Constitutive Activity of the Serotonin2C Receptor Inhibits In Vivo Dopamine Release in the Rat Striatum and Nucleus Accumbens

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Numerous research has pointed out that serotonin2c (5-HT2C) receptor, a subtype of 5-HT receptors belonging to the G-protein-coupled receptor superfamily, modulates the activity of mesencephalic dopamine (DA) neurons, the dysfunction of which is involved in devastating diseases such as schizophrenia, Parkinson’s disease, and drug addiction. In the present study, using in vivo intracerebral microdialysis and Chinese hamster ovary (CHO) cells expressing 5-HT2C receptors to identify appropriate 5-HT2C receptor ligands, we sought to determine whether the property of 5-HT2C receptors to spontaneously activate intracellular signaling pathways in vitro (constitutive activity) participates in the tonic inhibitory control that they exert on DA release in the rat striatum and nucleus accumbens in vivo. In CHO cells, the purported antagonist 5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-f]indole hydrochloride (SB 206553), but not 6-chloro-5-methyl-1-[6-(2-methylpropyl)pyridin-3-yl]carbamoyl] indole hydrochloride (SB 242084), decreased basal inositol phosphate accumulation, thus behaving as a 5-HT2C inverse agonist. Its effect was prevented by SB 242084.

206553), but not 6-chloro-5-methyl-1-(3-pyridylcarbamoyl) indole hydrochloride (SB 242084) decreased basal inositol phosphate accumulation, thus behaving as a 5-HT2C inverse agonist. Its effect was prevented by SB 242084. Pretreatment by SB 242084 reversed the change in DA release elicited by Ro-60-0175 and SB 206553. Furthermore, SB 206553-stimulated DA release was insensitive to reduction of 5-HT neuronal function induced by the 5-HT1A agonist (constitutive activity) participates in the tonic inhibitory control that they exert on DA release in the rat striatum and nucleus accumbens in vivo. In vivo electrophysiological and biochemical studies have shown that 5-HT2C agonists and antagonists inhibit and enhance, respectively, basal DA cell firing and DA release at terminals (Di Giovanni et al., 1999; Gobert et al., 2000; De Deurwaerdere and Spampinato, 2001). The tonic inhibitory control revealed by purported antagonists has been classically attributed to blockade of endogenous 5-HT action at 5-HT2C receptors (Di Giovanni et al., 1999). Nevertheless, the magnitude of this effect differs across the antagonists (De Deurwaerdere and Spampinato, 2001), and these different responses are not solely related to different selectivity of antagonists toward 5-HT2C receptors (Di Giovanni et al., 1999; Gobert et al., 2000). It is possible, as suggested previously (Willins and Meltzer, 1998), that the 5-HT2C receptor-dependent control of basal DA neuron activity is not related strictly to 5-HT extra- cellular levels.

In vivo dopamine release; striatum; nucleus accumbens; 5-HT2C receptor; rat; constitutive activity

Key words: DA release; striatum; nucleus accumbens; 5-HT2C receptor; rat; constitutive activity

Introduction

Precise control of ascending mesencephalic dopamine (DA) neurons is critical for normal brain function, and aberration in their functional status contributes to various devastating diseases and conditions such as schizophrenia, Parkinson’s disease, and drug addiction (Dunnett and Robbins, 1992; Di Chiara, 2002). It is well established that the central serotonin (5-HT) system controls DA neuron activity (Soubrie et al., 1984; Kapur and Remington, 1996). Evidence indicates that the 5-HT2C receptor, a 5-HT receptor subtype belonging to the G-protein-coupled receptor superfamily and expressed to a large extent along ascending DA pathways (Eberle-Wang et al., 1997; Barnes and Sharp, 1999), plays a prominent role in this interaction and represents a useful target for improved treatment of neuropsychiatric disorders related to DA neuron dysfunctions (De Deurwaerdere and Chesselet, 2000; Grottick et al., 2000; Wood et al., 2001).

In vivo electrophysiological and biochemical studies have shown that 5-HT2C agonists and antagonists inhibit and enhance, respectively, basal DA cell firing and DA release at terminals (Di Giovanni et al., 1999; Gobert et al., 2000; De Deurwaerdere and Spampinato, 2001). The tonic inhibitory control revealed by purported antagonists has been classically attributed to blockade of endogenous 5-HT action at 5-HT2C receptors (Di Giovanni et al., 1999). Nevertheless, the magnitude of this effect differs across the antagonists (De Deurwaerdere and Spampinato, 2001), and these different responses are not solely related to different selectivity of antagonists toward 5-HT2C receptors (Di Giovanni et al., 1999; Gobert et al., 2000). It is possible, as suggested previously (Willins and Meltzer, 1998), that the 5-HT2C receptor-dependent control of basal DA neuron activity is not related strictly to 5-HT extracellular levels.

Studies conducted in heterologous expression systems have demonstrated the considerable ability of the native 5-HT2C re-
ceptor to spontaneously activate intracellular signaling pathways, including phospholipase C (PLC) and phospholipase A2 (PLA2), in the absence of agonist stimulation (Barker et al., 1994; Berg et al., 1999; Niswender et al., 1999). Most drugs previously thought to be antagonists at the 5-HT$_{2C}$ receptor are capable of silencing its constitutive activity in vitro, behaving as inverse agonists (Berg et al., 1999; Herrick-Davis et al., 1999). Interestingly, the prototypical striatal DA pathways (Di Matteo et al., 2001).

Materials and Methods

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

Cell culture. CHO-1C19 and CHO-1C7 cells are CHO-K1-derived cell lines that stably express human 5-HT$_{2C}$ receptors at a density of ~250 pmol/mg protein and ~10–20 pmol/mg protein, respectively (Berg et al., 1999). Cells were maintained in α-MEM supplemented with 5% FBS and 300 μg/ml hygrocytom. For these experiments, the cells were seeded into multiwell tissue culture plates at a density of 4 × 10$^6$ cells/cm$^2$. After a 24 hr plating period, cells were washed with HBSS and placed into DMEM/F-12 (1:1) with 5 μg/ml insulin, 5 μg/ml transferrin, 30 μM selenium, 20 μM progesterone, and 100 μM putrescine (serum-free media). Cells were grown in serum-free media for 24 hr before experimentation.

Inositol phosphate accumulation and arachidonic acid release measurements. Cells were labeled with 1 μCi/ml myo-[3H]inositol in serum-free medium for 24 hr and 0.1 μCi/ml [3H]arachidonic acid (AA) for 4 hr. Total inositol phosphate accumulation (IP$_{1}$, IP$_{2}$, IP$_{3}$, collectively referred to as IP) and AA release were measured as described previously (Berg et al., 1999). Measurements of PLC-mediated IP accumulation and PLA$_2$-mediated AA release were made simultaneously from the same multiwell. The assay was begun by adding experimental media (HBSS containing calcium and magnesium supplemented with 20 mM HEPES, 20 mM LiCl, and 0.01% fatty-acid-free bovine serum albumin (BSA)). After a 25 min incubation, 200 μl aliquots of media from each well were added directly to scintillation vials for measurement of [3H] content (AA release) with liquid scintillation counting. The remaining media was aspirated quickly, and 2 ml of 10 mM formic acid was added to extract the accumulated [3H]-IPs. The [3H]-IPs were separated with ion exchange chromatography and quantified with liquid scintillation counting.

[y$^{35}$S]GTP binding. [y$^{35}$S]GTP binding was performed as described previously (Evans et al., 2001). Briefly, after 24 hr in serum-free media, cells in 15 cm plates (~320 μg of total protein) were washed twice with ice-cold HBSS, scraped, and pelleted. Pellets were flash frozen and stored in liquid nitrogen. Membranes were prepared by repeated trituration of thawed cell pellets through a 1 ml pipette in ice-cold wash buffer (20 mM HEPES, 3 mM MgCl$_2$, 0.2 mM EGTA, and 100 mM NaCl, pH 7.4 at 23°C). The homogenate was centrifuged (39,000 × g; 4°C; 10 min), and the pellet was washed two times by resuspension in 40 vol of the same buffer and centrifugation. Membranes were resuspended in assay buffer [wash buffer plus GDP (10 μM), okadaic acid (100 nM), and cypermethrin (10 μM)] at a protein concentration of 50 μg/ml. Aliquots (100 μl) of the membrane suspension were preincubated with the test compound or vehicle (assay buffer) in Millipore 96-well Multiscree filtration plates for 30 min at 37°C in triplicate. The assay was initiated by the addition of [y$^{35}$S]GTP (final concentration of 0.3 nM). The assay was terminated after 30 min by rapid filtration and subsequent washing of filters (eight times with 200 μl each) with ice-cold wash buffer. Filters from the plates were removed, placed in scintillation vials, and counted with a Beckman LS7500 liquid scintillation counter. 5-HT$_{2C}$ receptor-mediated [y$^{35}$S]GTP binding was completely abolished by pretreatment of cells with pertussis toxin (50 ng/ml, 24 hr). Nonspecific binding was determined in the presence of guanosine 5′-(β,γ-imido)triphosphate (1 μM). Protein determination was according to the method of Bradford.
5-HT transmission was evaluated in vivo by monitoring 5-hydroxyindolacetic acid (5-HIAA) extracellular levels, which have been shown to correlate positively with the magnitude of central 5-HT neuron depletion (Kirby et al., 1995; De Deurwaerdere et al., 1998). Only data obtained from lesioned rats having at least a 90% decrease in basal 5-HIAA extracellular levels compared with sham-lesioned rats were included in the results (five of eight animals).

**Chromatographic analysis.** Dialysate samples were immediately analyzed by reverse-phase HPLC coupled with electrochemical detection, as described previously (Porras et al., 2002). The mobile phase (containing in mM): 70 NaH2PO4, 0.1 Na2EDTA, 0.7 triethylamine, and 0.1 octylsulfonic acid plus 10% methanol, adjusted to pH 4.8 with orthophosphoric acid was delivered at 1 mL/min flow rate (system LC-10AD-VP, Shimadzu, Duisburg, France) through a Hypersyl column (C18; 4.6 × 150 mm; particle size 5 µm; Touzard and Matignon, Paris, France). Detection of DA, and 5-HIAA in some experiments was performed with a coulometric detector (Coulouchem II, ESA, Paris, France) coupled to a dual-electrode analytical cell (model 5014, ESA). The potential of the electrodes was set at −175 and +175 mV. Output signals were recorded on a computer (system class VP-4, Shimadzu). Under these conditions, the sensitivity for DA and 5-HIAA was 0.5 and 2 pg/30 µL, respectively, with a signal/noise ratio of 3:1.

**Pharmacological treatment.** 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 120 min after the beginning of the perfusion (stabilization period). In case of coadministration of SB 206553 or Ro-60–0175, SB 206553 was administered 30 min before Ro-60–0175, and (±)-8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT) was administered 5 min before SB 206553. Ro-60–0175 and 8-OH-DPAT were dissolved in a 99:1 v/v mixture of apyrogenic water and lactic acid, and SB 242084 was dissolved in a mixture of physiological saline (NaCl 0.9%) containing hydroxypropyl-β-cyclodextrin (8% by weight) plus citric acid (25 mM). All drugs were injected intraperitoneally with the exception of 8-OH-DPAT (subcutaneously), and all drug doses were calculated as the free base. In each experimental group, animals received either drugs or their appropriate vehicle.

**Statistical analysis.** 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 ± SEM values of the percentage obtained in each experimental group. The overall effect corresponds to the average of percentages of baseline for dialysates collected after the administration of pharmacological treatments.

The statistical analysis of the effect elicited by 5-HT2C agents alone on DA release was performed by a one-way ANOVA with time as repeated measures (10 samples). To determine an interaction between two drugs on DA release, a two-way ANOVA using the two treatments as the main factors and time as repeated measures (eight samples) was performed. The ability of 5,7-DHT lesion to modify basal DA and 5-HIAA dialysate content from the nucleus accumbens and striatum and the effect of SB 206553 was studied by using a one-way ANOVA. Also, a one-way ANOVA was performed for each experiment to determine whether absolute DA levels were homogenous across groups. Finally, in case of a significant result of the ANOVA (p < 0.05), the ANOVA was followed by the Fisher’s protected least significance difference post hoc test (Fisher’s PLSD) to allow adequate multiple comparisons between groups.

**Drugs.** The following compounds were used: Ro-60–0175 kindly donated by Dr. P. Weber (Hoffmann-La Roche, Basel, Switzerland); SB 242084 generously provided by Dr. M. Wood (Psychiatry, Centre of Excellence for Drug Discovery, GlaxoSmithKline, Harlow, UK); SB 206553, (±)-8-OH-DPAT hydrobromide, 5,7-DHT creatinine sulfate, and desmethylimipramine hydrochloride were purchased from Research Biochemicals (Natick, MA). All other chemicals and reagents were the purest commercially available (Sigma, St. Louis, MO; VWR, Strasbourg, France; Tocris, Strasbourg, France).

**Results**

**Pharmacological characterization of 5-HT2C agents in CHO cells expressing the human 5-HT2C receptor**

The objective of this set of experiments was to characterize the efficacy properties (positive = agonist, negative = inverse agonist, and 0 = neutral antagonist) of two purported 5-HT2C antagonists in CHO-1C7 cells. The efficacy of 5-HT2C ligands was measured on three independent signaling pathways: PLC was assessed by monitoring IP accumulation, PL2 was assessed by measuring AA release, and activation of Gαi was assessed with [γ35S]GTP binding. As expected (Berg et al., 1999; Price et al., 2001), SB 206553 decreased basal IP accumulation (−70%), [3H]-AA release (−25%), and [γ35S]GTP binding (−30%), indicating that SB 206553 behaves as an inverse agonist toward these three responses (Fig. 1). SB 242084 showed similar inverse agonist activity in reducing [3H]-AA release (−20%) and [γ35S]GTP binding (−30%); however, at variance with SB 206553, SB 242084 displayed weak agonist activity on IP accumulation. When tested further in a cell line with lower receptor expression levels and no receptor reserve (CHO-1C19; 250 fmol/mg protein) (Berg et al., 1999) in which the full agonist 5-HT increased IP accumulation 235 ± 27% above basal, SB 242084 did not change IP accumulation (5 ± 4%; n = 3). Moreover, SB 242084 (100 nM) induced a rightward shift of the SB 206553-induced decrease in IP accumulation (Fig. 1a).

**Basal extracellular DA concentrations in dialysates from nucleus accumbens and striatum**

All measurements were performed 120 min after the beginning of perfusion, by which time a steady state was achieved. Absolute basal levels of DA in dialysates, simultaneously collected from the striatum and the nucleus accumbens, did not differ between the different experimental groups throughout the course of the study and were (mean ± SEM, without adjusting for probe recovery) 14.7 ± 2.7 pg/30 µL and 4.3 ± 0.8 pg/30 µL, respectively (n = 16 animals chosen randomly from the cohort).

**Effect of 5-HT2C compounds on in vivo DA release in the rat striatum and nucleus accumbens**

The effect of the intraperitoneal administration of increasing doses of SB 206553 (1–10 mg/kg), SB 242084 (1–10 mg/kg), and Ro-60–0175 (0.3–3 mg/kg) on DA extracellular levels is shown in Figure 2. Systemic administration of purported 5-HT2C antagonists SB 206553 and SB 242084 differed regarding their effect on DA release. SB 206553 elicited a significant and dose-dependent
increase in DA efflux in the striatum ($F_{(3,21)} = 26.4; p < 0.001$) and the nucleus accumbens ($F_{(3,21)} = 10.5; p < 0.001$). This effect reached a maximal value within 30–45 min after injection (Fig. 2a,b); the overall magnitude reached approximately +20, +35, and +70% of baseline in both brain areas after 1, 5, and 10 mg/kg SB 206553 administration, respectively (Fig. 2a,b, insets).

SB 242084 elicited a significant and progressive enhancement of DA efflux in both the striatum ($F_{(3,21)} = 6.1; p < 0.01$) and nucleus accumbens ($F_{(3,21)} = 5.7; p < 0.01$) (Fig. 2c,d). The overall effect was small and reached a maximum at the dose of 3 mg/kg (+22 and +29% above vehicle-treated rats in the striatum and nucleus accumbens) (Fig. 2c,d, insets).

Intraperitoneal administration of 3 mg/kg, but not 0.3 or 1 mg/kg, of Ro-60–0175 elicited a significant decrease in DA efflux in the striatum ($F_{(3,10)} = 7; p < 0.01$) and the nucleus accumbens ($F_{(3,10)} = 9.7; p < 0.001$). This effect started 30 min after Ro-60–0175 injection and reached a maximal inhibition of 35 and 40% in the striatum and the nucleus accumbens, respectively (Fig. 2e,f). The overall inhibition induced by Ro-60–0175 was slightly more pronounced in the nucleus accumbens (−25% above control values) compared with the striatum (−19%) (Fig. 2e,f, insets).

**Effect of SB 242084 on Ro-60–0175- and SB 206553-induced changes in striatal and accumbal DA release**

Figure 3, a and b, reports that the overall increase in striatal and accumbal DA efflux induced by 5 mg/kg SB 206553 was prevented by 1 mg/kg SB 242084 ($F_{(1,23)} = 10.3$ and 13.98 for the striatum and the nucleus accumbens, respectively; $p < 0.01$). DA efflux in the SB 242084 + SB 206553 group was not different from that obtained in the SB 242084 + vehicle group in both regions (Fig. 3a,b, insets).

As shown in Figure 3, c and d, SB 242084 also impaired the inhibitory effect elicited by 3 mg/kg Ro-60–0175 in the nucleus accumbens ($F_{(1,24)} = 5.83; p < 0.05$). In the striatum, the two-way ANOVA failed to reach significance ($F_{(1,24)} = 2.62; p = 0.118$). Nevertheless, as for the nucleus accumbens, DA extracellular levels in the SB 242084 + Ro-60–0175 group were not significantly different from those obtained in the SB 242084 + vehicle group in the striatum (Fisher’s PLSD after significant one-way ANOVA; $F_{(3,24)} = 11; p < 0.01$).

Of note, the inhibitory effect elicited by 3 mg/kg Ro-60–0175 was also prevented by 5 mg/kg SB 206553 in the nucleus accumbens ($F_{(3,20)} = 28.95; p < 0.001$) and the striatum ($F_{(3,20)} = 9.69; p < 0.01$). DA extracellular levels in the SB 206553 + Ro-60–0175 group were not significantly different from those obtained in the SB 206553 + vehicle group in both brain regions (data not shown).
The present study provides the first in vivo evidence that constitutive activity of the 5-HT$_{2C}$ receptor tonically inhibits striatal and accumbal DA release, thus identifying a new modality of heterologous control regulating basal DA neuron activity.

Striatal and accumbal DA release were enhanced by the purported 5-HT$_{2C}$ antagonists SB 206553 and SB 242084 and inhibited by the 5-HT$_{2A}$ agonist Ro-60–0175. These results confirm previous findings proposing that 5-HT$_{2C}$ receptors exert both phasic and tonic inhibitory control on DA neuron activity (Di Giovanni et al., 1999; Gobert et al., 2000; De Deurwaerde and Spampinato, 2001). The performed dose–response experiments allow us to show first that the sensitivity of the nigrostriatal and mesoaccumbens DA pathways is similar to the acute administration of 5-HT$_{2C}$ ligands, dampening the proposal of a preferential role of 5-HT$_{2C}$ receptors in the control of the mesoaccumbens DA pathway (Di Matteo et al., 2001; Rocha et al., 2002).

Second, they confirm previous sporadic observations (De Deurwaerde and Spampinato, 2001) that SB 206553 elicited a marked increase in DA release compared with SB 242084 and clearly indicate that SB 206553 is more efficacious than SB 242084 in enhancing DA release. It is unlikely that the 5-HT$_{1A}$ component of SB 206553 may account for its higher efficacy, because selective 5-HT$_{2B}$ agents do not affect basal DA neuron activity (Gobert et al., 2000). The difference observed cannot be explained if both drugs act as simple 5-HT$_{2C}$ antagonists to block the effect of endogenous 5-HT. In line with this consideration, administration of the 5-HT$_{1A}$ agonist 8-OH-DPAT or selective lesion of 5-HT neurons located in the raphe nuclei, two conditions associated with decreased endogenous 5-HT tone (Sharp et al., 1989a,b), did not affect basal DA release in the nucleus accumbens or the striatum (De Deurwaerde et al., 1998; this study). Taken together, these data highlight the possibility that basal 5-HT$_{2C}$ receptor tone on DA release is not strictly related to extracellular levels of 5-HT and that the differential effect of SB 206553 and SB 242084 may be a consequence of distinct intrinsic pharmacological properties.

In favor of this possibility, studies in CHO cells expressing human 5-HT$_{2C}$ receptors expressed at a density to optimize its constitutive receptor activity (Berg et al., 1998) revealed that SB 206553 and SB 242084, although sharing the ability to antagonize IP accumulation induced by agonists (Kennett et al., 1996; 1997), possess distinct pharmacological properties. In agreement with previous data (Berg et al., 1998; Price et al., 2001), we found that SB 206553 behaves as a strong inverse agonist at PLC-, PLA$_2$-, and activation of G$_{o}$-$\Pi$-dependent responses coupled to the 5-HT$_{2C}$ receptor, respectively. At variance, SB 242084 appears to be a protean ligand (Kenakin, 2001) that is equally as efficacious as SB 206553 toward PLA$_2$ and G$_{o}$-$\Pi$ activation but displays low-efficacy agonism toward PLC. According to their pharmacological properties at PLC-dependent responses, SB 242084 induced a rightward shift of the inhibition of IP accumulation elicited by SB 206553. This pharmacological characterization toward three independent intracellular pathways provides further support for the hypothesis of agonist-directed trafficking of receptor stimuli (Kenakin, 1995; Clarke and Bond, 1998) and supports the pleiotropic behavior of ligands at 5-HT$_{2C}$ receptors (Berg et al., 1998; Clarke and Bond, 1998). Moreover, these results provide a mechanistic basis to explain the different responsiveness of DA neurons to these ligands and to further evaluate their interaction in vivo.

In line with the above consideration, SB 242084 significantly blocked the increase in striatal and accumbal DA release elicited by SB 206553. Also, SB 242084 reversed the decrease in DA release produced by Ro-60–0175 in both brain regions, although the interaction did not reach statistical significance in the striatum.
(see Results). These findings indicate that the opposite changes of DA release elicited by SB 206553 and Ro-60–0175 are mediated by their action at 5-HT2C receptors and suggest, furthermore, that the marked effect of SB 206553 on DA release is related to its inverse agonist activity at central 5-HT2C receptors. As anticipated from its inverse agonist profile (Lefkowitz et al., 1993; Morisset et al., 2000), we found that SB 206553 also prevented Ro-60–0175-induced inhibition of DA release in both brain areas. These findings together strongly suggest that endogenous 5-HT2C receptors have physiologically relevant constitutive activity in inhibiting both the nigrostriatal and the mesoaccumbens DA pathways. The existence of a constitutive activity of the 5-HT2C receptor in vivo, which is compatible with the idea that this receptor tonically controls the excitability of various neuronal networks (Tecott et al., 1995), is also in line with the occurrence of inverse agonism in the 5-HT2 regulation of the rabbit nictitating membrane reflex (Harvey et al., 1999).

Because SB 206553 and SB 242084 differ dramatically in their effect on the PLC response, it is tempting to suggest that the prominent increase in DA release produced by SB 206553 may be related to constitutive activity of the 5-HT2C receptor toward the PLC effector pathway. Of note, other 5-HT2C inverse agonists on PLC-dependent responses, such as mesulergine, ritanserin, or mianserin, may increase basal DA release in vivo (Andersson et al., 1995; Di Matteo et al., 2001), but the weak selectivity of these compounds toward 5-HT2C receptors deserves caution in interpreting the data (Di Giovanni et al., 1999). Moreover, the small increase in basal DA release induced by SB 242084 in our study could be consequent to its full inverse agonist activity toward the PLA2 and the Gq pathways or to its ability to selectively block a small endogenous inhibitory tone exerted by 5-HT itself at 5-HT2C receptors.

5-HT is constitutively released from terminal fields of 5-HT neurons (Sharp et al., 1989a,b), and its continuous presence in the synaptic cleft could confound our interpretation that the 5-HT2C receptor is constitutively active in vivo. Nonetheless, we found that SB 206553-stimulated DA release was insensitive to the decrease in 5-HT terminal activity induced by either 5,7-DHT destruction of central 5-HT neurons or 8-OH-DPAT administration. In line with these findings, 8-OH-DPAT did not modify the effect elicited by the µ opioid agonist morphine on DA release, although this latter was potentiated by SB 206553 (Williams and Meltzer, 1998; Porras et al., 2002). These findings, showing that the efficacy of SB 206653 on DA neuron activity is independent from changes in extracellular levels of 5-HT, corroborate a recent proposal that the low influence of changes of 5-HT extracellular levels in a biological response might be a direct consequence of the high constitutive tone of native 5-HT2C receptors (Niswender et al., 1999). In this respect, RNA editing of the transcript encoding 5-HT2C receptors (Burns et al., 1997), by lowering constitutive activity of the native product (Niswender et al., 1999), might represent a determining factor in organizing the strength of 5-HT2C receptor influence at central 5-HT synapses (Price et al., 2001).

An aberration in DA signaling in brain is associated with various devastating diseases and conditions such as schizophrenia, Parkinson’s disease, and drug addiction, and 5-HT2C receptors have been proposed as a useful target for improved treatments of these chronic diseases (Meltzer, 1999; De Deurwaerdere and Chesselet, 2000; Grottick et al., 2000; Wood et al., 2001; Rocha et al., 2002). The fact that inverse agonists effectively behave differently compared with neutral antagonists in vivo brings up important therapeutic perspectives. For instance, several antipsychotic drugs display inverse agonist activity at constitutive 5-HT2C receptors (Rauser et al., 2001), and this property could participate in the clinical superiority of some atypical antipsychotics, including clozapine or olanzapine (Rauser et al., 2001). Long-term treatment experiments are warranted to directly address this possibility.

In conclusion, we have shown that constitutively active 5-HT2C receptors are responsible for a tonic inhibitory control on nigrostriatal and mesolimbic DA neuronal pathways. These data extend the in vivo identification of constitutive activity of the 5-HT2C receptor (Harvey et al., 1999) and bring up additional neurochemical evidence for such an activity of G-protein-coupled receptors from the regulation of histamine metabolism exerted by histamine H3 autoreceptors (Morisset et al., 2000) to the heteroregulation of neuronal network excitability exerted by 5-HT2C receptors within the living brain. Our study underscores the need for a better understanding of the pathophysiological role of constitutive receptor activity and of the therapeutic potential of inverse agonism (Niswender et al., 2001; Rauser et al., 2001).

References


