

5-HT_{2A} and 5-HT_{2C/2B} Receptor Subtypes Modulate Dopamine Release Induced in Vivo by Amphetamine and Morphine in Both the Rat Nucleus Accumbens and Striatum

Grégory Porras, M.S., Vincenzo Di Matteo, Ph.D., Claudia Fracasso, B.S., Guillaume Lucas, Ph.D., Philippe De Deurwaerdère, Ph.D., Silvio Caccia, Ph.D., Ennio Esposito, M.D., Ph.D., and Umberto Spampinato, M.D., Ph.D.

In vivo microdialysis and single-cell extracellular recordings were used to assess the involvement of serotonin_{2A} (5-HT_{2A}) and serotonin_{2C/2B} (5-HT_{2C/2B}) receptors in the effects induced by amphetamine and morphine on dopaminergic (DA) activity within the mesoaccumbal and nigrostriatal pathways. The increase in DA release induced by amphetamine (2 mg/kg *i.p.*) in the nucleus accumbens and striatum was significantly reduced by the selective 5-HT_{2A} antagonist SR 46349B (0.5 mg/kg *s.c.*), but not affected by the 5-HT_{2C/2B} antagonist SB 206553 (5 mg/kg *i.p.*). In contrast, the enhancement of accumbal and striatal DA output induced by morphine (2.5 mg/kg *s.c.*), while insensitive to SR 46349B, was significantly increased by SB 206553. Furthermore, morphine (0.1–10 mg/kg *i.v.*)-induced increase in DA

neuron firing rate in both the ventral tegmental area and the substantia nigra pars compacta was unaffected by SR 46349B (0.1 mg/kg *i.v.*) but significantly potentiated by SB 206553 (0.1 mg/kg *i.v.*). These results show that 5-HT_{2A} and 5-HT_{2C} receptors regulate specifically the activation of midbrain DA neurons induced by amphetamine and morphine, respectively. This differential contribution may be related to the specific mechanism of action of the drug considered and to the neuronal circuitry involved in their effect on DA neurons. Furthermore, these results suggest that 5-HT_{2C} receptors selectively modulate the impulse flow-dependent release of DA.

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From the Laboratoire de Neuropsychobiologie des Désadaptations, UMR CNRS 5541-Université Victor Segalen Bordeaux 2, 146 rue Léo Saignat, Boîte Postale 31, 33076 Bordeaux Cedex, France (GP, GL, PD, US); Istituto di Ricerche Farmacologiche "Mario Negri", Consorzio "Mario Negri Sud", Santa Maria Imbaro, Chieti, Italy (VDM, EEE); and Istituto di Ricerche Farmacologiche "Mario Negri", via Eritrea 62, 20157 Milano, Italy (CF, SC)

Address correspondence to: Pr. U. Spampinato, Laboratoire de Neuropsychobiologie des Désadaptations, UMR CNRS 5541-Université Victor Segalen Bordeaux 2, 146 rue Léo Saignat, Boîte Postale 31, 33076 Bordeaux Cedex, France; E-mail: Umberto.Spampinato@lnpb.u-bordeaux2.fr

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Several recent studies have focused on the role of serotonin₂ (5-HT₂) receptors in the regulation of forebrain dopamine (DA) function and highlight their potential as a target for improved treatments of neuropsychiatric disorders related to central DA neuron dysfunction (Kapur and Remington 1996; Walsh and Cunningham 1997; Meltzer 1999). The involvement of 5-HT₂ receptor subtypes, that is, 5-HT_{2A} and 5-HT_{2C} receptors, in the control of mesolimbic and nigrostriatal

DA neuron activity is now well established (De Deurwaerdère and Spampinato 1999; Gobert and Millan 1999; Lucas et al. 2000a), and evidence has been provided that they exert opposite effects on DA release. It has also been suggested that these receptors may control DA release by acting at different levels of DA neuron regulatory mechanisms. On the one hand, 5-HT_{2A} receptors have been shown to facilitate stimulated but not basal DA release in both the rat nucleus accumbens (NAc) and the striatum. Compelling evidence from biochemical and electrophysiological studies has shown that 5-HT_{2A} receptors may modulate either impulse flow-independent or -dependent release of DA through mechanisms involving regulation of either DA synthesis or DA neuron firing rate, respectively (Schmidt et al. 1992; Gudelsky et al. 1994; Schmidt and Fadaye 1996). On the other hand, several neurochemical studies have shown that 5-HT_{2C} receptors exert inhibitory control on both basal and stimulated DA release (Di Giovanni et al. 1999; Hutson et al. 2000; Lucas et al. 2000a). In addition, electrophysiological studies have shown that basal firing rate of DA neurons located in both the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) is increased by 5-HT_{2C} receptor antagonists and inhibited by 5-HT_{2C} receptor agonists (Di Matteo et al. 2000; Gobert et al. 2000). Interestingly, it has recently been proposed that 5-HT_{2C} receptors may selectively regulate the impulse flow-dependent release of DA (Willins and Meltzer 1998; Lucas et al. 2000a).

The above-reported arguments that 5-HT_{2A} and 5-HT_{2C} receptors regulate DA release through different mechanisms may be of considerable interest when discussing the effect of drugs of abuse, such as psychostimulants or opiates, on DA function. Indeed, the fact that drugs of abuse increase DA release through different cellular mechanisms leads to the possibility that their effect on DA release could be modulated differentially by each of the 5-HT₂ receptor subtypes, depending on the mechanism of action of the drug considered. Thus, amphetamine-induced DA release, which occurs independently from DA neuron firing rate and involves increase in DA synthesis (Seiden et al. 1993; Cadoni et al. 1995), could be sensitive to 5-HT_{2A} but not 5-HT_{2C} receptor regulation. Conversely, morphine-stimulated DA release, thought to be a consequence of its excitatory effect on DA neuron firing rate (Di Chiara and North 1992), could be controlled by 5-HT_{2A} and 5-HT_{2C} receptors. Although some studies have already investigated the role of 5-HT₂ receptors on amphetamine- or morphine-induced DA release, the poor selectivity of the 5-HT compounds used does not allow a clear conclusion concerning this issue (Ichikawa and Meltzer 1992; Willins and Meltzer 1998).

Thus, in this study, we examined, by *in vivo* microdialysis, the effect of the selective 5-HT_{2A} receptor

antagonist SR 46349B and the 5-HT_{2C/2B} receptor antagonist SB 206553 on the release of DA induced by amphetamine or morphine in the NAc and striatum of halothane-anesthetized rats. This experimental procedure was chosen because it permits simultaneous monitoring of DA outflow in the ipsilateral NAc and striatum (De Deurwaerdère et al. 1998). To further explore the influence of each 5-HT₂ receptor subtype on the impulse flow-dependent DA release, we examined, by using single-unit extracellular recordings, the effect of SR 46349B and SB 206553 on the increase in VTA and SNc DA neuron firing rate induced by morphine. Finally, in a separate group of experiments, plasma and brain concentrations of amphetamine and morphine were measured to evaluate possible pharmacokinetic interactions between the probe drugs and the 5-HT-agents used.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (IFFA CREDO, Lyon, France and Consorzio Mario Negri Sud, Chieti, Italy) weighing 330–380 g were used. Animals were kept at constant room temperature (21 ± 2°C) and relative humidity (60%) with a 12-h light/dark cycle (dark from 8 P.M.) and had free access to water and food. All animals use procedures conformed to International European Ethical Standards (86/609-EEC) and the French National Committee (*décret* 87/848) for the care and use of laboratory animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Drugs

The following compounds were used: SR 46349B (trans-4-[(3Z)-3-[(2-dimethylaminoethyl)oximino]-3-(2-fluorophenyl)propen-1-yl]-phenol) hemifumarate (Sanofi Recherche, Montpellier, France) and SB 206553 (5-methyl-1-(3-pyridylcarbonyl)-1,2,3,5-tetrahydropyrrolo[2,3-f]indole) (SmithKline Beecham Pharmaceuticals, Harlow, UK), which were kindly provided by the producers. Morphine sulfate and *d*-amphetamine sulfate (amphetamine) were from Sigma (St Louis, MO). *d*-norfenfluramine hydrochloride was a generous gift from I.R.I.S. Laboratories (Courbevoie, France). All other chemicals and reagents were the purest commercially available (Merck; Sigma).

Microdialysis

Surgery and perfusion procedures were performed as previously described (De Deurwaerdère et al. 1998), with minor modifications. Briefly, rats were anesthetized with a mixture of halothane and nitrous oxide-oxygen (1%; 2:1 v/v). After tracheotomy for artificial

ventilation, the animals were placed in a stereotaxic frame, and their rectal temperature was monitored and maintained at $37.3^{\circ}\text{C} \pm 0.1$ with a heating pad. Two microdialysis probes, 2 and 3 mm long, (CMA/11, 240 μm outer diameter, Cuprophane; Carnegie Medicin, Phymep, Paris, France) were implanted simultaneously using a dual probe holder (Carnegie Medicin, Phymep) in the right NAc and striatum (coordinates from interaural point: anteroposterior [AP] = 11, lateral [L] = 1.3, ventral [V] = 2 and AP = 9.8, L = 3.3, V = 3, respectively) according to the atlas of Paxinos and Watson (1986). Probes were perfused at a constant flow rate of $2\mu\text{l}/\text{min}$ by means of a microperfusion pump (CMA 111; Carnegie Medicin, Phymep) with artificial cerebro spinal fluid (CSF) containing (in mM): 154.1Cl^{-} , 147Na^{+} , 2.7K^{+} , 1Mg^{2+} , and 1.2Ca^{2+} , adjusted to pH 7.4 with 2 mM sodium phosphate buffer. Dialysates (30 μl) were collected on ice every 15 min. The in vitro recoveries of the probes were $\sim 10\%$ for DA. At the end of each experiment, the brain was removed and fixed in NaCl (0.9%)/paraformaldehyde solution (10%). The location of the probes was determined histologically on serial coronal sections (100 μm) stained with cresyl violet, and only data obtained from rats with correctly implanted probes were included in the results.

Chromatographic Analysis

Dialysate samples were immediately analyzed by reverse-phase high-performance liquid chromatography (HPLC) coupled with electrochemical detection, as previously described (Bonhomme et al. 1995). The mobile phase (containing [in mM] $70\text{NaH}_2\text{PO}_4$, $0.1\text{Na}_2\text{EDTA}$, 0.7 triethylamine, and 0.1 octylsulfonic acid plus 10% methanol, adjusted to pH 4.8 with orthophosphoric acid) was delivered at $1\text{ml}/\text{min}$ flow rate (System Gold; Beckman, Paris, France) through a Hypersyl column (C18; $4.6 \times 150\text{mm}$, particle size $5\mu\text{m}$; Touzard & Matignon, Paris, France). Detection of DA was carried out with a coulometric detector (Coulchem II, ESA, Paris, France) coupled to a dual electrode analytic cell (model 5014). The potential of the electrodes was set at -175 and $+175\text{mV}$. Under these conditions, the sensitivity for DA was $0.5\text{pg}/30\mu\text{l}$ with a signal/noise ratio of 3:1.

Single-Cell Recording Procedures

As described previously (Di Giovanni et al. 1999), rats were anesthetized with chloral hydrate ($400\text{mg}/\text{kg}$ i.p.) and mounted on a stereotaxic instrument (SR-6; Narishige, Japan). Supplemental doses of anesthetic were administered *via* a lateral tail vein cannula. Throughout the experiment, the animal's body temperature was maintained at $36\text{--}37^{\circ}\text{C}$ by a thermostatically regulated heating pad. The coordinates, relative to the

interaural line, for placement of the recording electrode in the areas studied were (in millimeters), for the VTA, AP = 2.7–3.4, L = 0.3–0.5, V = 7–8 ventral to the level of exposed tissue; and for the SNc, AP = 2.7–3.4, L = 1.8–2.2, V = 6.5–7.5 (Paxinos and Watson 1986). Extracellular recordings were performed using single-barrel micropipettes (4–7 M Ω resistance containing 2% pontamine sky blue dye in 2 M NaCl). DA neurons were identified by their location, waveform, firing rate, and pattern (Bunney et al. 1973; Wang 1981). Electrical signals of spike activity were passed through a high-impedance amplifier whose output was led into an analog oscilloscope, audio monitor, and window discriminator. Unit activity was then converted to an integrated histogram by a rate-averaging computer and displayed as spikes per 10-s intervals.

After each experiment, the recording site was marked by the ejection of pontamine sky blue dye from the electrode using a $-20\mu\text{A}$ current for 10 min. Brains were removed and placed in 10% buffered formalin for 2 days before histological examination. Frozen sections were cut at $40\text{-}\mu\text{m}$ intervals and stained with Neutral Red. Microscopic examination of the sections was carried out to verify that the electrode tip was in the VTA or the SNc.

Pharmacological Treatments

Microdialysis Experiments. Pharmacological treatments were performed after the stabilization of DA levels in the perfusate. A stable baseline, defined as three consecutive samples in which DA contents varied by $<10\%$ in both structures, was generally obtained 135 min after the beginning of the perfusion (stabilization period).

Amphetamine ($0.5\text{mg}/\text{kg}$ and $2\text{mg}/\text{kg}$ i.p., diluted in NaCl 0.9%) and morphine ($2.5\text{mg}/\text{kg}$ s.c., diluted in a 50:50 v/v mixture of apyrogenic water and NaCl 0.9%) were administered at doses known to elicit a significant increase in DA outflow in both the rat NAc and the striatum (Di Chiara and Imperato 1988; Ichikawa and Meltzer 1992; Cadoni et al. 1995; Millan et al. 1999).

The 5-HT_{2A} receptor antagonist SR 46349B and the 5-HT_{2C/2B} receptor antagonist SB 206553, dissolved just before use in a mixture (50:50 v/v) of apyrogenic water and physiological saline (NaCl 0.9%) with a drop of lactic acid, were injected 15 min before amphetamine or morphine administration. SR 46349B was injected subcutaneously at $0.5\text{mg}/\text{kg}$, and SB 206553 was injected intraperitoneally at $5\text{mg}/\text{kg}$. These doses were chosen on the basis of previous studies, to keep both selectivity and efficiency toward the targeted sites (Schreiber et al. 1995; Kennett et al. 1996). All drug doses were calculated as the free base. In each experimental group, animals received either drugs or their appropriate vehicle.

Electrophysiological Experiments. Morphine ($0.1\text{--}10\text{mg}/\text{kg}$) was dissolved in 0.9% saline and administered

intravenously (via a lateral tail vein) in increasing doses every 2 min, and the effect on the activity of DA neurons was recorded. Only one cell per animal was studied. In one series of experiments, SR 46349B (100 $\mu\text{g}/\text{kg}$) and SB 206553 (100 $\mu\text{g}/\text{kg}$) were dissolved in 200 μl of 10% acetic acid, made up to almost the required volume with 0.9% saline and brought to pH 6.5 before injecting. Both SR 46349B and SB 206553 were administered i.v. 5–7 min before the first injection of morphine (0.1–10 mg/kg i.v.). All drug dosages refer to the weight of the salt.

Ex vivo Measurement of Plasma and Brain Concentrations of Amphetamine and Morphine

In a separate group of experiments, plasma and brain concentrations of amphetamine and morphine were measured to evaluate possible pharmacokinetic interactions between the probe drugs and the 5-HT agents used. As for the microdialysis studies, SR 46349B (0.5 mg/kg s.c.) or SB 206553 (5 mg/kg i.p.) were administered 15 min before amphetamine (2 mg/kg i.p.) or morphine (2.5 mg/kg s.c.). Rats were killed by decapitation 30 (amphetamine) or 75 min (morphine) later. Blood was collected in heparinized tubes and centrifuged, and the plasma was stored at -20°C . Brains were removed immediately after exsanguination, and brain areas were dissected (NAc, striatum, and the remaining part of the brain), blotted with paper to remove excess surface blood, and quickly frozen in dry ice.

Plasma and brain concentrations of amphetamine were analyzed by a modification of the electron capture gas liquid chromatography method previously described for *d*-fenfluramine (Caccia et al. 1995), using *d*-norfenfluramine as an internal standard. Briefly, 0.5 ml of plasma or homogenated tissues (hydrochloric acid, 5 ml/g) were alkalized and extracted with toluene. After derivatization with pentafluoropropionyl anhydride, the samples were washed with ammonia solution and water, and 1–2 μl of the toluene phase was injected into a CP-Sil-8 CB capillary column (25 m \times 0.25 mm i.d.). The chromatographic conditions were 2 ml/min carrier gas head pressure, total injection, PTV injector temperatures ranging from 50 to 300°C . The temperature gradient was $2.8^{\circ}\text{C}/\text{min}$ ranging from 70 to 120°C and then a second gradient of $30^{\circ}\text{C}/\text{min}$ ranging from 120 to 200°C .

Morphine was extracted from plasma by a solid-liquid extraction procedure and quantified by a minor modification of the HPLC with electrochemical detection described by Svensson et al. (1995). Brain tissues were homogenized (5 ml/g) in 0.1 M KH_2PO_4 , pH 7.4, and centrifuged at 5000 g for 10 min (at 4°C), and the supernatants were processed as the plasma samples.

Standard curves were prepared daily by adding known concentrations of amphetamine or morphine to blank plasma or brain tissue. Linear regression analyses

revealed coefficients of correlation between 0.993 and 0.998 for both drugs. For amphetamine, the lower point of the calibration graph (100 ng/ml, using 0.5 ml of plasma or brain homogenate) was well above the limit of quantification. At this concentration, coefficients of variation (CV) for the precision of the assay were usually $<10\%$. For morphine, the limit of quantification in plasma was ~ 10 ng/ml, using 0.5 ml of plasma. In brain it was 50 ng/g, using 0.5 ml of brain homogenate or ~ 100 mg of tissue; this precluded the quantification of morphine in the NAc. The CV were between 12 and 18% in both tissues.

Statistical Analysis

Microdialysis Experiments. DA content in each sample was expressed as the percentage of the average baseline level calculated from the three fractions preceding any treatment. Data correspond to the mean \pm SEM values of the percentage obtained in each experimental group.

The statistical analysis of the effect of the 5-HT₂ antagonists alone on DA outflow was assessed by a one-way analysis of variance (ANOVA) with time as repeated measures, performed for the 13 samples that followed their administration (i.e., time fractions 0–180 min in Figures 1 and 2). A similar procedure was used to assess the effect of amphetamine and morphine on basal DA release, but in this case, the repeated measures were performed for the 12 samples that followed their administration (i.e., time fractions 15–180 min in Figures 1 and 2). The ability of 5-HT₂ antagonists (pretreatment) to modify the effect of either amphetamine or morphine (treatment) on DA release was studied by a two-way ANOVA (pretreatment \times treatment) with time as repeated measures, performed for the 12 samples that followed the administration of amphetamine or morphine (i.e., time fractions 15–180 min in Figures 1 and 2). This statistical procedure takes into account an eventual effect of the pretreatment on its own by comparing the difference between the treatment (amphetamine or morphine) alone and control groups with that between the treatment plus pretreatment and the pretreatment alone groups. When two-way ANOVA results were significant ($p < .05$), a post hoc Tukey's test was performed to permit adequate multiple comparison among groups.

Electrophysiological Experiments. Data acquisition and analysis were accomplished using an 83286-based PC and an integrated software package for electrophysiology (RISI; Symbolic Logic, Dallas, TX). Cumulative dose–response curves were constructed by comparing the mean firing rate of 500 consecutive spikes, starting immediately after the injection of each drug with the basal firing rate. The data obtained were subjected to

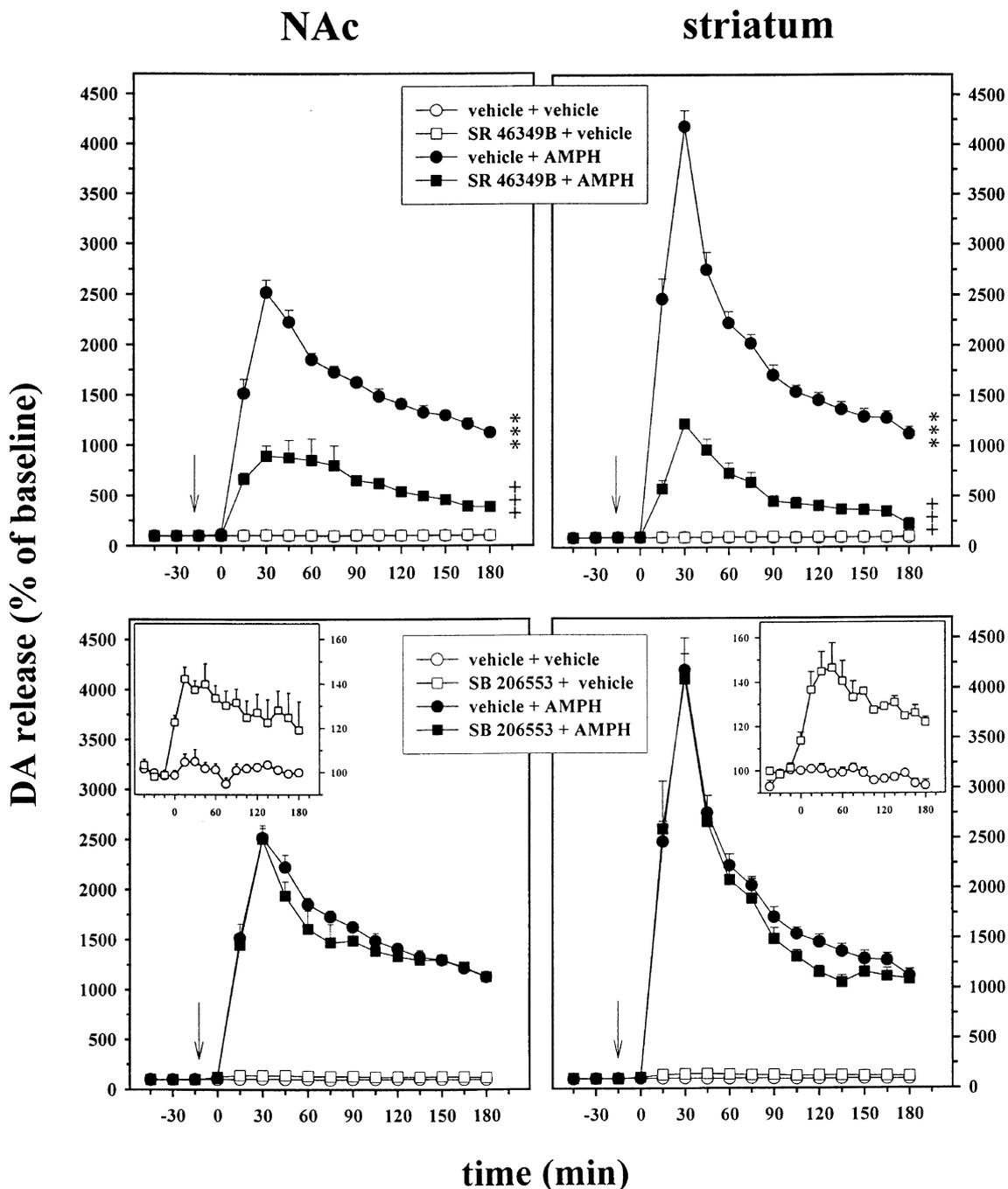


Figure 1. Time course of the effect of the 5-HT_{2A} antagonist SR 46349B (0.5 mg/kg s.c.; top) and the 5-HT_{2C/2B} antagonist SB 206553 (5 mg/kg i.p.; bottom) administration on basal and amphetamine-stimulated DA release in the NAc and striatum. The 5-HT₂ antagonists were injected (vertical arrows) 15 min before the intraperitoneal administration of 2 mg/kg amphetamine (time zero). Data, obtained from 5–11 animals per group, are presented as the mean ± SEM percentages of the baseline calculated from the three samples preceding the first drug administration. ****p* < .001 versus the vehicle + vehicle group; +++*p* < .01 versus the vehicle + amphetamine group (Tukey’s test). **Inset:** Time course of the effect of the 5-HT_{2C/2B} antagonist SB 206553 on basal DA release in the NAc (left) and striatum (right). AMPH, amphetamine.

one-way ANOVA followed by Tukey’s test. The interactions between SR 46349B or SB 206553 and morphine were analyzed by ANOVA (two-factor mixed design) followed by Tukey’s test.

Ex vivo Measurement of Amphetamine and Morphine Concentrations.

Concentrations of amphetamine and morphine were expressed as the mean ± SD of nanomoles/gram of

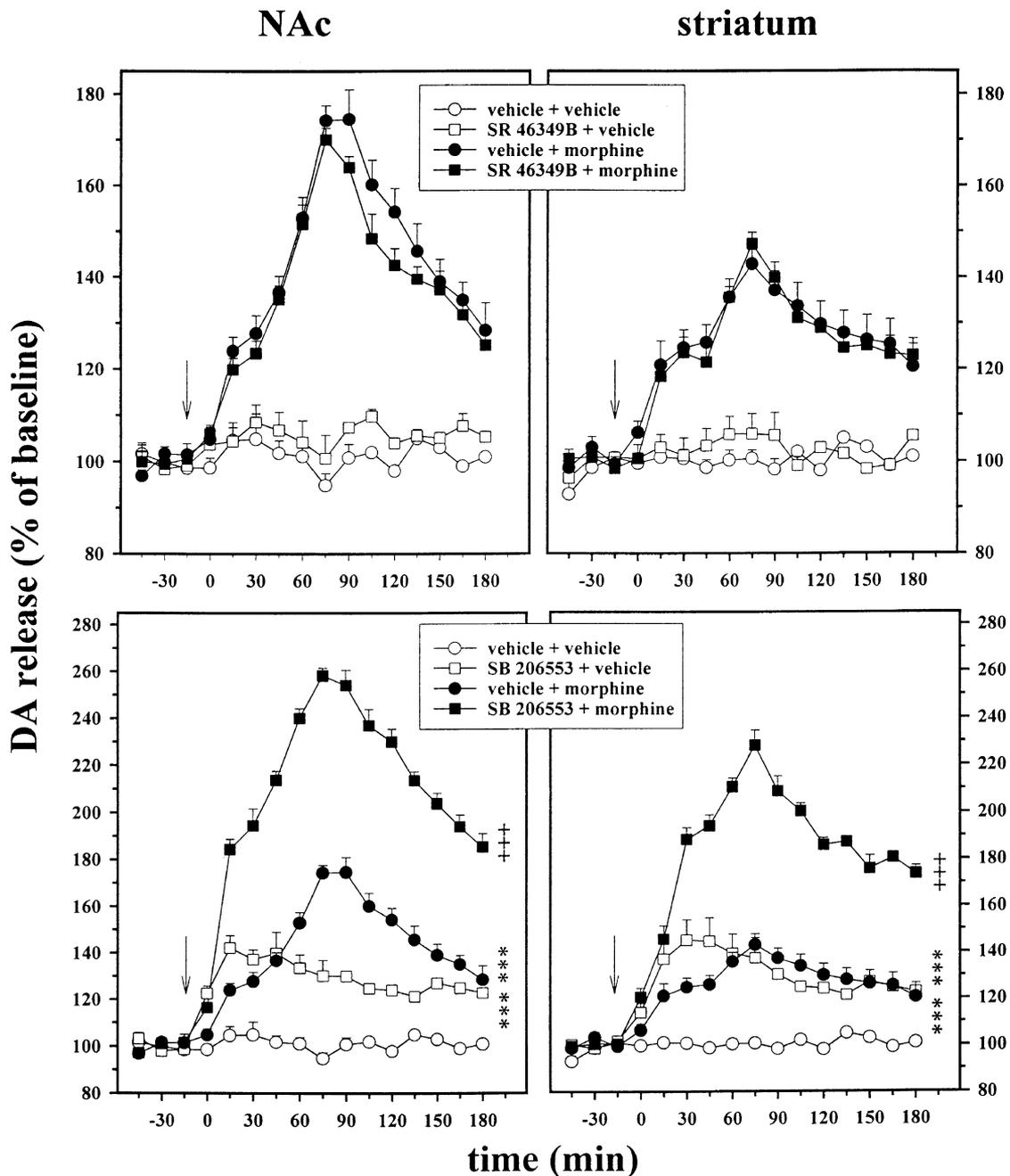


Figure 2. Time course of the effect of the 5-HT_{2A} antagonist SR 46349B (0.5 mg/kg s.c.; top) and the 5-HT_{2C/2B} antagonist SB 206553 (5 mg/kg i.p.; bottom) administration on basal and morphine-stimulated DA release in the NAc and striatum. The 5-HT₂ antagonists were injected (vertical arrows) 15 min before the subcutaneous administration of 2.5 mg/kg morphine (time zero). Data, obtained from four to nine animals per group, are presented as the mean \pm SEM percentages of the baseline calculated from the three samples preceding the first drug administration. *** p < .001 versus the vehicle + vehicle group; +++ p < .01 versus the vehicle + morphine group (Tukey's test).

striatum or as the mean \pm SD of tissue-to-plasma ratio obtained in each experimental group. The data were analyzed by a Student's *t*-test to determine whether one 5-HT agent may affect amphetamine and morphine concentrations with respect to its vehicle.

In all cases, p < .05 was chosen as the criterion for significance.

RESULTS

Basal Extracellular DA Concentrations in Dialysates from NAc and Striatum

All measurements were performed 150 min after the beginning of perfusion, by which time a steady state was achieved. Absolute basal levels of DA in dialysate si-

multaneously collected from the NAc and the striatum were (without adjusting for probes recovery) 4.6 ± 0.6 pg/30 μ l ($n = 15$) and 13.3 ± 1.6 pg/30 μ l ($n = 15$), respectively.

Effect of Amphetamine and Morphine on Basal DA Outflow

The effect of the intraperitoneal administration of amphetamine on extracellular concentrations of DA in the NAc and in the striatum is shown in Figure 1. Amphetamine (2 mg/kg) elicited a strong increase in DA outflow, which reached $\sim 2500\%$ of basal values in the NAc (one-way ANOVA, $F_{1,14} = 434.8$, $p < .001$) and 4200% in the striatum (one-way ANOVA, $F_{1,14} = 424.5$, $p < .001$) 30 min after drug injection. Thereafter, in both brain regions, DA outflow decreased progressively but remained significantly elevated (~ 1500 – 2000% of basal values) during the entire experimental period. In a separate set of experiments, the effect of the intraperitoneal administration of 0.5 mg/kg amphetamine was also assessed. In this case, amphetamine enhanced significantly DA outflow in both brain regions (one-way ANOVA, $F_{1,11} = 281.8$ and 83.3 in the NAc and in the striatum, respectively; $p < .001$), but its effect was of lower magnitude than that induced by the higher dose. Its maximum, observed 45 min after amphetamine injection, reached 957 and 1800% of baseline in the NAc and the striatum, respectively ($p < .001$). Thereafter, DA outflow decreased progressively to reach 387 and 562% of baseline 3 h after its administration (data not shown).

As illustrated in Figure 2, the subcutaneous administration of 2.5 mg/kg morphine enhanced DA outflow in both the NAc and the striatum (one-way ANOVA, $F_{1,10} = 191$, $p < .001$ and $F_{1,11} = 61.3$, $p < .001$, respectively). The maximal increase was observed 75 min after drug injection in each brain region ($+75\%$ and $+37\%$ over baseline values for the NAc and striatum, respectively).

Effect of SR 46349B and SB 206553 on Basal DA Outflow

As previously reported (De Deurwaerdère and Spampinato 1999), selective blockade of central 5-HT_{2A} receptors induced by SR 46349B (0.5 mg/kg s.c.) did not affect basal DA outflow in either the NAc or the striatum (one-way ANOVA, $F_{1,13} = 3.5$, not significant (n.s.), and $F_{1,13} = 4$, n.s., respectively). On the other hand, the intraperitoneal administration of the 5-HT_{2C/2B} receptor antagonist SB 206553 elicited a long-lasting and significant increase in DA outflow over control levels in both regions (one-way ANOVA, $F_{1,9} = 25.6$, $p < .001$ for the NAc and $F_{1,9} = 114.1$, $p < .001$ for the striatum, see insets in Figure 1 and lower panels in Figure 2). As shown previously (De Deurwaerdère and Spampinato 1999),

the maximal effect was observed 30 and 45 min after its administration in the NAc ($+ 42\%$) and the striatum ($+ 46\%$), respectively.

Effect of SR 46349B and SB 206553 on Amphetamine-Induced DA Outflow

The administration of SR 46349B (0.5 mg/kg s.c.) significantly reduced the increase in DA outflow induced by amphetamine in the NAc (two-way ANOVA, treatment \times pretreatment, $F_{1,27} = 122$, $p < .001$) and the striatum (two-way ANOVA, pretreatment \times treatment, $F_{1,27} = 188$, $p < .001$). In the NAc, DA outflow reached only 800% of baseline in the SR 46349B + amphetamine group 30 min after the administration of amphetamine, and thereafter it decreased progressively to 500% of baseline at the end of the experimental period (Tukey's test, $p < .001$ versus the vehicle + amphetamine group; Figure 1, top left). In the striatum, amphetamine-induced DA output was also reduced by SR 46349B. DA extracellular levels reached only 1500% of basal values at their maximum and thereafter decreased progressively to $\sim 250\%$ of baseline at the end of the experience (Tukey's test, $p < .001$ versus the vehicle + amphetamine group; Figure 1, top right).

In contrast, SB 206553 (5 mg/kg i.p.) failed to modify amphetamine-induced increase of DA outflow in both the NAc (two-way ANOVA, treatment \times pretreatment, $F_{1,22} = 1.3$, n.s.) and the striatum (two-way ANOVA, treatment \times pretreatment, $F_{1,22} = 1.32$, n.s.) (Figure 1, bottom). As well, SB 206553 did not modify the increase in accumbal and striatal DA outflow induced by 0.5 mg/kg amphetamine (two-way ANOVA, treatment \times pretreatment, $F_{1,18} = 2.7$ and 1.7 in the NAc and the striatum, respectively, n.s.) (data not shown).

Effect of SR 46349B and SB 206553 on Morphine-Induced DA Outflow

SR 46349B failed to alter the facilitatory effect of morphine on DA outflow in either brain region (two-way ANOVA, pretreatment \times treatment, $F_{1,24} = 5.5$, n.s., and $F_{1,24} = 1.7$, n.s., for the NAc and the striatum, respectively) (Figure 2, top).

On the other hand, SB 206553 significantly potentiated morphine-stimulated DA outflow in both the NAc and the striatum (two-way ANOVA, $F_{1,18} = 28.8$, $p < .001$ and $F_{1,18} = 15.9$, $p < .001$, respectively). Accumbal and striatal DA outflow in the SB 206553 + morphine group was increased up to 260% and 230% of baseline, respectively, 75 min after morphine administration. Thereafter, DA outflow remained significantly higher than values found in the SB 206553 + vehicle and vehicle + morphine groups throughout the entire experimental period (Tukey's test, $p < .001$ versus the vehicle + morphine group) (Figure 2, bottom).

Effect of SB 206553 and SR 46349B on Basal and Morphine-Stimulated Activity of DA Neurons in the VTA and the SNc

Cumulative intravenous administration of morphine (0.1–10 mg/kg i.v.) caused a dose-dependent increase in the basal activity of DA neurons both in the VTA (one-way ANOVA, $F_{1,5} = 14.32$, $p < .01$, $n = 5$) and the SNc (one-way ANOVA, $F_{1,5} = 22.21$, $p < .01$, $n = 6$). The maximal excitatory effect of morphine was reached at the cumulative dose of 14.4 mg/kg, which enhanced by ~40% the basal firing rate of VTA and SNc DA neurons (Tukey's test, $p < .01$) (Figures 3B and C and 4B and C, see the different morphine-alone groups).

As previously shown (Di Giovanni et al. 1999), pretreatment with SR 46349B (100 μ g/kg i.v.) had no influence on basal DA neuron activity in both brain regions. Although a transient and slight reduction was observed in some neurons, basal firing rate returned to baseline levels before the first morphine injection (Figures 3A

and 4A). Also, SR 46349B did not affect the morphine-induced excitation of DA neurons in either the VTA (two-way ANOVA, $F_{1,9} = 0.13$, n.s., $n = 5$) or the SNc (two-way ANOVA, $F_{1,10} = 0.08$, n.s., $n = 5$) (Figures 3C and 4C).

Injection of a single dose of SB 206553 (100 μ g/kg i.v.) caused in some neurons a slight and transient increase in the basal firing rate of VTA and SNc DA neurons, whereas it did not affect the activity of other DA neurons tested (not shown). However, the firing rate of neurons that were excited by SB 206553 returned close to baseline levels before the first injection of morphine. Pretreatment with SB 206553 (100 μ g/kg i.v.) potentiated the stimulatory effect of morphine on VTA DA neurons (two-way ANOVA, $F_{1,10} = 1.91$, $p < .05$, $n = 6$). Although this potentiating effect reached statistical significance only at the dose of 0.3 mg/kg morphine (Tukey's test, $p < .05$), there was also a tendency toward potentiation at the dose of 1 mg/kg. However,

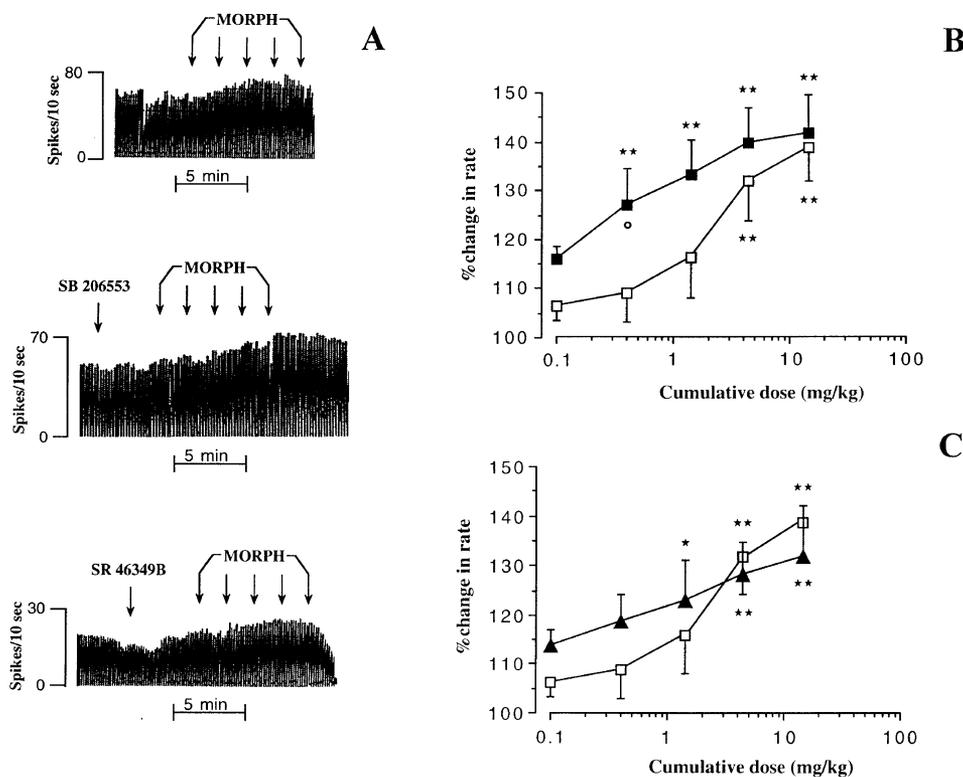


Figure 3. Effect of the 5-HT_{2A} antagonist SR 46349B and the 5-HT_{2C/2B} antagonist SB 206553 administration on basal and morphine-stimulated activity of DA neurons in the VTA. **A)** Representative rate histograms showing the effects of i.v. morphine (MORPH) (0.1, 0.3, 1, 3, and 10 mg/kg, at arrows) administered alone (top) and after pretreatment with SB 206553 (100 μ g/kg i.v.) (middle) or SR 46349B (100 μ g/kg i.v.) (bottom). **B)** Cumulative dose–response curves showing that pretreatment with SB 206553 (100 μ g/kg i.v.) (■) potentiates the excitatory effect of morphine (□) on DA neurons in the VTA. **C)** Cumulative dose–response curves showing the lack of effect of SR 46349B (100 μ g/kg i.v.) (▲) pretreatment on morphine (□)-induced increase in the activity of VTA DA neurons. Data, obtained from five to six animals per group, are presented as the mean \pm SEM percentages of the basal firing rate. * $p < .05$, ** $p < .01$ versus the respective basal firing rate of morphine alone, SB 206553 + morphine, and SR 46349B + morphine groups (Tukey's test). ° $p < .05$ versus the corresponding dose of the morphine-alone group (Tukey's test).

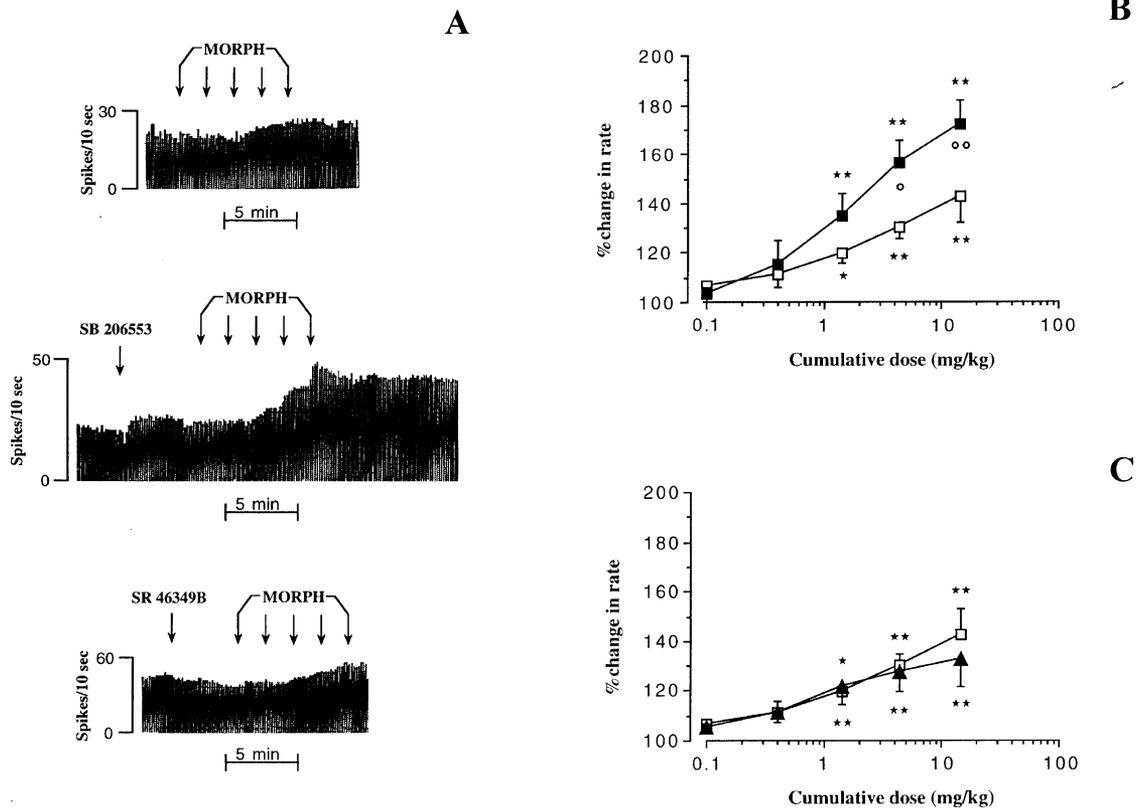


Figure 4. Effect of the 5-HT_{2A} antagonist SR 46349B and the 5-HT_{2C/2B} antagonist SB 206553 administration on basal and morphine-stimulated activity of DA neurons in the SNc. **A)** Representative rate histograms showing the effects of i.v. morphine (MORPH) (0.1, 0.3, 1, 3, and 10 mg/kg, at arrows) administered alone (top) and after pretreatment with SB 206553 (100 μg/kg i.v.) (middle) or SR 46349B (100 μg/kg i.v.) (bottom). **B)** Cumulative dose–response curves showing that pretreatment with SB 206553 (100 μg/kg i.v.) (■) potentiates the excitatory effect of morphine (□) on DA neurons in the SNc. **C)** Cumulative dose–response curves showing the lack of effect of SR 46349B (100 μg/kg i.v.) (▲) pretreatment on morphine (□)-induced increase in the activity of SNc DA neurons. Data, obtained from five to six animals per group, are presented as the mean ± SEM percentages of the basal firing rate. **p* < .05, ***p* < .01 versus the respective basal firing rate of morphine alone, SB 206553 + morphine, and SR 46349B + morphine groups (Tukey’s test). °*p* < .05, °°*p* < .01 versus the corresponding dose of the morphine-alone group (Tukey’s test).

the maximal effect of 14.4 mg morphine was almost superimposable in the two experimental groups (Figure 3B). Moreover, pretreatment with SB 206553 (100 μg/kg i.v.) clearly potentiated the excitatory effect of morphine on DA neurons in the SNc (two-way ANOVA, $F_{1,11} = 3.1, p < .01, n = 5$). Thus, the maximal excitatory effect of morphine in the control group was $42.3 \pm 10.4\%$, whereas it reached the value of $72.0 \pm 10.0\%$ in the SB 206553-pretreated group (Figure 4B).

Effects of 5-HT Pretreatments on Amphetamine and Morphine Brain Concentrations

Table 1 reports the mean ± SD striatal concentrations of amphetamine and morphine in vehicle-, SR 46349B- (0.5 mg/kg s.c.) and SB 206553- (5 mg/kg i.p.) treated rats. Measurements were performed 30 min after amphetamine (2 mg/kg i.p.) and 75 min after morphine

(2.5 mg/kg s.c.) administration, a time corresponding approximately to the peak effect induced by these drugs on accumbal and striatal DA release. None of the 5-HT agents used in this study affected amphetamine striatal concentrations (Table 1). Similar results were observed in the Nac, where amphetamine concentrations were 22 ± 5 nmol/g in controls and 24 ± 6 and 24 ± 7 nmol/g in SR 46349B- and SB 206553-treated rats respectively (data not shown). Mean plasma concentrations of amphetamine were $1.2 \pm 0.5, 1.1 \pm 0.4,$ and 1.2 ± 0.3 nmol/ml in vehicle-, SR 46349B- and SB 206553-treated rats, respectively. The mean tissue-to-plasma ratios were not significantly affected by 5-HT₂ antagonists (Table 1).

Mean striatal concentrations of morphine in SR 46349B- and SB 206553-pretreated rats were also comparable with those in controls (Table 1). As mentioned, morphine concentrations in the NAc could not be quan-

tified because of methodological limitations. However, whole-brain concentrations of morphine (~ 0.2 nmol/g) were also similar among the different experimental groups (data not shown). Morphine mean plasma concentrations were 0.31 ± 0.08 , 0.33 ± 0.11 , and 0.27 ± 0.08 nmol/ml in vehicle-, SR 46349B- and SB 206553-treated rats, with mean tissue-to-plasma ratios close to unity in all groups (Table 1). The mean distribution ratios are similar to those observed in rats under other experimental conditions (Bolander et al. 1983; Bhargava et al. 1992).

DISCUSSION

This study confirms and extends previous findings showing that 5-HT_{2A} and 5-HT_{2C} receptors serve opposite roles in the control of activated DA release in both the NAc and striatum. Furthermore, the obtained results indicate that the recruitment of these receptor subtypes in the control of amphetamine- or morphine-induced DA release is different and may depend on the mechanism of action and/or on the neuronal circuitry involved in the DA effect elicited by each drug considered. Finally, they provide evidence in support of the proposal that 5-HT_{2C} receptors selectively control the impulse flow-dependent release of DA.

As expected, we found that 5-HT_{2A} and 5-HT_{2C} receptors exert different roles in the control of basal DA neuron activity. Thus, selective blockade of central 5-HT_{2A} receptors by SR 46349B has no effect on its own on either mesoaccumbal or nigrostriatal DA neuron activity. This finding supports the conclusion initially proposed by Schmidt and coworkers (1992), and confirmed by others (Ichikawa and Meltzer 1995; De Deurwaerdère and Spampinato 1999; Gobert and Millan 1999; Lucas et al. 2000a), that 5-HT_{2A} receptors are unable to modulate DA function under resting conditions. In contrast, the 5-HT_{2C/2B} antagonist SB 206553 enhanced by itself both basal DA release in the NAc and the striatum and basal firing rate of DA neurons in the VTA and SNc. Similar biochemical and electrophysiological results have been obtained recently in the presence of the selective 5-HT_{2C}

antagonist SB 242084 (Di Matteo et al. 2000; Gobert et al. 2000). Considering that selective blockade of central 5-HT_{2B} receptors has no influence on DA cell function (Gobert et al. 2000) and that extremely low levels of 5-HT_{2B} receptor mRNA and protein are found in any case only in restricted rat brain regions (Pompeiano et al. 1994; Duxon et al. 1997), it is likely that the effects we observed in the presence of SB 206553 are mediated by 5-HT_{2C} receptors. Thus, in contrast to 5-HT_{2A} receptors, 5-HT_{2C} receptors exert a tonic inhibitory control on basal DA neuron activity in the brain (Di Giovanni et al. 1999).

As previously reported (Di Chiara and Imperato 1988; Ichikawa and Meltzer 1992; Millan et al. 1999), systemic administration of amphetamine and morphine elicits a significant and long-lasting enhancement of DA release in both the NAc and the striatum. We found that the effects of amphetamine and morphine on DA release are sensitive to the peripheral administration of 5-HT_{2A} and 5-HT_{2C/2B} receptor antagonists, respectively. Although the clearance of the P₄₅₀ substrate amphetamine may be affected by drugs that undergo oxidative metabolism (Garattini and Samanin 1981), we found that its brain concentrations in SR 46349B- and SB 206553-pretreated rats were not different from those in controls. Morphine is cleared mainly by glucuronide conjugation, and like amphetamine, its transport to the brain is thought to occur mainly by passive diffusion (Murphey and Olsen 1994). Recent studies have shown that morphine is also a P-glycoprotein substrate and that inhibition of this protein may alter its blood-to-brain distribution, thereby enhancing its brain availability and increasing its pharmacological action (Callaghan and Riordan 1993; Letrent et al. 1998). However, the brain concentrations and the brain-to-plasma distribution ratios of morphine in 5-HT agent-treated rats were not different from controls at the time considered. Thus, changes of amphetamine- and morphine-stimulated DA output observed in the presence of the 5-HT₂ antagonists used are not due to metabolic interference but are based on pharmacodynamic interactions between the 5-HT and DA systems in the brain.

Central 5-HT_{2A} receptor blockade by SR 46349B strongly reduces the facilitatory effect of 2 mg/kg amphetamine on accumbal and striatal DA outflow. This result confirms and extends previous findings suggesting, on the basis of experiments performed with the non selective 5-HT₂ antagonist amperozide, that 5-HT_{2A} receptors facilitate amphetamine-induced DA release (Ichikawa and Meltzer 1992). In line with these results, it has been reported that preferential or selective 5-HT_{2A} antagonists such as ketanserin or MDL 100907 reduce the increase in DA outflow induced by 3,4-methylenedioxymethamphetamine (MDMA) (Nash 1990; Schmidt et al. 1992). A facilitatory action of 5-HT_{2A} receptors on DA synthesis has been shown to be critical to sustain

Table 1. Mean Striatal Concentrations of Amphetamine and Morphine

Treatment (mg/kg)	Striatal Concentrations (nmol/g)			
	Amphetamine		Morphine	
Vehicle	16 ± 5	(16 ± 8)	0.31 ± 0.08	(1.1 ± 0.3)
SR 46349B (0.5)	16 ± 4	(12 ± 4)	0.28 ± 0.05	(0.9 ± 0.4)
SB 206553 (5)	19 ± 7	(18 ± 5)	0.34 ± 0.02	(1.1 ± 0.1)

Rats were given the vehicle, SR 46349B, or SB 206553 at the doses indicated 15 min before amphetamine (2 mg/kg i.p.) or morphine (2.5 mg/kg s.c.) and were killed by decapitation 30 min (amphetamine) or 75 min (morphine) later. The tissue-to-plasma ratios are shown in parentheses. Each value is the mean ± SD of four to five rats.

the impulse flow-independent release of DA induced by MDMA (Schmidt et al. 1992). Amphetamine also increases the efflux of DA by a mechanism independent from DA neuron impulse flow and in part related to the mobilization of the newly synthesized pool of DA (Hurd and Ungerstedt 1989; Seiden et al. 1993; Cadoni et al. 1995). Thus, as in the case of MDMA, the inhibitory effect of SR 46349B on amphetamine-stimulated DA outflow may occur through an inhibition of DA synthesis.

Conversely, we found that 5-HT_{2A} antagonism has no influence on the enhancement of DA release induced by morphine in either the NAc or the striatum. In agreement with this result, previous studies in freely moving rats have shown that morphine-induced accumbal DA release is insensitive to 5-HT_{2A} receptor modulation (Willins and Meltzer 1998). In addition, and in agreement with previous electrophysiological findings (Gysling and Wang 1983), we found that SR 46349B does not modify morphine-stimulated firing rate either in VTA or in SNc DA neurons. Hence, it appears that an increase of DA release and/or transmission does not necessarily allow per se the occurrence of the 5-HT_{2A}-mediated control of DA function. It is unlikely that the lack of effect of SR 46349B could be explainable only by the less marked increase in DA release induced by morphine with respect to amphetamine (175–137% versus 2500–4200% of baseline, respectively). Indeed, although it has been proposed that 5-HT_{2A} receptors control DA neuron activity in conditions in which DA release and/or synthesis is markedly increased (Schmidt et al. 1992; Gudelsky et al. 1994), we and others have already shown that an increase in DA release of similar or lower magnitude than that induced by morphine is also sensitive to 5-HT_{2A} antagonism (Schmidt and Fadaye 1996; De Deurwaerdere and Spampinato 1999; Liégeois et al. 2000; Lucas et al. 2000a). It is also unlikely that the impulse flow-dependent nature of the DA released by morphine (Di Chiara and North 1992) may account for the failure of SR 46349B to modulate the effect of morphine. 5-HT_{2A} receptors are able to modulate the neurochemical effects induced by drugs thought to induce an impulse flow-dependent release of DA, such as the uncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 (Schmidt and Fadaye 1996) and haloperidol (Imperato and Di Chiara 1985). Thus, MDL 100907 reduces the increase in both accumbal DA release and VTA DA neuron firing rate induced by MK-801 (Sorensen 1995; Schmidt and Fadaye 1996). Moreover, we have recently found that SR 46349B counteracts both the increase in striatal DA release (Lucas et al. 2000a) and in SNc DA neuron firing rate (unpublished results) induced by haloperidol. Considering the above-discussed data, the failure of 5-HT_{2A} receptors to control morphine-stimulated DA function indicates that, in addition to DA neuron activity level (increased DA synthesis

and/or release/transmission) (Schmidt et al. 1992; Millan et al. 1999; Lucas et al. 2000a), factors other than the magnitude and/or the nature of the DA released are required to trigger the control exerted by 5-HT_{2A} receptors. These factors may be related to the specific mechanism of action of a given drug and to the neuronal circuitry involved in its effect on DA neurons. In line with this idea, the fact that 5-HT_{2A} antagonism modifies the increase in accumbal DA release elicited by the uncompetitive NMDA receptor antagonists MK-801 but not phencyclidine (Schmidt and Fadaye 1996; Millan et al. 1999) may reflect the different neuronal mechanisms underlying the DA effects elicited by these NMDA receptor antagonists (Ögren and Goldstein 1994). Further studies are needed to determine the specific neurochemical states in which central 5-HT_{2A} receptors control DA neuron activity.

A different picture emerges when looking at the influence of 5-HT_{2C} receptors on morphine- and amphetamine-induced effects on DA neuron activity. Thus, we found that SB 206553 administration potentiates the enhancement of DA release elicited by morphine in both the NAc and striatum. Consistent with this finding, stimulation of central 5-HT_{2C} receptors has been shown to inhibit morphine-induced increase in DA release in the NAc of freely moving rats (Willins and Meltzer 1998). Thus, our results confirm previous work showing that morphine-induced DA release is sensitive to 5-HT modulation (Carboni et al. 1989; Pozzi et al. 1995; Willins and Meltzer 1998) and indicate that endogenous 5-HT via 5-HT_{2C} receptors inhibits stimulated DA release. In line with this, it has been reported that the increase in accumbal or striatal DA release elicited, respectively, by phencyclidine or haloperidol is further enhanced by the blockade of 5-HT_{2C} receptors (Hutson et al. 2000; Lucas et al. 2000a). Interestingly, we found that SB 206553 also potentiates the increase in VTA and SNc DA neuron firing rate induced by morphine. This finding is consistent with previous electrophysiological data showing that 5-HT_{2C} receptors exert an inhibitory control on mesencephalic DA neuron firing rate (Di Matteo et al. 2000; Gobert et al. 2000). Although the distinct temporal and spatial resolution, the different anesthetics, and the different doses and routes of drug administration make it difficult to directly compare data from *in vivo* microdialysis and single-unit recordings, it is tempting to suggest that 5-HT_{2C} receptors exert their control on DA release via a modulation of DA neuron firing rate. This possibility is further supported by recent data from our laboratory showing that the facilitatory effect of the SB 206553 on haloperidol-stimulated striatal DA release is no longer observed when haloperidol is administered at doses inducing a maximal increase in DA neuron firing rate (Lucas et al. 2000a).

On the other hand, SB 206553 did not modify the increase in DA release elicited by 2 mg/kg amphet-

amine. It is unlikely that the failure of SB 206553 to potentiate amphetamine-induced DA release is due to a loss of reactivity of DA neurons related to the high magnitude of the increase in DA release elicited by 2 mg/kg amphetamine. Indeed, the lower increase in DA outflow induced by 0.5 mg/kg amphetamine is also insensitive to 5-HT_{2C} antagonism. Thus, our findings provide the first evidence that amphetamine-induced DA release is insensitive to 5-HT_{2C} receptor modulation. Also, they support Willins and Meltzer's (1998) proposal that impulse flow-dependent and -independent release of DA is regulated differently by 5-HT and that 5-HT_{2C} receptors preferentially act on the release of DA originating from DA nerve impulse flow. This regulatory process is likely to occur indirectly given that 5-HT_{2C} receptor mRNA is expressed by GABA-containing cells (γ -amino butyric acid) but not by DA neurons within the SN and the VTA (Eberle-Wang et al. 1997; Di Giovanni et al. 1999).

Finally, it is important to note that, although halothane is known to modify DA neuron activity (Bunney et al. 1973), it is unlikely that the anesthetized preparation used in the present study may have modified the outcome of the performed experiments. Indeed, previous studies reporting similar modulatory effects of the 5-HT system on striatal and accumbal DA release in either freely moving (De Simoni et al. 1987; Ichikawa and Meltzer 1992; Schmidt and Fadaye 1996; Steward et al. 1996; Willins and Meltzer 1998; Millan et al. 1999; Lucas and Spampinato 2000; Lucas et al. 2000a, 2000b) or anaesthetized (Benloucif et al. 1993; Bonhomme et al. 1995; Kankaanpää et al. 1996; De Deurwaerdère and Spampinato 1999; Di Matteo et al. 2000; Lucas et al. 2000b) rats strongly suggest that anesthesia does not alter the responsiveness of midbrain DA neurons to 5-HT modulation.

In summary, this study confirms that 5-HT_{2A} and 5-HT_{2C} receptor subtypes exert opposite influence on central DA function and provides new evidence that the recruitment of these receptors in the control of DA neuron activity occurs under different conditions. 5-HT_{2A} and 5-HT_{2C} receptors specifically regulate the activation of midbrain DA neurons induced by amphetamine or morphine, respectively. This differential contribution may be conditioned by the specific mechanism of action of the drug considered and/or by the neuronal circuitry involved in its effect on DA neurons. Furthermore, the obtained results suggest that 5-HT_{2C} receptors selectively modulate the impulse flow-dependent release of DA, probably by acting on DA neuron firing rate. From a therapeutic point of view, given the importance of the mesolimbic DA system in the neurobiological mechanisms leading to drug addiction (Koob and Le Moal 2001), our findings add further support to the proposal that 5-HT agents may have some relevance for the treatment of this pathological condition (Walsh and Cunningham 1997). Also, they highlight the potential of 5-HT₂

receptor subtypes as a target for improved pharmacotherapies acting selectively on the DA-stimulating effects induced by the different drugs of abuse. Further studies are therefore warranted to provide a more thorough appreciation of the role of central 5-HT receptors in drug addiction.

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