SB 242084: A Selective 5-HT\textsubscript{2C} Receptor Antagonist

Vincenzo Di Matteo, Giuseppe Di Giovanni, and Ennio Esposito

*Istituto di Ricerche Farmacologiche “Mario Negri,”
Consorzio “Mario Negri” Sud, Santa Maria Imbaro (Chieti), Italy

Key Words: 5-HT\textsubscript{2C} receptors — Anxiety — Depression — Dopaminergic function — SB 242084 — Schizophrenia.

ABSTRACT

SB 242084 is the most potent and selective 5-HT\textsubscript{2C} receptor antagonist thus far available. Thus, SB 242084 has high affinity for the cloned human 5-HT\textsubscript{2C} receptor with a pK\textsubscript{i} of 9.0, a much lower affinity for the human cloned 5-HT\textsubscript{2B} (pK\textsubscript{i} 7.0) and 5-HT\textsubscript{2A} (pK\textsubscript{i} 6.8) receptors, and low affinity for other 5-HT, dopamine, and adrenergic receptors. In the 5-HT-stimulated PI hydrolysis model of 5-HT\textsubscript{2C} receptor function, SB 242084 was found to be a competitive antagonist with a pK\textsubscript{B} of 9.3. A series of in vivo studies have shown that SB 242084 is a very effective antagonist of behavioral responses mediated by 5-HT\textsubscript{2C} receptors such as penile erections, and the hypophagic and hypolocomotor effect of mCPP in rats. In addition, this compound has anxiolytic-like properties. Moreover, SB 242084 increases the basal activity of dopaminergic neurons in the VTA and the in vivo DA release in the nucleus accumbens, and it is capable of blocking the inhibitory effects of mCPP and RO 60-0175 on mesolimbic dopaminergic activity. These data are consistent with the evidence that 5-HT\textsubscript{2C} receptors exert an inhibitory control upon the mesolimbic dopaminergic system.

Taken together, the available data on SB 242084 might have implication for the possible use of this compound in the treatment of anxiety, depression, and the negative symptoms of schizophrenia.

INTRODUCTION

An increasing amount of evidence indicates that serotonin (5-HT) is involved in the regulation of several physiological functions, including affective behavior, sleep, food...
intake, sexual behavior, memory, and thermoregulation (47). Moreover, a dysregulation of the serotonergic system is thought to underly the pathogenesis of psychiatric illnesses, such as depression, anxiety, obsessive compulsive disorder (OCD), eating disorders, and schizophrenia (12,13,48). 5-HT probably attains such a variety of functions by acting on distinct receptor subtypes, most of which have been sequenced and cloned (7,20,41). The availability of compounds that act on various 5-HT receptor subtypes has allowed progress in the understanding of the relative contribution of these receptors in the control of various aspects of behavior. For example, 5-HT$_{1A}$ receptor agonists stimulate locomotor activity and increase food intake, whereas 5-HT$_{2C}$ agonists cause hypolocomotion and reduce eating (6,26,28,31). Recently, 5-HT$_{2C}$ receptor antagonists have been proposed to be potential anxiolytic and antidepressant agents (4,27), whereas 5-HT$_{2A}$ blockers might be helpful in the treatment of psychotic disorders (21). Most of the serotonergic agents used in pharmacological tests, however, are not selective enough to permit an accurate investigation of the role of various 5-HT receptors. The lack of selectivity is probably due to structural similarities between 5-HT receptor subtypes. This fact is particularly evident in the case of 5-HT$_{2}$ receptors, which form a closely related subgroup (4).

5-HT$_{2}$ receptors are G-protein-coupled receptors, functionally linked to the phosphatidylinositol (PI) hydrolysis pathway and are currently classified as 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ subtypes (3,7,23) based on their close structural homology and pharmacology (3,7,23). The amino acid sequence of the 5-HT$_{2}$ receptors shares a high degree of homology within the seven transmembrane domains (3,7,23); thus, it is not surprising that many compounds bind with high affinity to all three of these receptor subtypes. Presently, there is evidence that the 5-HT$_{2C}$ receptor is located mainly postsynaptically, with a high degree of cellular heterogeneity within dopaminergic, GABAergic, cholinergic, substance P, dynorphin, and other neuronal systems (3,18,46). There is also high sequence homology (>80% in the transmembrane regions) between mouse, rat, and human 5-HT$_{2C}$ receptors (3). They are widely distributed throughout the brain and have been proposed to be mediators of the different actions of 5-HT in the central nervous system (CNS) (3,7,23). High levels of 5-HT$_{2C}$ mRNA or protein expression have been found in the choroid plexus, rat frontal cortex, hippocampus, septum, hypothalamus, striatum, rhombencephalon, and spinal cord (36,42,44), while the 5-HT$_{2A}$ receptor showed a more widespread localization in the CNS, with higher levels in the cortical areas (42). Recently several groups have shown, using more sensitive techniques, the presence of both 5-HT$_{2B}$ receptor mRNA (19) and protein (17) in the rat brain.

The regional and cellular distribution of 5-HT$_{2C}$ receptors has been investigated also in the human brain. The main sites of mRNA 5-HT$_{2C}$ receptors or protein expression are the choroid plexus, cerebral cortex, hippocampus, amygdala, some components of the basal ganglia, and other limbic structures (1,39), suggesting that this receptor may be involved in the regulation of different human brain functions. The presence of 5-HT$_{2C}$ receptors in the prefrontal cortex and in different components of the limbic system suggests this receptor has a direct involvement in anxiety and/or mood disorders. Moreover, this receptor has been implicated in the serotonergic control of appetite (27,45). Studies of conventional tricyclic and nontricyclic antidepressants suggest that a number of these drugs display considerable pharmacological activity at 5-HT$_{2C}$ receptors in the brain (24,25), and the possible involvement of 5-HT$_{2C}$ receptors in the pathogenesis of depressive disorders and in the mechanism of action of antidepressants is further substantiated by several other studies (37,38). In addition, selective serotonin reuptake inhibitors (SSRIs)
decrease 5-HT\textsubscript{2C} receptor responsiveness (27). Other pharmacological studies have shown that central 5-HT\textsubscript{2C} receptors are involved in the pathogenesis of panic disorder, OCD, eating disorders, alcoholism, migraine, sleep disorders, and obesity (for review see ref. 27). Therefore, 5-HT\textsubscript{2C} receptor ligands may have therapeutic applications in a variety of illnesses (27). In particular, preclinical and early phase clinical studies have shown that 5-HT\textsubscript{2C} antagonists may be useful in the treatment of anxiety, depression, and sleep disorders (4).

SB 242084 is the most selective and brain penetrant 5-HT\textsubscript{2C} receptor antagonist (8,29). Herein its preclinical pharmacology and its possible applications in human neuropsychiatric disorders are reviewed.

**CHEMISTRY**

SB 242084, (C\textsubscript{21}H\textsubscript{19}ClN\textsubscript{4}O\textsubscript{2}), 6-chloro-5-methyl-1-\{[2-(2-methyl-3-pyridyl)oxy]-5-pyridyl carbamoyl\} indoline (Fig. 1) has a molecular weight of 394.860 (free base). The compound is a white powder, soluble in dimethyl sulfoxide or in N\textsubscript{2}N\textsubscript{-}dimethylformamide. SB 242084 was prepared by coupling 6-chloro-5-methylindoline with phenyl carbamate in a four-step synthesis described by Bromidge et al. (8). For \textit{in vivo} studies, SB 242084 was dissolved in 0.9% saline containing 8% hydroxypropyl-\beta\text{-}cyclodextrin by weight and 25 mM citric acid and was given either i.p. or i.v.(16,29).

**SELECTIVITY FOR 5-HT\textsubscript{2C} RECEPTORS AND IN VITRO PHARMACOLOGY**

In the 5-HT-stimulated PI hydrolysis model of 5-HT\textsubscript{2C} receptor function using human cloned receptors expressed in HEK 293 cells, SB 242084 was found to be a competitive antagonist with a pK\textsubscript{B} of 9.3, which is consistent with its binding affinity (pK\textsubscript{i} 9.0) (8). Thus, SB 242084 has an increased affinity for cloned human 5-HT\textsubscript{2C} receptors compared with previously available compounds, such as SB 206553 (5-methyl-1-\{(3-pyridyl)carba-
moyl)-1,2,3,5-tetrahydropyrrolo(2,3-f)indole]] (pKi 7.9) and SB 200646A [N-(1-methyl-5-indolyl)-N’-(3-pyridyl) urea hydrochloride] (pKi 6.9) (8,29,30). Moreover, SB 242084 shows at least 160- and 100-fold selectivity over the 5-HT2A and 5-HT2B receptor subtypes, respectively (8,29). In addition, SB 242084 displays a greater than 100-fold selectivity over a range of other 5-HT, dopamine, and adrenergic receptors (8,29). Similar results were obtained in another binding study using human neuroblastoma cells (SH-SY5Y) transfected with the human cloned 5-HT2C receptor (29). SB 242084 showed high affinity for the cloned human 5-HT2C receptor with a pKi of 9.0, a much lower affinity for the human cloned 5-HT2B (pKi 7.0) and 5-HT2A (pKi 6.8) receptors, and low affinity for all other receptors tested (29). In the same cellular line, 5-HT caused a concentration-dependent increase in PI hydrolysis that was fully antagonized by SB 242084 (29).

In a 5-HT2C receptor model constructed on the basis of the structure of bacteriorhodopsin, the central pyridyl ring of SB 242084 occupies a lipophilic pocket surrounded by Phe 508 and Phe 616, while the terminal pyridyl ring occupies another lipophilic pocket formed by Ile 305 and Ile 410 (8). In this model, the terminal pyridyl nitrogen may form a hydrogen bond with Ser 413; this interaction is not possible in the case of the 5-HT2B receptor since the corresponding residue in the protein sequence is alanine (8). In addition, the substituted indoline is placed in another pocket, the boundary of which is defined by residues Val 212, Val 608, Met 612, and Tyr 715. In the 5-HT2B receptor model, the corresponding 608 residue is leucine, and in the 5-HT2A receptor both the 212 and 608 residues are leucine (8). These differences are expected to lead to smaller binding pockets. Consequently, steric differences among receptors of the 5-HT2 family may contribute to the observed 5-HT2C selectivity of SB 242084 (8).

**ELECTROPHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION**

Electrophysiological techniques and in vivo microdialysis were used to investigate the effect of SB 242084 in the control of nigrostriatal and mesolimbic dopaminergic function in rat brain. Extracellular single unit recordings were performed from neurochemically identified dopamine neurons in the substantia nigra pars compacta (SNc), and the ventral tegmental area (VTA). SB 242084 dose-dependently (160–640 µg/kg, i.v.) and significantly increased the basal firing rate of VTA dopaminergic neurons, and the bursting activity was also enhanced in the same area (Fig. 2) (16). On the other hand, SB 242084 did not cause any significant change in the basal firing rate and bursting activity of dopaminergic neurons in the SNc (Fig. 2) (16). Consistently, basal dialysate dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) were significantly increased in the nucleus accumbens of anesthetized rats after intraperitoneal injection of SB 242084 (5–10 mg/kg) (Fig. 3) (16). Moreover, the inhibitory effects of m-chlorophenylpiperazine (mCPP) and MK 212 [6-chloro-2-(1-piperazinyl)piperazine], two nonselective 5-HT2C receptors agonists, were prevented by SB 242084, suggesting an inhibitory action of 5-HT on mesolimbic dopaminergic activity through stimulation of 5-HT2C receptors (15). Interestingly, SB 242084 also prevented the inhibitory influence of RO 60-0175 [(S)-2-(chloro-5-fluoro-indol-1-yl)-1–methylethylamine 1:1 C4H4O4], a selective 5-HT2C agonist (32), on me-
Fig. 2. Effect of SB 242084 on the firing rate of SNc and VTA DA neurons (16). (A, C) Histograms showing mean percentage of change (±S.E.M.) in firing rate of DA neurons in the SNc (A) and the VTA (C) after i.v. SB 242084 ($n = 6–8$). (B, D) Representative rate histograms showing the lack of effect of i.v. SB 242084 (640 μg/kg) in the SNc (B) and the typical excitatory response in the VTA (D). *$P < 0.05$, **$P < 0.01$ vs. control group; one-way ANOVA followed by Tukey's test.
solimbic dopaminergic activity (Figs. 4 and 5). RO 60-0175 (2.5 mg/kg, s.c.) markedly reduced DA and noradrenaline (NA) release in the frontal cortex of freely moving rats without affecting 5-HT release (35). In contrast, SB 242084 (10 mg/kg, i.p.) markedly increased dialysate DA and NA levels without affecting 5-HT outflow in the frontal cortex, suggesting a tonic inhibitory influence of 5-HT upon frontocortical dopaminergic and noradrenergic transmission by stimulating 5-HT$_{2C}$ receptors (35).

**BEHAVIORAL STUDIES**

To evaluate its *in vivo* antagonist activity, SB 242084 was used in a model of penile erection in rats, which is mediated by activation of 5-HT$_{2C}$ receptors (35). SB 242084
dose-dependently antagonized penile erections induced by RO 60-0175 (2.5 mg/kg, s.c.) with an ID$_{50}$ of 0.7 mg/kg, i.p. (35). Moreover, pretreatment with SB 242084 completely attenuated the reduction in locomotor activity induced by m-chlorophenylpiperazine (mCPP), a behavioral response which is mediated by the stimulation of central 5-HT$_{2C}$ receptors (29). Thus, administration of mCPP (7 mg/kg, i.p.) caused a marked reduction in locomotor activity, and pretreatment with SB 242084 either i.p. (0.01–0.3 mg/kg) or p.o. (0.5–5 mg/kg) completely blocked the effect of mCPP (29). In the social interaction test, SB 242084 (0.1–1 mg/kg, i.p.) increased the amount of time rats spent in social interaction without affecting locomotor activity and also markedly increased punished responses in a rat Geller–Seifter conflict test (0.1–3 mg/kg, i.p.), suggesting anxiolytic-like properties of this compound (29). Furthermore, the ability of SB 242084 (2 and 6 mg/kg, p.o.) to antagonize the hypophagic action of mCPP (5 mg/kg, i.p.) (29) confirms the hypothesis that 5-HT$_{2C}$ receptors are involved in the control of food intake (27). Finally, SB 242084 had no effect on convulsant activity in the rat maximal electroshock seizure threshold test (29).

**DISCUSSION**

SB 242084 is the most potent and selective 5-HT$_{2C}$ receptor antagonist that has been developed to date (8,29). Receptor binding studies have shown that this compound has
100- and 160-fold selectivity over the closely related, cloned human 5-HT\textsubscript{2B} and 5-HT\textsubscript{2A} receptor subtypes, respectively (8,29). Moreover, systemic administration of SB 242084 blocks the hypolocomotor response to mCPP (29), an effect which is mediated by stimulation of 5-HT\textsubscript{2C} receptors in the CNS. This finding indicates that SB 242084 is a brain-penetrant compound which is capable of blocking central 5-HT\textsubscript{2C} receptors \textit{in vivo}.

A series of experiments conducted in our laboratory have shown that SB 242084 causes a dose-dependent increase in the basal firing rate of DA neurons in the VTA (16). In addition, SB 242084 increases the bursting activity of DA neurons in the VTA. In agreement with these data, basal DA output is also enhanced in the nucleus accumbens following the administration of SB 242084 (16). Consistent with the fact that bursting activity of midbrain DA neurons is associated with a much larger increase in DA output in the terminal regions than single-spike firing (5,22), a robust enhancement of basal DA release was found in the nucleus accumbens (16). Moreover, a recent report has shown that selective 5-HT\textsubscript{2C} blockade by SB 242084 causes a marked increase in dialysate levels of DA in the rat prefrontal cortex (35). The finding that SB 242084 is capable of changing the basal activity of mesocorticolimbic DA neurons is consistent with the presence of 5-HT\textsubscript{2C} receptors within these brain regions (1,33,36,40,42,46). Furthermore, it has re-
recently been shown that 5-HT\textsubscript{2C} receptor mRNA is expressed by γ-aminobutyric acid (GABA)-containing cells but not by DA neurons within the SN and the VTA (18). It is, therefore, conceivable that the effect of SB 242084 on DA neuronal activity would be indirect and resulting from the removal of a tonic inhibitory action of GABAergic neurons. Nevertheless, the extent that 5-HT\textsubscript{2C} receptors located in other brain areas, such as the nucleus accumbens, participate in the observed excitatory effect on DA neurons remains to be established.

In view of the hypothesis that disinhibition of the mesolimbic DA system underlies the mechanism of action of several antidepressant drugs (9,10,11), the stimulatory effect of SB 242084 on the mesolimbic DA system may open new possibilities for the employment of 5-HT\textsubscript{2C} receptor antagonists as antidepressants. This hypothesis is consistent with the suggestion that 5-HT\textsubscript{2C} receptor blockers may exert antidepressant activity (4,27). In this respect, it is interesting to note that several antidepressant drugs have been shown to bind with submicromolar affinity to 5-HT\textsubscript{2C} receptors in the pig brain and to antagonize mCPP-induced penile erections in rats, an effect mediated through the stimulation of central 5-HT\textsubscript{2C} receptors (24,25). However, the hypothesis that selective 5-HT\textsubscript{2C} receptor antagonists may have antidepressant properties is apparently in contrast with the evidence that 5-HT\textsubscript{2C} agonists show antidepressant activity in animal models of depression (37). The authors suggest that the potential antidepressant effect of 5-HT\textsubscript{2C} agonists may result from their ability to down-regulate 5-HT\textsubscript{2C} receptors following chronic treatment (37). Interestingly, chronic administration of both agonists and antagonists of 5-HT\textsubscript{2C} receptors causes a down-regulation of this 5-HT receptor subtype (2,43). Therefore, it is possible to argue that a reduced function of 5-HT\textsubscript{2C} receptors may be the common mechanism by which 5-HT\textsubscript{2C} agonists and antagonists may alleviate depression.

It has recently been reported that SB 242084 is active in various animal tests predictive of anxiolytic activity in humans, such as the social interaction test and the Geller–Seifter procedure (29). The ability of SB 242084 to enhance mesolimbic DA function is of interest, especially in view of the evidence that increased DA release may be involved in the anxiolytic effect of buspirone and low doses of sulpiride and haloperidol (34). Interestingly, it has been proposed that 5-HT\textsubscript{2C} receptor antagonists might be provided with therapeutic activity in several clinical forms of anxiety, such as generalized anxiety, panic disorder, and OCD (27). In addition, selective 5-HT\textsubscript{2C} receptor antagonists may be useful in the treatment of the negative symptoms of schizophrenia, a clinical condition in which a reduced function of the mesocorticolimbic DA system has been hypothesized (14).

In conclusion, preclinical studies with SB 242084 indicate that selective 5-HT\textsubscript{2C} receptor antagonists may be useful in the treatment of neuropsychiatric disorders, such as anxiety, depression, and the negative symptoms of schizophrenia.

REFERENCES


42. Pompeiano M, Palacios JM, Mengod G. Distribution of the serotonin 5-HT\textsubscript{2} receptor family mRNAs: Comparison between 5-HT\textsubscript{2A} and 5-HT\textsubscript{2C} receptors. *Mol Brain Res* 1994;23:163–78.

43. Pranzatelli MR, Murthy JN, Tailor PT. Novel regulation of 5-HT\textsubscript{1C} receptors: Down-regulation induced both by 5-HT\textsubscript{1C/2C} receptor agonists and antagonists. *Eur J Pharmacol* 1993;244:1–5.


