysiological reports showed the existence of a functional relationship between 5-hydroxytryptamine (serotonin, 5-HT) and dopamine in the mesolimbic dopamine system (De Deurwaerdere and Spampinato, 1999; Di Matteo et al., 1998; Hallbus et al., 1997; Parsons and Justice, 1993; Yan, 2000).

Dopamine transmission in the mesolimbic dopamine system could be affected by 5-HT both at the level of cell bodies and at the level of the nerve terminals. Several in vivo studies suggest that the serotonergic modulation of the mesolimbic dopamine system has a facilitatory character. Yoshimoto and McBride (1992) have shown that stimulation and inhibition of the raphe nucleus neurons produced a corresponding increase and decrease in the release of dopamine in the nucleus accumbens. In addition, direct administration of 5-HT into the ventral tegmental area (Guan and McBride, 1989) or nucleus accumbens (Parsons and Justice, 1993; Hallbus et al., 1997) all increased extracellular dopamine in the terminal areas of the mesolimbic dopamine system.

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The increase in extracellular dopamine in the nucleus accumbens produced by focally applied 5-HT is related to the stimulation of various 5-HT receptors. It has been shown that 5-HT-induced dopamine release in the nucleus accumbens was antagonized by co-infusion of 5-HT_1, 5-HT_2 or 5-HT_3 receptor antagonists (Parsons and Justice, 1993). Furthermore, local application of (±)-1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane (DOI, a 5-$HT_{2A/2C}$ receptor agonist) (Yan, 2000) or 1-phenylbiguanide (a 5-HT_3 receptor agonist) (Chen et al., 1991) increased extracellular dopamine in the nucleus accumbens. Taken together, the results suggest that 5-HT_1, 5-HT_2 and 5-HT_3 receptors within the nucleus accumbens are all involved in the regulation of dopamine release in this region.

Several subtypes of the 5-HT_1 receptor have been identified. One of the 5-HT_1 receptor subtypes suggested to have a modulating effect on dopamine release is the 5-HT_{1B} receptor. Most studies of 5-HT and dopamine interactions via the 5-HT_{1B} receptor have been focused on the prefrontal cortex and nigrostriatal system. Recent microdialysis studies showed that local infusion of the selective 5-HT_{1B} receptor agonist CP 93129 {3-(1,2,5,6-tetrahydro-4-pyridyl)pyrrolo[3,2-*b*]pyrid-5-one} or CP 94253 {3-(1,2,5,6-tetrahydro-4-pyridyl)-5-propoxypyrrolo[3,2-*b*]-pyridine} into the prefrontal cortex (Iyer and Bradberry, 1996), substantia nigra (Thorre et al., 1998) or striatum (Galloway et al., 1993) increased dopamine release in these regions. In addition, increases in dopamine release in the prefrontal cortex produced by perfusion with either 5-HT, 5-HT uptake blockers or 5-HT_{1B} receptor agonists were attenuated or abolished by co-perfusion with the 5-$HT_{1B/1D}$ receptor antagonist GR 127935 {*N*-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-[1,1-*b*iphenyl]-4-carboxamide]} (Iyer and Bradberry, 1996; Matsumoto et al., 1999), suggesting that 5-$HT_{1B/1D}$ receptors may be associated with the facilitation of dopamine release in the prefrontal cortex.

The nucleus accumbens contains a high density of 5-HT_{1B} receptors (Bruinvels et al., 1993). However, the significance of this receptor subtype in the regulation of accumbal dopaminergic transmission remains largely unexplored. There are only a few reports in the literature showing the potential involvement of 5-HT_{1B} receptors in the regulation of accumbal dopamine release. Hallbus et al. (1997) observed that the elevation of accumbal dopamine produced by focal 5-HT perfusion was antagonized by co-perfusion of GR 127935, consonant with the involvement of 5-$HT_{1B/1D}$ receptors in modulation of accumbal dopaminergic transmission. In the study by Hallbus et al. (1997), however, stimulation of dopamine release was induced by perfusion with 5-HT which can act at all 5-HT receptors including 5-HT_2 and 5-HT_3 subtypes. Activation of 5-HT_2 (Yan, 2000) or 5-HT_3 receptors (Chen et al., 1991) can increase dopamine release in the nucleus accumbens. Therefore, in the study by Hallbus et al. (1997), the effects of GR 127935 were tested under conditions in which synaptic 5-HT as well as dopamine levels were already enhanced. As a result, the findings by Hallbus et al. (1997) suggest that 5-$HT_{1B/1D}$ receptors in the nucleus accumbens may take part in the regulation of dopamine release under certain specific conditions. Furthermore, there are no reports to date regarding the effects of 5-HT_{1B} receptors in the ventral tegmental area on dopamine release in the ipsilateral nucleus accumbens although a moderately high density of 5-HT_{1B} binding sites has been found to exist in the ventral tegmental area (Bruinvels et al., 1993).

This study was designed to investigate the role of 5-HT_{1B} receptors located in both the ventral tegmental area and the nucleus accumbens for the modulation of dopaminergic transmission in the nucleus accumbens. Towards this aim, CP 93129 was administered into the ventral tegmental area and nucleus accumbens via retrograde microdialysis to minimize the effects of the compound on the structures other than ventral tegmental area or nucleus accumbens. The effects of intra-accumbal or intra-tegmental CP 93129 on extracellular dopamine levels in the nucleus accumbens were assessed using one- and dual-probe microdialysis, respectively. For dual-probe microdialysis, rats were equipped with two probes, one in the ventral tegmental area for drug administration and the other in the ipsilateral nucleus accumbens for dopamine measurement. CP 93129 was chosen because this agent is a putatively specific 5-HT_{1B} receptor agonist (Chopin et al., 1994; Macor et al., 1990).

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats, weighing 250–300 g at the time of surgery, were obtained from Harlan Sprague–Dawley (Indianapolis, IN, USA). They were housed at 21 ± 3°C, 40–60% relative humidity and were maintained under 12-h light/12-h dark condition with ad libitum access to food and water. All animal care and experimentation were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Illinois College of Medicine at Peoria.

2.2. Drugs

Tetrodotoxin was purchased from Sigma (St. Louis, MO, USA) and dissolved with artificial cerebrospinal fluid (ACSF) to 1 μM for perfusion. *S*(−)-cyanopindolol hemifumarate and WAY-100635 maleate {*N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-2-pyridinyl-cyclohexanecarboxamide maleate} were purchased from RBI (Natick, MA, USA). CP 93129 {3-(1,2,5,6-tetrahydro-4-pyridyl)pyrrolo[3,2-*b*]pyrid-5-one} was generously provided by

Pfizer (Groton, CT, USA). The drugs were dissolved in water and diluted to desired concentrations with ACSF for perfusion. The pH values of all perfusion solutions were within the range of 7.21–7.35. Reagents used in chemical assays were of analytical grade.

2.3. Microdialysis

Surgery was conducted on a Kopf stereotaxic instrument under anesthesia with a combination of sodium pentobarbital (35 mg/kg, i.p.) and halothane (5% in oxygen). Dialysis guide cannulae (Harvard Apparatus, S. Natick, MA, USA) were stereotaxically implanted over the nucleus accumbens (one-probe design) or over the both ventral tegmental area and the ipsilateral nucleus accumbens (two-probe design) and attached to the skull with dental acrylic and machine screws. The coordinates relative to bregma and skull surface were as follows: the ventral tegmental area: AP −5.2 mm, L 3 mm (at an angle of 14° from the sagittal plane to avoid rupture of the sagittal sinus), DV 8.0 mm and the nucleus accumbens: AP 1.7 mm, L 1.0 mm, DV 8.0 mm according to the atlas of Paxinos and Watson (1998). The period of post-surgical recovery was at least 5 days. On the evening of the day before the experiment, each rat was placed in a plexiglas chamber and a dialysis probe (2 mm in length), made from cellulose acetate hollow fibers (I.D. 215 ± 15 μm, molecular weight cutoff = 6000; Spectrum Medical Industries, Los Angeles, CA, USA), was inserted into the guide and directed to the nucleus accumbens while gently restraining the freely behaving rat. ACSF, which contained (in mM) Na^+ (150), K^+ (3.0), Ca^{2+} (1.2), Mg^{2+} (0.8), Cl^- (155), was perfused at 0.2 μl/min overnight. On the experimental day, the ACSF flow rate was increased to 1.5 μl/min. Meanwhile, for dual-probe microdialysis, another probe (1 mm in length) was inserted into the ventral tegmental area and perfused with ACSF at a rate of 1.5 μl/min. After 2–3 h, dialysate samples from the nucleus accumbens were collected at 20-min intervals by a CMA/170 refrigerated fraction collector into vials containing 5 μl 0.1 N HCl, and stored at −80°C until analysis. Frozen samples showed no signs of degradation for up to 1 month in our previous studies (Yan, 1999a,b, 2000). Five consecutive samples were collected for determination of basal dopamine concentrations. All treatments were administered via a dialysis probe.

In order to evaluate the implantation of the probe functionally, each dual-probe experiment was finished with infusion of 50 μM of baclofen, a $GABA_B$ receptor agonist, into the ventral tegmental probe and the response of extracellular dopamine in the ipsilateral nucleus accumbens was determined. A significant decrease (≥ 50% deduction) in extracellular dopamine in the ipsilateral nucleus accumbens after perfusion with baclofen was considered an appropriate implantation of the probe.

2.4. Analytical and histological procedure

Dialysate samples were injected onto a high performance liquid chromatography (HPLC) system with electrochemical detection for determination of dopamine. This system consisted of an ESA solvent delivery system (model 580), an ESA microbore column (MD-150 × 1/RP-C18, 3 μM), and an ESA coulochem II electrochemical detector equipped with a dual electrode analytical cell (Model 5041) and a guard cell (Model 5020). The guard cell was set at 400 mV, electrode 1 at −100 mV, and electrode 2 at 175 mV with respect to palladium reference electrodes. A VICI micro-electric two-position valve actuator with a 5-μl injection loop was used for sample injection. The mobile phase contained 75 mM Na_2HPO_4, 1.53 mM sodium dodecyl sulfate, 25 μM EDTA, 100 μl/l triethylamine, 11.5% acetonitrile and 11.5% methanol (pH 5.6 with H_3PO_4), and was pumped through the system at 0.07 ml/min. Chromatograms were integrated, compared with standards run separately on each experimental day, and analyzed using a computer-based data acquisition system (EZChrom Chromatography Data System, Scientific Software, San Ramon, CA, USA). The detection limit for dopamine was 0.5–1 fmol at a 2:1 signal-to-noise ratio.

After completion of the dialysis, the animals were given an intracardiac perfusion with buffered saline and 10% formalin solutions under anesthesia with sodium pentobarbital, and then decapitated. The brains were removed quickly, and 40-μm thick coronal sections were cut on a freezing microtome, stained with neutral red and analyzed in the light microscope. The heavy staining of gliosis along the guide cannula track permitted reliable location of the deepest point of penetration. A 2-mm-long (in the nucleus accumbens) or 1-mm-long (in the ventral tegmental area) dialysis membrane extended below the tip of the guide cannula. The point of the probe tip was then marked on coronal sections from the atlas of Paxinos and Watson (1998).

2.5. Data analysis

All values of dopamine reported herein represent uncorrected dialysate levels, expressed as fmol/μl of dialysate, and calculated as means ± S.E.M. A *t*-test, one-way or two-way analysis of variance (ANOVA) followed by Newman–Keuls or Tukey's tests were applied. All analyses were performed through computer-based software (SigmaStat). The criterion of significance was set at $P < 0.05$.

3. Results

Only data from animals with correct probe placements (and appropriate accumbal dopamine responses to perfusion of the ventral tegmental area with baclofen in dual-probe experiments) were included in data analyses. In the

dual-probe procedure, approximately 75% of the animals that had undergone surgery had both probes correctly implanted in the ventral tegmental area and nucleus accumbens and met the functional criterion. Fig. 1 shows representative probe tip placements in the ventral tegmental area and the nucleus accumbens.

3.1. Effects of infusion of CP 93129 into the nucleus accumbens on extracellular concentrations of dopamine in this region

The control group showed that switching between syringes containing ACSF did not alter significantly the dialysate dopamine levels in the nucleus accumbens (Fig. 2). In another three groups, three concentrations of CP 93129 (2, 5, and 10 μM in ACSF) were administered through a probe into the nucleus accumbens for 60 min, respectively. Concentrations of CP 93129 were chosen based on the previous studies by Galloway et al. (1993) who showed that local infusion of 10 μM CP 93129 caused striatal dopamine to increase to approximately 219% of baseline, and by Hjorth and Tao (1991) who showed that infusion of 10 μM CP 93129 into the hippocampus produced a reduction of basal 5-HT by 40%. The basal extracellular concentrations of dopamine in the nucleus accumbens were (fmol/μl of dialysate, mean ± S.E.M., the same below) 1.83 ± 0.16 (the control group, $n = 5$), 1.67 ± 0.18 (the 2 μM group, $n = 5$), 1.77 ± 0.11 (the 5 μM group, $n = 7$) and 1.64 ± 0.14 (the 10 μM group, $n = 7$), and did not differ significantly among the groups ($P = 0.811$, one-way ANOVA). As shown in Fig. 2, infusion of CP 93129 into the nucleus accumbens produced a concentration-dependent increase in extracellular levels of dopamine. Perfusion with 2 μM CP 93129 did not cause dopamine to change significantly when compared with the ACSF alone group (Fig. 2). However, at 40 and 60 min after perfusion with higher concentrations of CP 93129 (5 and 10 μM), extracellular dopamine levels were significantly greater than those in response to perfusion with either 2 μM or ACSF alone ($P < 0.05$, one-way ANOVA followed by Newman–Keuls test). The maximum increases of dopamine levels produced by 5 and 10 μM were 149% and 190% of baseline, respectively. Extracellular dopamine concentrations fell rapidly and reached the pre-treatment basal values within 20 min after discontinuation of 5 or 10 μM CP 93129 perfusion.

3.2. Effects of cyanopindolol or WAY-100635 on the CP 93129 (10 μM)-induced dopamine release in the nucleus accumbens

In another two groups of rats, cyanopindolol (5 μM) and WAY-100635 (5 μM) were infused into the nucleus accumbens for 40 min, and then co-infused with 10 μM CP 93129 for another 1 h, respectively. The concentrations of cyanopindolol and WAY-100635 were chosen based on their affinity for 5-HT_{1A} and/or 5-HT_{1B} receptors (for cyanopindolol, pK_i values: 5-HT_{1A} 8.3, 5-HT_{1B} 8.3, Chopin et al., 1994; for WAY-100635, pK_i values: 5-HT_{1A} 8.87, Forster et al., 1995). Prior to the treatments with cyanopindolol and WAY-100635, the basal dopamine values were 1.73 ± 0.23 ($n = 5$) and 1.61 ± 0.21 ($n = 5$), respectively, and were not significantly different from that (1.64 ± 0.14) of the group of CP 93129 (10 μM) alone

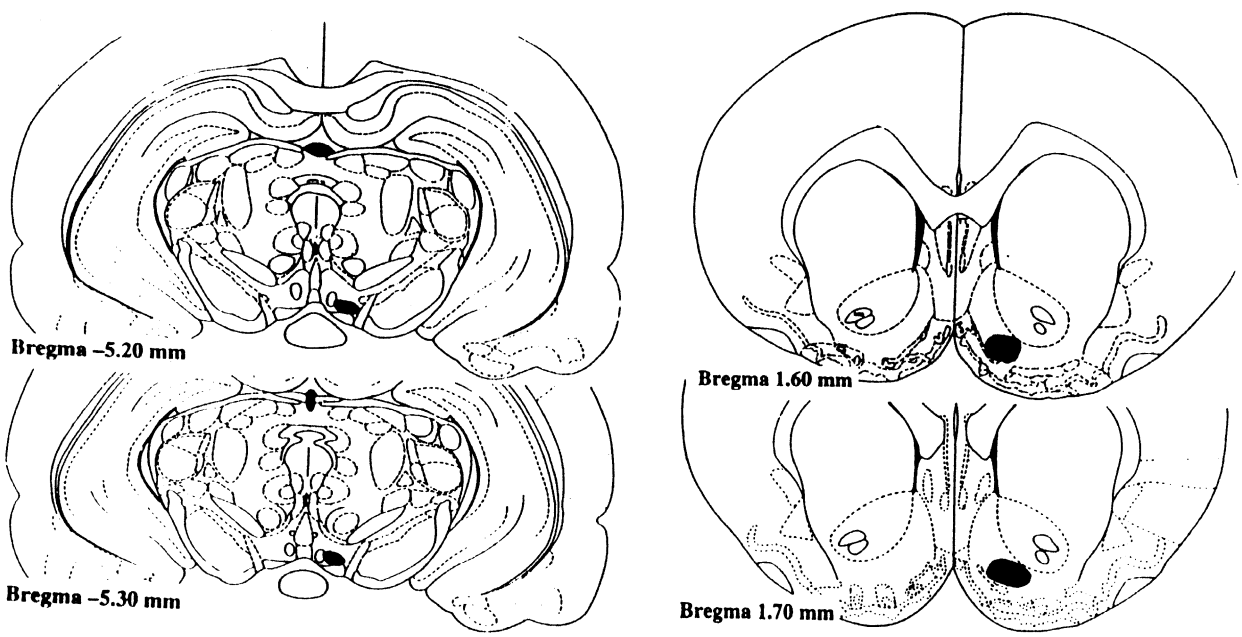

Fig. 1. Diagrammatic representation of microdialysis probe placements in the ventral tegmental area (left panels) and the nucleus accumbens (right panels) (adapted from the atlas of Paxinos and Watson, 1998). The shadowed area represents placements of the probe tips, but not the whole dialysis membrane.

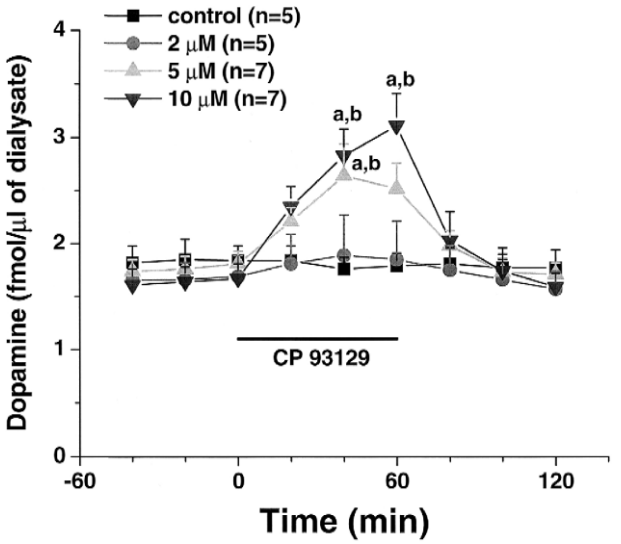

Fig. 2. Effects of local infusion of CP 93129 into the nucleus accumbens on dopamine release in this region. CP 93129 (2, 5, and 10 μM) was administered via the probe into the nucleus accumbens during the period indicated by the bar. Results are mean ± S.E.M. $^{a}P < 0.05$ as compared with the control group; $^{b}P < 0.05$ as compared with the 2 μM group (one-way ANOVA followed by Newman–Keuls test).

($P = 0.686$). Pre-treatment with either cyanopindolol or WAY-100635 for 40 min did not alter significantly extracellular dopamine levels (data not shown). Fig. 3 shows comparisons of CP 93129 (10 μM)-induced dopamine release in the presence and absence of cyanopindolol or WAY-100635. As shown in this figure, the CP 93129-induced dopamine release in the nucleus accumbens was attenuated by co-infusion of cyanopindolol. In the presence of cyanopindolol, CP 93129 (10 μM)-induced dopamine outputs were significantly lower than those of the CP 93129 alone group at both the time points of 40 and 60 min ($P < 0.05$, two-way ANOVA followed by Tukey's test). However, administration of WAY-100635 had no significant effects on the CP-93129-induced dopamine release. There are no significant differences in extracellular dopamine levels at any time points after treatments between the groups of CP-93129 alone and CP 93129 plus WAY-100635 (Fig. 3).

3.3. Effects of perfusion with tetrodotoxin on the CP 93129 (10 μM)-induced dopamine release in the nucleus accumbens

To test the sensitivity of CP 93129-induced dopamine release to the blockade of Na^{+} channels, after basal dopamine was stable, tetrodotoxin (1 μM) was infused via a probe into the nucleus accumbens for 1 h and then co-infused with CP 93129 (10 μM) for another 1 h. In another group of rats, tetrodotoxin (1 μM) was administered through a probe for 2 h.

Prior to the tetrodotoxin treatment, the basal levels of dopamine were 2.07 ± 0.33 (the tetrodotoxin alone group, $n = 4$) and 1.92 ± 0.36 (the tetrodotoxin plus CP 93129 group, $n = 4$). As shown in Fig. 4, perfusion with 1 μM tetrodotoxin caused extracellular dopamine concentrations in the nucleus accumbens to decrease dramatically. By 1 h, extracellular dopamine decreased by more than 80%. From this time point, CP 93129 (10 μM) was co-infused with

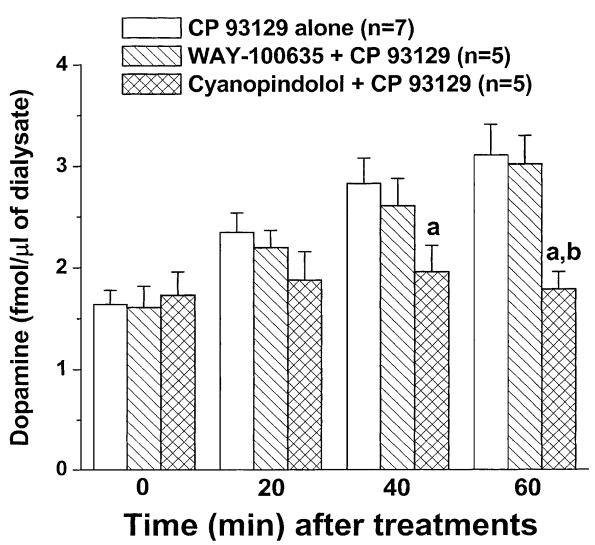

Fig. 3. Comparisons of CP 93129-induced dopamine release from the nucleus accumbens in the presence and absence of cyanopindolol or WAY-100635. Cyanopindolol (5 μM) and WAY-100635 (5 μM) were administered through the probe into the nucleus accumbens for 40 min, and then co-infused with CP 93129 (10 μM) for another 60 min, respectively. Results are mean ± S.E.M. $^{a}P < 0.05$ as compared with the group of CP 93129 alone; $^{b}P < 0.05$ as compared with the group of CP 93129 plus WAY-100635 (two-way ANOVA followed by Tukey's test).

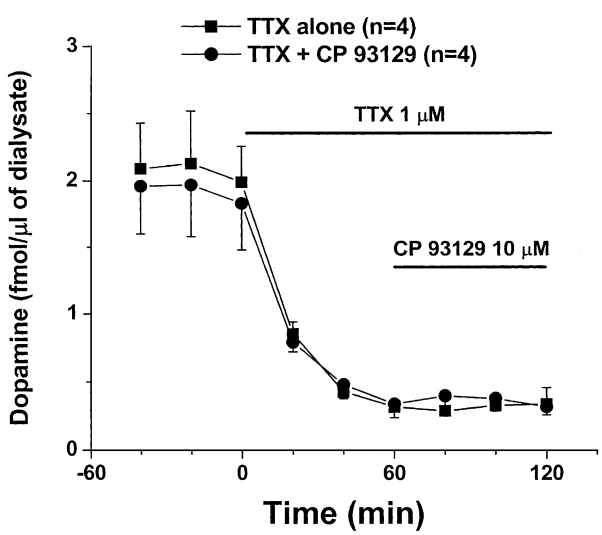

Fig. 4. Effects of perfusion with tetrodotoxin on CP 93129-induced dopamine release in the nucleus accumbens. Tetrodotoxin (1 μM) and CP 93129 (10 μM) were administered via the probe into the nucleus accumbens indicated by the bars, respectively. Results are mean ± S.E.M. There are no significant differences in extracellular dopamine levels at any time points between the groups of tetrodotoxin alone and tetrodotoxin plus CP 93129 (*t*-test).

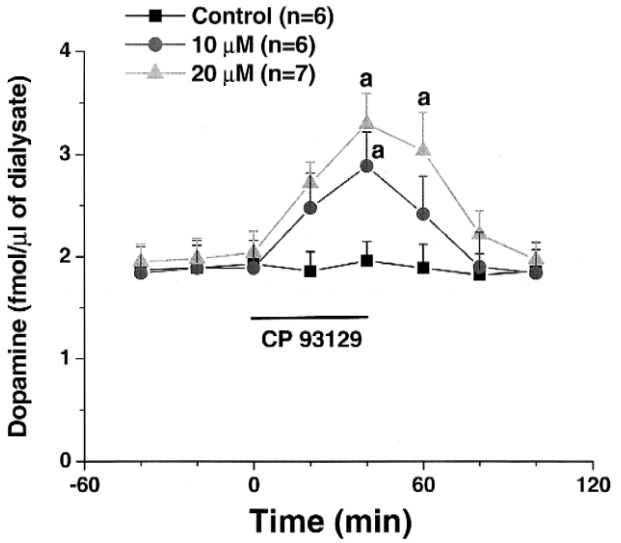

Fig. 5. Effects of infusion of CP 93129 into the ventral tegmental area on dopamine release in the ipsilateral nucleus accumbens. CP 93129 (10 and 20 μM) was administered through a probe into the ventral tegmental area indicated by the bar. Extracellular dopamine in the ipsilateral nucleus accumbens was monitored by a second probe in this region. Results are mean ± S.E.M. [a]$P < 0.05$ as compared with the control group (one-way ANOVA followed by Newman–Keuls test).

tetrodotoxin for another 1 h. As shown in Fig. 4, perfusion with tetrodotoxin blocked the ability of CP 93129 to increase extracellular dopamine in the nucleus accumbens. Statistical analyses showed that there are no differences in extracellular dopamine levels at any time points between the groups of tetrodotoxin alone and CP 93129 plus tetrodotoxin ($P > 0.05$, *t*-test).

3.4. Effects of infusion of CP 93129 into the ventral tegmental area on extracellular dopamine concentrations in the ipsilateral nucleus accumbens

In this experiment, two concentrations of CP 93129 (10 and 20 μM) were administered via a probe into the ventral tegmental area of two groups of rats for 40 min, respectively, and extracellular levels of dopamine in the ipsilateral nucleus accumbens were monitored. The concentrations (10 and 20 μM) of CP 93129 used here were higher than those (2, 5, and 10 μM) used in the nucleus accumbens based on considerations that smaller probes (1 mm long) were used for drug perfusion. In another group of rats (the control group), ACSF was infused into the ventral tegmental area for the same period as the drug groups and switching between syringes containing ACSF in this group was found to have no significant effects on the dialysate dopamine levels in the ipsilateral nucleus accumbens (Fig. 5). The basal dopamine values in the nucleus accumbens were 1.90 ± 0.22 (the control group, $n = 6$), 1.87 ± 0.27 (the 10 μM group, $n = 6$) and 1.99 ± 0.19 (the 20 μM group, $n = 7$), and did not differ significantly among the groups ($P = 0.933$, one-way ANOVA). As shown in Fig. 5, infusion of CP 93129 into the ventral tegmental area for 40 min increased significantly extracellular levels of dopamine in the ipsilateral nucleus accumbens when compared with the control group ($P < 0.05$ at the time point of 40 min, one-way ANOVA followed by Newman–Keuls test). The maximum increases of nucleus accumbens dopamine produced by 10 and 20 μM were 155% and 166% of baseline, respectively.

3.5. Effects of cyanopindolol or WAY-100635 on intra-tegmental CP 93129-induced dopamine release in the ipsilateral nucleus accumbens

In another two groups of rats, cyanopindolol (5 μM) and WAY-100635 (5 μM) were infused into the ventral tegmental area for 40 min and then co-infused with CP 93129 (20 μM) for another 40 min, respectively. Prior to cyanopindolol ($n = 6$) and WAY-100635 ($n = 6$), the basal dopamine values in the ipsilateral nucleus accumbens were 1.86 ± 0.13 and 1.82 ± 0.17, respectively, and not significantly different from those (1.99 ± 0.19) of the group of CP 93129 (20 μM) alone ($P = 0.768$, one-way ANOVA). Pre-treatment with either cyanopindolol or WAY-100635 for 40 min did not alter significantly extracellular dopamine levels in the ipsilateral nucleus accumbens (data not shown). Fig. 6 shows comparisons of intra-tegmental CP 93129 (20 μM)-induced accumbal dopamine release in the presence and absence of cyanopindolol or WAY-100635.

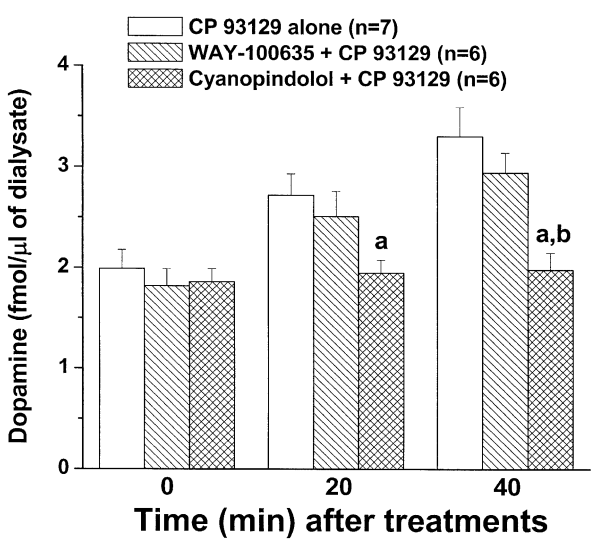

Fig. 6. Comparisons of intra-tegmental CP 93129-induced dopamine release from the ipsilateral nucleus accumbens in the presence and absence of cyanopindolol or WAY-100635. Cyanopindolol (5 μM) and WAY-100635 (5 μM) were administered through a probe into the ventral tegmental area for 40 min, and then co-infused with CP 93129 (20 μM) for another 40 min, respectively. Extracellular dopamine in the ipsilateral nucleus accumbens was monitored by a second probe in this region. Results are mean ± S.E.M. [a]$P < 0.05$ as compared with the group of CP 93129 alone; [b]$P < 0.05$ as compared with the group of CP 93129 plus WAY-100635 (two-way ANOVA followed by Tukey's test).

As shown in this figure, the CP 93129-induced dopamine release in the ipsilateral nucleus accumbens was attenuated by co-infusion of cyanopindolol. In the presence of cyanopindolol, CP 93129 (20 μM)-induced dopamine outputs in the ipsilateral nucleus accumbens were significantly lower than those of the CP 93129 alone group at both the time points of 20 ($P < 0.05$) and 40 min ($P < 0.05$, two-way ANOVA followed by Tukey's test) after co-administration. However, administration of WAY-100635 had no significant effects on the CP 93129-induced dopamine release. There are no significant differences in extracellular dopamine levels at any time points after treatments between the groups of CP-93129 alone and CP 93129 plus WAY-100635 (Fig. 6).

At the end of each experiment with the dual-probe design, baclofen (50 μM) was infused into the ventral tegmental area. Infusion of baclofen caused extracellular dopamine in the ipsilateral nucleus accumbens to decrease to 26–40% of baseline (data not shown). The observed effects of baclofen on nucleus accumbens dopamine were consistent with those reported in the literature (Westerink et al., 1996; Yoshida et al., 1994), suggesting the functional integrity of the circuitry studied in our dual-probe design.

4. Discussion

The major findings of this study are that (1) infusion of CP 93129 into the nucleus accumbens and the ventral tegmental area enhanced dopamine release in the terminal area of the mesolimbic dopamine system, respectively; (2) augmentations of dopamine produced by intra-tegmental or intra-accumbal infusion of CP 93129 were antagonized by co-infusion with cyanopindolol but not with WAY-100635, and (3) the Na^+ channel blockade with tetrodotoxin abolished the effects of intra-accumbal CP 93129 on dopamine release in this region.

5-HT_{1B} receptors are predominantly located on axon terminals (Barnes and Sharp, 1999; Boschert et al., 1994; Sari et al., 1999). In keeping with this location, activation of 5-HT_{1B} autoreceptors residing on serotonergic terminals resulted in inhibition of 5-HT release from the hippocampus and frontal cortex in rats (Hjorth and Tao, 1991; Martin et al., 1992) and mice (Trillat et al., 1997). It has also been reported that 5-HT_{1B} receptors can function as inhibitory heteroreceptors modulating the release of other neurotransmitters such as γ-aminobutyric acid (GABA) (Cameron and Williams, 1994, 1995).

The nucleus accumbens contains a high density of 5-HT_{1B} receptors (Bruinvels et al., 1993). In agreement with existence of 5-HT_{1B} receptors in this region, the previous reports by Hallbus et al. (1997) showed that elevation of accumbal dopamine produced by focal 5-HT perfusion was antagonized by co-perfusion of the 5-$HT_{1B/1D}$ antagonist GR 127935. The present data extend these findings and provide the first direct evidence that local application of CP 93129 (2–10 μM) into the nucleus accumbens produced a concentration-dependent augmentation of extracellular levels of dopamine.

CP 93129 is a putatively specific 5-HT_{1B} receptor agonist, with ≥150-fold higher affinity for 5-HT_{1B} vs. other 5-HT_1 and 5-HT_2 ligand binding sites (IC_{50} values: 5-HT_{1A} 3000 ± 400, 5-HT_{1B} 15 ± 5, 5-HT_{1D} 2200 ± 700, and 5-HT_2 > 10,000 nM) (Chopin et al., 1994; Macor et al., 1990). It is also claimed to lack substantial affinity for dopamine, noradrenaline or opiate receptors (Macor et al., 1990). The concentrations of CP 93129 used for nucleus accumbens and the ventral tegmental area were 2–10 and 10–20 μM, respectively. It should be pointed out that, unlike in vitro conditions, actual drug concentrations around receptors after retrograde dialysis are much lower than those present in the perfusion medium due to (1) the probe efficiency and (2) diffusion through the interstitial space from the probe into the synaptic site. Considering approximately 5–10% of the efficiency of the probe (1–2 mm long) used, it is estimated that actual concentrations of CP 93129 in the extracellular fluid may be in the range of 200–1000 nM. Therefore, it is likely that CP 93129 acts mainly at 5-HT_{1B} receptors under the present experimental conditions.

To further assess the involvement of 5-HT_{1B} receptors in CP 93129's actions, cyanopindolol and WAY-100635 were used. Cyanopindolol is a 5-$HT_{1A/1B}$ receptor antagonist. Its pK_i values for 5-HT_{1A} (8.3) and 5-HT_{1B} (8.3) receptors are similar (Chopin et al., 1994). WAY-100635 is a highly selective and "silent" 5-HT_{1A} receptor antagonist. It binds with a greater than 100-fold selectivity and affinity to the 5-HT_{1A} receptor relative to binding at other 5-HT receptor subtypes (Forster et al., 1995). The affinity ($pK_i = 8.87$) of WAY-100635 for 5-HT_{1A} receptors is approximately the same as that ($pK_i = 8.3$) of cyanopindolol. If 5-HT_{1A} receptors are involved in the CP 93129's actions, both WAY-100635 and cyanopindolol, at the same concentration used (5 μM), should antagonize the effects of CP 93129 in a similar magnitude. However, this is not the case. The data presented here show that augmentations of dopamine release produced by CP 93129 were antagonized by cyanopindolol but not by WAY-100635. These results are in opposition with the involvement of 5-HT_{1A} receptors in the observed CP 93129's effects. Recent microdialysis studies by Ichikawa and Meltzer (1999, 2000) showed that systemic administration of 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin), a selective 5-HT_{1A} receptor agonist, decreased (at the dose of 0.2 mg/kg) or had no effects (at the dose of 0.05 mg/kg) on basal dopamine release in the nucleus accumbens, suggesting that activation of 5-HT_{1A} receptors may not be associated with facilitation of nucleus accumbens dopamine release. Therefore, it is unlikely that the observed enhancement of nucleus accumbens dopamine by CP 93129 was produced by an interaction of the drug with 5-HT_{1A} receptors. Taken

together, the present results showing augmentations of dopamine release following local CP 93129 application are not in opposition to the concept that increased dopamine may be associated with the drug-induced activation of 5-HT_{1B} receptors. This hypothesis is supported by the observation by Boulenguez et al. (1996) who reported that subcutaneous injections of RU 24969 (5-methoxy-3-(1,2,5,6-tetrahydro-4-pyridinyl)-1*H*-indole), a 5-$HT_{1B/1A}$ receptor agonist, but not 8-OH-DPAT, produced increases of extracellular dopamine in the nucleus accumbens, in agreement with facilitation of nucleus accumbens dopamine by 5-HT_{1B} receptors.

As mentioned above, both CP 93129 and cyanopindolol also have certain affinities for 5-HT_{1D} receptors although their affinities for this receptor subtype are all 100-fold lower than those for 5-HT_{1B} receptors (Chopin et al., 1994). At present, there is lack of direct evidence showing the existence of the 5-HT_{1D} receptor binding site either in the nucleus accumbens or in the ventral tegmental area, but the possibility that 5-HT_{1D} receptors may also be involved in CP 93129's actions warrants further investigation.

In view of the inhibitory property of 5-HT_{1B} receptors (Barnes and Sharp, 1999), it seems unlikely that the receptors of interest are located on dopamine terminals in the nucleus accumbens. The present results that tetrodotoxin blocked the ability of CP 93129 to increase nucleus accumbens dopamine suggest that CP 93129 may modulate accumbal dopaminergic transmission by influencing transsynaptic regulation of ventral tegmental area dopamine neuron projecting to the nucleus accumbens.

There is a moderately high density of 5-HT_{1B} binding sites in the ventral tegmental area (Bruinvels et al., 1993). In agreement with this evidence, the present data show that infusion of CP 93129 into the ventral tegmental area produced increases of dopamine levels in the ipsilateral nucleus accumbens and that the effect of intra-tegmental CP 93129 was antagonized by co-infusion of cyanopindolol but not by WAY-100635. To our knowledge, the present results provide the first evidence for the involvement of 5-$HT_{1B/1D}$ receptors within the ventral tegmental area in the modulation of dopamine release in the ipsilateral nucleus accumbens.

It has been reported that a large proportion of the 5-HT_{1B} receptor within the ventral tegmental area are probably located on the terminal of afferent GABAergic cells (Bruinvels et al., 1994), which provide substantial inhibitory regulation to mesoaccumbens dopamine cells (Johnson and North, 1992; Sugita et al., 1992). Recent evidence suggests that activation of 5-HT_{1B} receptors in the ventral tegmental area reduces ventral tegmental area GABA release, resulting in a disinhibition of mesolimbic dopamine cells (Cameron and Williams, 1994, 1995). Based on these reports, we speculate that intra-tegmental infusion of CP 93129 increases dopamine release in the ipsilateral nucleus accumbens via an indirect GABA mechanism.

The interaction between 5-HT and dopamine via the 5-HT_{1B} receptor may play an important role in the physiopathology of several behavioral disorders such as drug abuse. 5-HT_{1B} receptors have been shown to enhance the reinforcing properties of both cocaine and the selective dopamine reuptake inhibitor GBR 12909 (Parsons et al., 1996, 1998; Scearce-Levie and Hen, 2000). It has been demonstrated that the potentiation of cocaine reinforcement by 5-HT_{1B} receptor agonists is mediated at least in part by a potentiation of cocaine-induced increases in mesolimbic dopamine transmission (Parsons et al., 1999). Interestingly, activation of 5-HT_{1B} receptors has also been reported to influence brain stimulation reward (Harrison et al., 1999) or amphetamine-induced enhancement of responding for conditioned reward (Fletcher and Korth, 1999). Studies carried out in mice lacking 5-HT_{1B} receptors indicated that 5-HT_{1B} receptors are also important for ethanol's rewarding effects (Crabbe et al., 1996; Risinger et al., 1996) and for 3,4-methylenedioxy-*N*-methamphetamine (MDMA)-induced locomotion (Scearce-Levie et al., 1999). Taken together, the results suggest that 5-HT_{1B} receptors are involved in regulation of locomotor activity and rewarding effects of cocaine as well as other drugs of abuse. Investigation of the interaction between 5-HT and dopamine within the mesolimbic dopamine system via the 5-HT_{1B} receptor would provide further insight into mechanisms regulating behavioral responses to abused drugs, thereby, providing for alternative approaches to treatment of drug addiction.

In summary, the present study shows that local infusion of the 5-HT_{1B} receptor agonist CP 93129 into the ventral tegmental area or nucleus accumbens all increased dopamine release in the mesolimbic dopamine terminal area. The increased dopamine release produced by CP 93129 was antagonized by the 5-$HT_{1B/1A}$ receptor antagonist cyanopindolol but not by the 5-HT_{1A} receptor antagonist WAY-100635, and sensitive to the Na^+ channel blockade with tetrodotoxin. The results are not in opposition to the involvement of 5-HT_{1B} receptors in the modulation of dopamine transmission in the mesolimbic dopamine system.

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References

Barnes, N.M., Sharp, T., 1999. A review of central 5-HT receptors and their function. Neuropharmacology 38, 1083–1152.

Boschert, U., Amara, A., Segu, L., Hen, R., 1994. The mouse 5-hydroxy-

tryptamine$_{1B}$ receptor is localized predominantly on axonterminals. Neuroscience 58, 167–182.

Boulenguez, P., Rawlins, J.N.P., Chauveau, J., Joseph, M.H., Mitchell, S.N., Gray, J.A., 1996. Modulation of dopamine release in the nucleus accumbens by 5-HT$_{1B}$ agonists: involvement of the hippocampal–accumbens pathway. Neuropharmacology 35, 1521–1529.

Bruinvels, A.T., Palacios, J.M., Hoyer, D., 1993. Autoradiographic characterisation and localisation of 5-HT$_{1D}$ compared to 5-HT$_{1B}$ binding sites in rat brain. Naunyn-Schmiedeberg's Arch. Pharmacol. 347, 569–582.

Bruinvels, A.T., Landwehrmeyer, B., Gustafson, E.L., Durkin, M.M., Mengod, G., Branchek, T.A., Hoyer, D., Palacios, J.M., 1994. Localization of 5-HT$_{1B}$, 5-HT$_{1D\alpha}$, 5-HT$_{1E}$ and 5-HT$_{1F}$ receptor messenger RNA in rodent and primate brain. Neuropharmacology 33, 367–386.

Cameron, D.L., Williams, J.T., 1994. Cocaine inhibits GABA release in the ventral tegmental area through endogenous 5-HT. J. Neurosci. 14, 6763–6767.

Cameron, D.L., Williams, J.T., 1995. Opposing roles for dopamine and serotonin at presynaptic receptors in the ventral tegmental area. Clin. Exp. Pharmacol. Physiol. 22, 841–845.

Chen, J., Van Praag, H.M., Gardner, E.L., 1991. Activation of 5-HT$_3$ receptor by 1-phenylbiguanide increases dopamine release in the rat nucleus accumbens. Brain Res. 543, 354–357.

Chopin, P., Moret, C., Briley, M., 1994. Neuropharmacology of 5-hydroxytryptamine$_{1B/D}$ receptor ligands. Pharmacol. Ther. 62, 385–405.

Crabbe, J.C., Phillips, T.J., Feller, D.J., Hen, R., Wenger, C.D., Lessov, C.N., Schafer, G.L., 1996. Elevated alcohol consumption in null mutant mice lacking 5-HT$_{1B}$ serotonin receptors. Nat. Genet. 14, 98–101.

De Deurwaerdere, P., Spampinato, U., 1999. Role of serotonin$_{2A}$ and serotonin$_{2B/2C}$ receptor subtypes in the control of accumbal and striatal dopamine release elicited in vivo by dorsal raphe nucleus electrical stimulation. J. Neurochem. 73, 1033–1042.

Di Matteo, V., Di Giovanni, G., Di Mascio, M., Esposito, E., 1998. Selective blockade of serotonin2C/2B receptors enhances dopamine release in the rat nucleus accumbens. Neuropharmacology 37, 265–272.

Fletcher, P.L., Korth, K.M., 1999. Activation of 5-HT$_{1B}$ receptors in the nucleus accumbens reduces amphetamine-induced enhancement of responding for conditioned reward. Psychopharmacology 142, 165–174.

Forster, E.A., Cliffe, I.A., Bill, D.J., Dover, G.M., Jones, D., Reilly, Y., Fletcher, A., 1995. A pharmacological profile of the selective silent 5-HT1A receptor antagonist, WAY-100635. Eur. J. Pharmacol. 281, 81–88.

Galloway, M.P., Suchowski, C.S., Keegan, M.J., Hjorth, S., 1993. Local infusion of the selective 5-HT-1b agonist CP-93,129 facilitates striatal dopamine release in vivo. Synapse 15, 90–92.

Guan, X.M., McBride, W.J., 1989. Serotonin microinfusion into the ventral tegmental area increases accumbens dopamine release. Brain Res. Bull. 23, 541–547.

Hallbus, M., Magnusson, T., Magnusson, O., 1997. Influence of 5-HT$_{1B/1D}$ receptors on dopamine release in the guinea pig nucleus accumbens: a microdialysis study. Neurosci. Lett. 225, 57–60.

Harrison, A.A., Parsons, L.H., Koob, G.F., Markou, A., 1999. RU 24969, a 5-HT$_{1A/1B}$ agonist, elevates brain stimulation reward thresholds: an effect reversed by GR 127935, a 5-HT$_{1B/1D}$ antagonist. Psychopharmacology 141, 242–250.

Herve, D., Pickle, V.M., Hoh, T.H., Beaudet, A., 1987. Serotonin axon terminals in the ventral tegmental area of the rat: fine structure and synaptic input to dopaminergic neurons. Brain Res. 435, 71–83.

Hjorth, S., Tao, R., 1991. The putative 5-HT$_{1B}$ receptor agonist CP-93129 suppresses rat hippocampal 5-HT release in vivo: comparison with RU 24969. Eur. J. Pharmacol. 209, 249–252.

Ichikawa, J., Meltzer, H.Y., 1999. *R*(+)-8-OH-DPAT, a serotonin-1A receptor agonist, potentiated *S*(−)-sulpiride-induced dopamine release in rat medial prefrontal cortex and nucleus accumbens, but not striatum. J. Pharmacol. Exp. Ther. 291, 127–1232.

Ichikawa, J., Meltzer, H.Y., 2000. The effect of serotonin(1A) receptor anonism on antipsychotic drug-induced dopamine release in rat striatum and nucleus accumbens. Brain Res. 858, 252–263.

Iyer, R.N., Bradberry, C.W., 1996. Serotonin-mediated increase in prefrontal cortex dopamine release: pharmacological characterization. J. Pharmacol. Exp. Ther. 277, 40–47.

Johnson, S.W., North, R.A., 1992. Two types of neurone in the rat ventral tegmental area and their synaptic inputs. J. Physiol. (London), 450, 455–468.

Macor, J.E., Burkhart, C.A., Heym, J.H., Ives, J.L., Lebel, L.A., Newman, M.E., Nielsen, J.A., Ryan, K., Schulz, D.W., Torgersen, L.K., Koe, B.K., 1990. 3-(1,2,5,6-Tetrahydropyrid-4-yl)pyrrolo[3,2-*b*]pyrid-5-one: a potent and selective serotonin (5-HT$_{1B}$) agonist and rotationally restricted phenolic analogue of 5-methoxy-3-(1,2,5,6-tetrahydropyrid-4-yl)indole. J. Med. Chem. 33, 2087–2093.

Martin, K.F., Hannon, S., Phillips, I., Heal, D.J., 1992. Opposing roles for 5-HT$_{1B}$ and 5-HT$_3$ receptors in the control of 5-HT release in rat hippocampus in vivo. Br. J. Pharmacol. 106, 139–142.

Matsumoto, M., Togashi, H., Mori, K., Ueno, K., Miyamoto, A., Yoshioka, M., 1999. Characterization of endogenous serotonin-mediated regulation of dopamine release in the rat prefrontal cortex. Eur. J. Pharmacol. 383, 39–48.

Moore, R.Y., Halaris, A.E., Jones, B.E., 1978. Serotonin neurons in the midbrain raphe: ascending projections. J. Comp. Neurol. 180, 417–438.

Parsons, L.H., Justice Jr., J.B., 1993. Perfusate serotonin increases extracellular dopamine in the nucleus accumbens as measured by in vivo microdialysis. Brain Res. 606, 195–199.

Parsons, L.H., Weiss, F., Koob, G.F., 1996. Serotonin$_{1B}$ receptor stimulation enhances dopamine-mediated reinforcement. Psychopharmacology 128, 150–160.

Parsons, L.H., Weiss, F., Koob, G.F., 1998. Serotonin$_{1B}$ receptor stimulation enhances cocaine reinforcement. J. Neurosci. 18, 10078–10089.

Parsons, L.H., Koob, G.F., Weiss, F., 1999. RU 24969, a 5-HT$_{1B/1A}$ receptor agonist, potentiates cocaine-induced increases in nucleus accumbens dopamine. Synapse 32, 132–135.

Paxinos, G., Watson, C., 1998. The Rat Brain in Stereotaxic Coordinates. 4th edn. Academic Press, Orlando, Florida, USA.

Risinger, F.O., Bormann, N.M., Oakes, R.A., 1996. Reduced sensitivity to ethanol reward, but not ethanol aversion, in mice lacking 5-HT1B receptors. Alcohol.: Clin. Exp. Res. 20, 1401–1405.

Sari, Y., Miquel, M.C., Brisorgueil, M.J., Ruiz, G., Doucet, E., Hamon, M., Verge, D., 1999. Cellular and subcellular localization of 5-hydroxytryptamine$_{1B}$ receptors in the rat central nervous system: immunocytochemical, autoradiographic and lesion studies. Neuroscience 88, 899–915.

Scearce-Levie, K., Hen, R., 2000. The 5-HT$_{1B}$ knockout mouse. In: Reith, M.E.A. (Ed.), Cerebral Signal Transduction, From First to Fourth Messengers. Humana Press, Totowa, NJ, pp. 341–373.

Scearce-Levie, K., Viswanathan, S.S., Hen, R., 1999. Locomotor response to MDMA is attenuated in knockout mice lacking the 5-HT$_{1B}$ receptor. Psychopharmacology 141, 154–161.

Sugita, S., Johnson, S.W., North, R.A., 1992. Synaptic inputs to GABA$_A$ and GABA$_B$ receptors originate from discrete afferent neurons. Neurosci. Lett. 134, 207–211.

Thorre, K., Ebinger, G., Michotte, Y., 1998. 5-HT$_4$ receptor involvement in the serotonin-enhanced dopamine efflux from the substantia nigra of the freely moving rat: a microdialysis study. Brain Res. 796, 117–124.

Trillat, A.C., Malagie, I., Scearce, K., Pons, D., Anmella, M.C., Jacquot, C., Hen, R., Gardier, A.M., 1997. Regulation of serotonin release in the frontal cortex and ventral hippocampus of homozygous mice lacking 5-HT1B receptors: in vivo microdialysis studies. J. Neurochem. 69, 2019–2025.

Vertes, R.P., Martin, G.F., 1988. Autoradiographic analysis of ascending projections from the pontine and mesencephalic reticular formation and the median raphe nucleus in the rat. J. Comp. Neurol. 275, 511–541.

Westerink, B.H.C, Kwint, H.F., De Vries, J.B., 1996. The pharmacology of mesolimbic dopamine neurons: a dual-probe microdialysis study in the ventral tegmental area and nucleus accumbens of the rat brain. J. Neurosci. 16, 2605–2611.

Yan, Q.S., 1999a. Extracellular dopamine and serotonin after ethanol monitored with 5-minute microdialysis. Alcohol 19, 1–7.

Yan, Q.S., 1999b. Focal bicuculline increases extracellular dopamine concentration in the nucleus accumbens of freely moving rats as measured by in vivo microdialysis. Eur. J. Pharmacol. 385, 7–13.

Yan, Q.S., 2000. Activation of 5-$HT_{2A/2C}$ receptors within the nucleus accumbens increases local dopaminergic transmission. Brain Res. Bull. 51, 75–81.

Yoshida, M., Yokoo, H., Tanaka, T., Emoto, H., Tanaka, M., 1994. Opposite changes in the mesolimbic dopamine metabolism in the nerve terminal and cell body sites induced by locally infused baclofen in the rat. Brain Res. 636, 111–114.

Yoshimoto, K., McBride, W.J., 1992. Regulation of nucleus accumbens dopamine release by the dorsal raphe nucleus in the rat. Neurochem. Res. 17, 401–407.