Serotonin/dopamine interaction—Focus on 5-HT$_{2C}$ receptor, a new target of psychotropic drugs

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Several hypotheses regarding physiopathology of major psychiatric diseases exist. Attention has been focused on cerebrospinal monoaminergic systems, the dysfunction of which is thought to underlie various aspects of their symptomatology. There are reports describing the involvement of serotonergic and dopaminergic systems in the mechanism of action of psychotropic drugs. This article reviews current knowledge on interaction between 5-hydroxytryptamine (5-HT), acting at 5-HT$_{2C}$ receptors in the central dopamine (DA) systems. Since 90s, a growing body of behavioural, neurochemical and electrophysiological evidence from animal studies have demonstrated a clear role for 5-HT$_{2C}$ receptors in modulation of activity of dopamine neurons. This evidence has led to the suggestion that drugs acting on 5-HT$_{2C}$ receptors have potential as novel antipsychotic and antidepressant agents and may also be used in the treatment of other neuropsychiatric disorders such as Parkinson’s disease and psychoactive substance abuse.

Serotonin (5-HT) containing neurons originating from mid-brain raphe nuclei innervate both substantia nigra (SN) and ventral tegmental area (VTA). Thus, neuroanatomical studies have shown a high density of 5-HT immunoreactive fibers both in substantia nigra pars compacta (SNC), pars reticulata (SNr), and VTA.$^{1,2}$ Serotonergic terminals make synaptic contacts with both dopaminergic (DA) and non-DA neurons in SNC, SNr, and the VTA.$^{1,2}$ Interestingly, ventral mesencephalic tegument including SN contains the highest brain concentrations of 5-HT, and both SNC and SNr receive a dense 5-HT input, which is higher in SNr ($9 \times 10^{4}$ varicosities/mm$^2$) than in SNC ($6 \times 10^{4}$ varicosities/mm$^2$). Moreover, virtually all 5-HT varicosities form synaptic specialization in SNr, whereas only 50% do so in SNC.$^{3}$ In addition, terminal areas of SNC and VTA, such as striatum or nucleus accumbens, receive an input from serotonergic neurons originating in raphe nuclei.$^{3}$ Several 5-HT$_{2}$ receptor subtypes have been shown to be present in the basal ganglia. Thus, a high density of 5-HT$_{1A}$ receptors has been found in SN, VTA, globus pallidus, and entopeduncular nucleus.$^{4,5}$ In contrast, levels of 5-HT$_{1A}$ binding sites and mRNA encoding 5-HT$_{1A}$ receptor are barely detectable in basal ganglia.$^{6}$ On the other hand, high to moderate levels of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor binding and corresponding mRNA are present in several fore-brain areas including basal ganglia and the limbic system. Thus, high levels of 5-HT$_{2A}$ and 5-HT$_{2C}$ binding sites are found in caudate nucleus, nucleus accumbens, olfactory tubercle, and pyriform cortex.$^{4}$ There is a good concordance between distribution of 5-HT$_{2A}$ and 5-HT$_{2C}$ binding sites and their relative mRNA.$^{6}$ However, relative distribution of mRNA for 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors is different. Thus, moderate levels of both 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor mRNA have been detected in SN, while VTA contains 5-HT$_{2C}$ but not 5-HT$_{2A}$ receptor mRNA.$^{6,8}$ There are reports concerning the role of different 5-HT receptor subtype in the control of brain DA transmission. For example, there is evidence that 5-HT$_{1B}$ Receptors underlie 5-HT-induced inhibition of GABA$_B$ receptor mediated IPSPs in rat mid-brain DA neurons in vitro.$^{9}$ However, in vivo electrophysiological experiments have shown that selective activation of 5-HT$_{1B}$ receptors does not cause any significant change in the basal activity of VTA DA-containing neurons, thus suggesting that 5-HT$_{1B}$ receptors do not play a relevant role in the control of mesolimbic DA system in vivo.$^{10}$ Moreover, systemic administration of potent and selective 5-HT$_{1A}$ receptor agonist, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), causes a pronounced excitatory effect on firing and bursting activity of neurons within a subpopulation of VTA DA-containing neurons.$^{10}$ Microiontophoretic
application of 8-OH-DPAT into VTA does not have any effect on basal firing rate of DA-containing neurons, and selective lesions of serotonergic neurons by 5,7-dihydroxytryptamine (5,7-DHT) abolish the excitatory effects of systemic 8-OH-DPAT\(^\text{10}\). These data indicate that stimulation of 5-HT\(_{1A}\) receptors by 8-OH-DPAT does not inhibit DA-containing neurons by reducing a tonic inhibitory activity exerted by serotonergic system\(^\text{10}\). A series of studies have shown that 5-HT exerts a tonic and phasic inhibitory control mainly on mesolimbic and mesocortical DA system, by stimulating 5-HT\(_{2C}\) receptors. On the other hand, 5-HT\(_{2A}\) receptors seem to have an opposite effect on these systems, and evidence is there that 5-HT\(_{2A}\) receptor agonists enhance 3,4-methylenedioxymethamphetamine-induced DA release\(^\text{11}\). In this paper, the most relevant findings regarding role of 5-HT\(_{2C}\) receptors in the control of nigrostriatal and mesocorticolimbic DA functions have been reviewed.

Effects of drugs enhancing 5-HT synaptic levels on electrical activity of DA neurons in SNc and VTA—Acute administration of fluoxetine causes a dose-dependent inhibition of firing rate of VTA DA neurons, but it does not affect the activity of DA cells in SNc\(^\text{12}\) (Fig. 1). A similar effect, though less pronounced, has been observed with citalopram\(^\text{12}\). Furthermore, mesulergine, an unselective 5-HT\(_{2C}\) receptor antagonist\(^\text{13}\), as well as destruction of 5-HT neurons by 5,7-DHT, prevents the fluoxetine-induced inhibition of VTA DA cells\(^\text{12}\). These results indicate that fluoxetine inhibits the mesolimbic DA pathway by enhancing the extracellular level of 5-HT, which would act through 5-HT\(_{2C}\) receptors\(^\text{12}\). This study also demonstrates that fluoxetine-induced inhibition of DA neurons in VTA is no longer observed after chronic treatment (21 days) with this drug (Fig. 2).

![Fig. 1 —Effect of fluoxetine on the firing rate of VTA dopaminergic neurons.—(A) representative rate histogram showing the typical inhibitory effect of intravenous fluoxetine (20, 20, 40, 80, 160, 320 \(\mu\)g/kg, at arrows); (B) Cumulative dose-response curve showing mean percentage of change (± S.E.M.) in firing rate of VTA dopaminergic neurons after intravenous fluoxetine. [*P < .05; ** < .01 compared to basal firing rate (one-way analysis of variance, followed by Tukey’s test). (Taken from Prisco and Esposito, 1995.1)]

![Fig. 2 —Effects of chronic treatment with intraperitoneal fluoxetine (10 mg/kg, for 21 days) on the response of VTA dopaminergic neurones to acute intravenous fluoxetine.—(A) Representative rate histogram showing the typical inhibitory effect of acute intravenous fluoxetine (20, 20, 40, 80, 160, 320 \(\mu\)g/kg, at arrows) in a control rat; (B) Typical rate histogram showing prevention by chronic intraperitoneal fluoxetine (20, 20, 40, 80, 160, 320, 640, 1280, 2560 \(\mu\)g/kg, at arrows); (C) Cumulative dose-response curves showing mean percentage of change (± S.E.M.) in the firing rate of VTA dopaminergic neurones after acute intravenous fluoxetine in control rats (\(\square\)) and in animals treated chronically with fluoxetine (\(\blacksquare\)). Complete tolerance developed after chronic fluoxetine administration. [*P < .05 \(F(6,84)=6.27\), two-way analysis of variance, split-plot design, followed by Tukey’s test]. (Taken from Prisco and Esposito, 1995.1)
ingly, m-chlorophenylpiperazine (mCPP), a mixed 5-HT$_{2A/B/C}$ receptor agonist, inhibits the firing activity of VTA DA neurons in control animals but not in those chronically treated with fluoxetine. The authors suggest that 5-HT$_{2C}$ receptors may be down-regulated after repeated fluoxetine administration. Consistent with this hypothesis, evidence is there that chronic treatment with sertraline and citalopram, two selective serotonin re-uptake inhibitors (SSRIs), induces tolerance to hypolocomotor effect of mCPP.

This hypersensitivity of 5-HT$_{2C}$ receptors may be a key step for achievement of an antidepressant effect. Indeed, it is possible to argue that acute inhibitory effect of fluoxetine on mesolimbic DA system would mask its clinical efficacy in the early stage of treatment. This masking effect may disappear when hypersensitivity of 5-HT$_{2C}$ receptors occurs. A series of studies carried out in our laboratory have shown that acute administration of SSRIs such as paroxetine, sertraline, and fluoxetine causes a slight but significant decrease in basal firing rate of VTA DA neurons. Therefore, it is conceivable that, similar to fluoxetine, these three SSRIs can reduce mesocorticolimbic DA transmission by activating 5-HT$_{2C}$ receptors.

**Effects of various 5-HT$_{2C}$ receptor agonists and antagonists on nigrostriatal and mesocorticolimbic DA function**—Series of studies carried out in our laboratory have clearly shown that 5-HT$_{2C}$ receptors play a prominent role in the control of mesocorticolimbic DA function. Initially, it has been found that the firing rate of DA neurons in VTA is reduced by mCPP and trifluoromethylphenylpiperazine (TFMPP), two mixed 5-HT$_{2A/B/C}$ receptor agonists, whereas these neurons are stimulated by mesulergine. Based on these findings, it has been suggested that 5-HT may exert an inhibitory action on DA neurons in VTA by acting through 5-HT$_{2}$ receptors. However, these data do not allow to distinguish the relative contribution of 5-HT$_{2}$ receptor subtype in the control of central DA function. Subsequent studies clearly indicate a differential involvement of 5-HT$_{2C}$ receptors on the basis of evidence that the inhibitory effect of mixed 5HT$_{2}$ receptor agonists mCPP and 6-chloro-2-(1-piperaziny1) piperazine (MK 212) on the activity of VTA DA-containing neurons and on accumbal DA release is completely prevented by 6-chloro-5-methyl-1-[2-(2-methylpyridyl)-3-oxy]-pyrid-5-yl carbamoyl] indoline (SB 242084), a selective 5-HT$_{2C}$ receptor antagonist.

Moreover, SB 242084 blocks the inhibitory action of (S)-2-(chloro-5-flouro-indo-1-yl)-1-methyllethylamine 1:1 C4 H4 O4 (RO 60-0175), a selective 5-HT$_{2C}$ receptor agonist (Figs 3, 4). Another series of studies have shown that 5-methyl-1-(3-pyriddylcarbamoil)-1,2,3,5-tetrahydropyrryrolo[2,3-f]indole (SB 206553), a selective 5-HT$_{2C}$ receptor antagonist, increases the basal firing rate and the bursting activity of VTA DA neurons and enhances DA release in the rat nucleus accumbens and prefrontal cortex. Consistent with these findings, indoline (SB 242084), the most powerful and selective 5-HT$_{2C}$ receptor antagonist, selectively enhances the mesocorticolimbic DA function (Figs 5, 6), while RO 60-0175 and MK 212, two 5-HT$_{2C}$ receptor agonists, reduce it. Moreover, SB 242084 has been found to potentiate the phenycyclidine-induced increase in accumbal DA release.

On the one hand, it does not seem that 5-HT$_{2C}$ receptors exert a relevant role in the control of nigrostriatal DA system. Thus, there is evidence that 5-HT$_{2C}$ receptor agonists such as mCPP, MK 212, and RO 60-0175 do not significantly affect the activity of SNc DA neurons, and *in vivo* DA release in striatum.

On the other hand, mixed 5-HT$_{2A/B/C}$ antagonist SB 206553 causes only a slight increase in the basal activity of DA neurons in SNc and striatal DA release. Therefore, on the basis of the above mentioned data, it is possible to conclude that serotonergic system exerts both phasic and tonic control of mesocorticolimbic DA function by acting through 5-HT$_{2C}$ receptors. A recent study carried out in our laboratory has shown that mCPP excites non-DA (presumably GABA-containing) neurons both in SNr and VTA by activating 5-HT$_{2C}$ receptors. One interesting finding of that study is the differential effect exerted by mCPP on subpopulations of SNr neurons. Thus, mCPP causes a marked excitation of so-called P(0) SNr non-DA neurons, whereas it does not affect P(+) neurons. These neurons are identified on the basis of presence [P(0)] or the absence [P(0)] of an excitatory response to a noxious stimulus (footpinch). There is evidence that P(+) SNr neurons are GABA-containing interneurons those exert a direct inhibitory influence on DA neurons in SN, whereas P(0) cells represent GABA-ergic and SNr projection neurons. On the other hand, all non-DA neurons in the VTA are equally excited by mCPP. It is tempting to speculate that this differential response to mCPP may be the basis of the preferential inhibitory effect of 5-HT$_{2C}$ agonists on the mesocorticolimbic versus nigrostriatal DA function.
Therapeutic potential of drugs acting through 5-HT\textsubscript{2c} receptors—In view of the hypothesis that disinhibition of the mesolimbic DA system underlies the mechanism of action of several antidepressant drugs\textsuperscript{27}, the disinhibitory effect of SB 206553 and 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\textsuperscript{28}. In this respect, it is interesting to note that several antidepressant drugs have been shown to bind with submi-

![Diagram](image-url)
Fig. 4.—Time course of the effect of intraperitoneal administration of 1 mg/kg of RO 60-0175 (A), 1 mg/kg of (±)-DOI (C), and 1mg/kg of BW 723C86 (D) on extracellular DA levels in the rat nucleus accumbens. (i) control groups treated with the vehicle. Drugs were administered at the time indicated by vertical arrows. Each data point represents mean percentage ± SEM of the baseline value calculated from three samples before drug injection. Each experiment was carried out on five to six animals per group, **P<.01 vs control group (two-way analysis of variance followed by Tukey’s test). (B) Time course of the effect of RO 60-0175 (■) (1 mg/kg, ip) and pretreatment of SB 242084 (A) (2.5 mg/kg, ip) on extracellular DA levels in the nucleus accumbens. RO 60-0175 was administered at the time indicated by vertical arrow. SB 242084 was given 10 min before RO 60-0175. Each data point represents mean percentage ± SEM of the baseline value calculated from three samples before RO 60-0175 injection. Each experiment was carried out on five to six animals per group. [F(1,10) = 10.252, *P<.05, **P<.01 RO 60-0175 versus SB 242084 + RO 60-0175 (two-way analysis of variance, split-plot design, followed by Tukey’s test). (Taken from Di Matteo et al., 2000)]

cromolar affinity to 5-HT2C receptors in the pig brain and antagonize mCPP-induced penile erections in rats, an effect mediated through stimulation of central 5-HT2C receptors27. Based on these findings, Di Matteo et al.80 have carried out experiments showing that acute administration of amitriptyline and mianserin, two antidepressants with high affinity for 5-HT2C receptors, enhances DA release in the rat nucleus accumbens probably by blocking these receptor subtypes. Interestingly, amitriptyline and mianserin have been tested in chronic, mild, stress-induced anhedonia model of depression and are found to be effective in reversing the stress effects3,12. Antianhedonic effects of tricyclic antidepressants, mianserin, and fluoxetine
abolish by pretreatment with D2/D3 receptor antagonists, thus indicating an involvement of DA in the antidepressant effect of various drugs in this model. Although, DA has received little attention in biological research on depression, with respect to other monoamines such as 5-HT and noradrenaline, it is now well established that disturbances of mesolimbic DA function are implied in the pathophysiology of depression. However, future experiments aimed at investigating the effects of chronic administration of 5-HT2C receptor antagonists on mesolimbic DA function will help to clarify the role played by this receptor subtype in their putative antidepressant action. Preferential disinhibition of mesocorticolimbic DA function by 5-HT2C antagonists may be relevant for the possible use of these compounds in the treatment of negative symptoms of schizophrenia, a clinical condition in which a reduced function of mesocorticolimbic DA system has been hypothesized. Moreover, it is noteworthy to mention recent data showing that atypical antipsychotic drugs (clozapine, sertindole, olanzapine, ziprasidone, risperidone, zotepine, tiotiaprine, fluperlapine, tenilapine), which produce little or no extra-pyramidal side effects while improving negative symptoms of schizophrenia, exert substantial inverse agonist activity at 5HT2C receptors. Thus, 5-HT2C receptor inverse agonism may underlie the unique clinical properties of atypical antipsychotics drugs. However, in vivo experiments are necessary to confirm the relevance of this action of atypical antipsychotics on 5-HT2C receptors. In addition, the evidence of selective reduction of mesocorticolimbic DA function by 5-HT2C receptor agonists may be exploited for therapeutic purposes. Thus, it has recently been found that RO 60-0175 reduces cocaine-reinforced behaviour by stimulating 5-HT2C receptors. Moreover, these authors have also shown that RO 60-0175 reduces ethanol- and nicotine-induced self-administration and hyperactivity. These data are consistent with biochemical studies showing that 5-HT2C receptor agonists inhibit morphine-induced DA release in the rat nucleus accumbens. Therefore, it is
conceivable that 5-HT$_2C$ receptor agonists may be useful for the treatment of drug addiction. Another interesting application of the data regarding functional role of 5-HT$_2C$ receptors in basal ganglia is the possible use of 5-HT$_2C$ receptor antagonists in the treatment of Parkinson’s disease. The neural mechanisms underlying the generation of parkinsonian symptoms are thought to involve reduced activation of primary motor and premotor cortex and supplementary motor areas, secondary to over-activation of the output regions of basal ganglia, i.e., SNr and globus pallidus internus (Gpi)\textsuperscript{12}, largely because of excessive excitatory drive from sub-thalamic nucleus (STN). Therapy of Parkinson’s disease consists mainly of amelioration of symptoms with classical dopaminomimetics\textsuperscript{43}. This treatment, however, is characterized by declining efficacy and occurrence of disabling side-effects\textsuperscript{45}. Functional inhibition of GPi or STN, has provided an alternative to lesion, by deep brain stimulation associated with modest side-effects\textsuperscript{45}. As already mentioned, 5-HT$_2C$ receptors are located in SNr and medial segment of pallidal complex in rat and human brain\textsuperscript{8,46}. In addition, 5-HT$_2C$-like receptor binding is increased in a rat model of parkinsonism\textsuperscript{47} and in human parkinsonian patients\textsuperscript{48}. Given that 5-HT$_2C$ receptor activation leads to excitation of SNr, it is tempting
to speculate that excessive 5-HT2c receptor stimulation may contribute to increased activity of output regions of basal ganglia and thus to symptoms of parkinsonism. In this respect, it is noteworthy that preliminary experiments carried out in our laboratory show that SB 242084 reduces basal firing rate of P(0) neurons in SNr. Thus, based on these findings, it would be very interesting to test the effects of 5-HT2c receptor antagonists on basal activity of GABA-ergic neurons in SNr, and on in vivo GABA release in SNr and thalamus (which is a projection area of SNr) of normal and 6-OHDA-lesioned rats, which represent a suitable animal model of Parkinson’s disease.

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