Clozapine and Haloperidol Differentially Alter the Constitutive Activity of Central Serotonin$_{2C}$ Receptors In Vivo

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Background: *Central serotonin$_{2C}$ (5-HT$_{2C}$) receptors are known to play a role in the mechanism of action of the antipsychotic drugs (APDs) clozapine and haloperidol. However, evidence for the involvement of the constitutive activity of 5-HT$_{2C}$ receptors in the dopamine (DA)ergic effects of APDs is lacking in vivo.*

Methods: *Using in vivo microdialysis in halothane-anesthetized rats, we assessed the ability of selective 5-HT$_{2C}$ compounds to modulate the release of DA induced by haloperidol and clozapine in the nucleus accumbens and striatum.*

Results: *Both APDs induced a dose-dependent increase in accumbal and striatal DA extracellular levels. The effect of .01 mg/kg haloperidol was potentiated by the 5-HT$_{2C}$ inverse agonist SB 206553 (5 mg/kg) but unaltered by the 5-HT$_{2C}$ antagonists SB 243213 and SB 242084 (1 mg/kg). Conversely, the effect of 1 mg/kg clozapine, a dose able to reverse the decrease in DA outflow induced by the 5-HT$_{2C}$ agonist Ro 60-0175 (3 mg/kg), was unaffected by SB 206553 but blocked by SB 243213 (1 mg/kg) and SB 242084 (.3 and 1 mg/kg).*

Conclusions: *These results show that clozapine and haloperidol differentially alter the constitutive activity of 5-HT$_{2C}$ receptors and suggest that clozapine behaves as a 5-HT$_{2C}$ inverse agonist in vivo.*

Key Words: Dopamine release, 5-HT$_{2C}$ receptor, constitutive activity, clozapine, haloperidol, inverse agonist

The serotonin$_{2C}$ (5-HT$_{2C}$) receptor, a member of the G-protein coupled receptors superfamily (Hoyer et al 2002), is widely expressed within the central nervous system (Pazos et al 1985), where it is thought to play a major role in the regulation of neuronal network excitability (Tecott et al 1995). Central 5-HT$_{2C}$ receptors are known to exert tonic and phasic inhibitory control on mesencephalic dopamine (DA) neuronal activity in vivo (Gobert et al 2000; Grottick et al 2000; Navailles et al 2004), and numerous studies have proposed the use of 5-HT$_{2C}$ antagonists for improved treatments of disorders associated with a dysfunction of DA neurons (Di Giovanni et al 2002; Jones and Blackburn 2002). Recently, the constitutive activity of 5-HT$_{2C}$ receptors has been demonstrated to be a new form of heteroregulation of DA neuron activity in vivo (De Deurwaerdère et al 2004). However, this property of the 5-HT$_{2C}$ receptor has not yet been taken into consideration in evaluating the mechanism of action of therapeutic drugs in vivo.

The 5-HT$_{2C}$ receptors are known to play a role in the DAergic effects of antipsychotic drugs (APDs) (Meltzer et al 2003; Wood et al 2001). Most atypical APDs, like clozapine, show high affinity for 5-HT$_{2C}$ receptors (Roth et al 1992). Specifically, evidence suggests that clozapine may behave as a 5-HT$_{2C}$ antagonist in vivo. Indeed, clozapine reverses the inhibition of accumbal DA release induced by the 5-HT$_{2C}$ agonist Ro 60-0175 (Di Matteo et al 2002) and blocks the hypolocomotion induced by the 5-HT$_{2C}$ agonist m-chlorophenylpiperazine (mCPP) (Prinssen et al 2000). It is noteworthy that clozapine, like several APDs, behaves as a 5-HT$_{2C}$ inverse agonist in heterologous expression systems in vitro (Herrick-Davies et al 2000; Rauser et al 2001). Although this pharmacological profile is also compatible with the above reported effects of clozapine, evidence for the involvement of 5-HT$_{2C}$ inverse agonism in clozapine's effects is lacking in vivo.

The 5-HT$_{2C}$ receptors may also participate in the DAergic effects of the typical APD haloperidol. Indeed, it has been reported that the increase in striatal DA release induced by haloperidol is dramatically potentiated by the purported 5-HT$_{2C}$ antagonist SB 206553 (Lucas et al 2000). However, the recent finding that SB 206553 behaves as a 5-HT$_{2C}$ inverse agonist in vivo (De Deurwaerdère et al 2004) raises the possibility that the potentiating effect reported above is not strictly related to the simple blockade of 5-HT$_{2C}$ receptors by SB 206553. Altogether, these findings suggest that the constitutive activity of 5-HT$_{2C}$ receptors may participate in the 5-HT$_{2C}$ receptor-dependent modulation of clozapine- and haloperidol-stimulated DA release, an effect that, as mentioned above, is currently attributed to the blockade of the action of endogenous 5-HT at 5-HT$_{2C}$ receptors.

Thus, the present study was designed to determine whether the constitutive activity of 5-HT$_{2C}$ receptors participates in the DAergic effects of APDs in vivo. For this purpose, we assessed the influence of the 5-HT$_{2C}$ inverse agonist SB 206553 (De Deurwaerdère et al 2004), the well-known neutral 5-HT$_{2C}$ antagonist SB 242084 (De Deurwaerdère et al 2004), and the recently available neutral 5-HT$_{2C}$ antagonist SB 243213 (Shilliam and Dawson 2005; Navailles, unpublished results, 2005) on clozapine- and haloperidol-induced DA release. Experiments were performed using in vivo microdialysis in halothane-anesthetized rats, an experimental procedure permitting simultaneous monitoring of DA outflow in the ipsilateral nucleus accumbens (NAc) and striatum, to evaluate possible differences in the 5-HT$_{2C}$ receptor control of the mesoaccumbal and the nigrostriatal DA pathways (De Deurwaerdère and Spampinato 2001; Di Matteo et al 2001).

Methods and Materials

Animals

Male Sprague Dawley rats (IFFA CREDO, Lyon, France) weighing 330 g to 380 g were used. Animals were kept at

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constant room temperature (21°C ± 2°C) and relative humidity (60%) with a 12-hour light/dark cycle (dark from 8:00 PM) 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: Ro 60-0175.HCl (S-2-(6-chloro-5-fluoroindol-1-yl)-1-methylethylamine.hydrochloride) kindly donated by Dr. P. Weber (F Hoffmann-La Roche, Basel, Switzerland); SB 243213 (5-methyl-1-[[2-[(2-methyl-3-pyridyl)oxy]-5-pyridyl]carbamoyl]-6-trifluoromethylindoline) generously provided by Dr. M. Wood (Psychiatry CEDD, Glaxo-SmithKline, Harlow, United Kingdom); SB 242084.2HCl (6-chloro-5-methyl-1-[6-(2-methylpiridin-3-yloxy)pyridin-3-yl carbamoyl] indoline dihydrochloride), SB 206553.HCl (5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-f] indole hydrochloride), and clozapine (8-chloro-11-(4methyl-1-piperazinyl)-5H6-dibenzo[b,e][1,4]-diazepine) (Sigma-RBI, Saint Quentin Fallavier, France); and haloperidol (4-[4-(p-chlorophenyl)-4-hydroxypiperidino]-4'-fluorobutyrophenone) as the commercially available solution (Haldol 5 mg/mL) (Janssen Pharmaceutica, Beerse, Belgium). All other chemicals and reagents were the purest commercially available (VWR, Strasbourg, France; Sigma, Illkirch, France).

Microdialysis

Surgery and perfusion procedures were performed as previously described (De Deurwaerdère et al 2004), with minor modifications. Briefly, rats were anesthetized with a mixture of halothane and nitrous oxide-oxygen (2%; 2:1 vol/vol). After tracheotomy for artificial ventilation, the animals were placed in a stereotaxic frame, and their rectal temperature was monitored and maintained at 37.3°C ± .1°C with a heating pad. Two microdialysis probes, 2 and 4 mm long (CMA/11, 240 μm outer diameter, Cuprophan) (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 = 2.7, respectively) according to the atlas of Paxinos and Watson (1986). Probes were perfused at a constant flow rate of 2 μL/min by means of a microperfusion pump (CMA 111, Carnegie Medicin, Phymep) with artificial cerebrospinal fluid (aCSF) containing (in mmol/L): 154.1 Cl^-, 147 Na^+, 2.7 K^+, 1 Mg^{2+}, and 1.2 Ca^{2+}, adjusted to pH 7.4 with 2 mmol/L sodium phosphate buffer. Dialysates (30 μL) were collected on ice every 15 minutes. The in vitro recoveries of the probes were about 10% for DA. At the end of each experiment, the brain was removed and fixed in sodium chloride (NaCl) (.9%)/paraformaldehyde solution (10%). The location of the probes was determined histologically on serial coronal sections (60 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 mmol/L] 70 sodium dihydrogen phosphate (NaH_2PO_4), .1 disodium ethylenediamine-tetraacetate (Na_2EDTA), .7 triethylamine, and .1 octylsulfonic acid plus 10% methanol, adjusted to pH 4.8 with ortophosphoric acid) was delivered at 1 mL/min flow rate (system LC-10AD-*VP*, Shimadzu, Champs sur Marne, France) through a Hypersyl column (C18; 4.6 × 150 mm, particle size 5 μm) (Touzard & Matignon, Paris, France). Detection of DA was carried out with a coulometric detector (Coulochem 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 France). Under these conditions, the sensitivity for DA was .5 ρg/30 μL with a signal/noise ratio of 3:1.

Pharmacological Treatments

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 less than 10% in both structures, was generally obtained 135 minutes after the beginning of the perfusion (stabilization period).

Clozapine, diluted in a 99:1 vol/vol mixture of apyrogenic water and lactic acid, was administered subcutaneously at .3, 1, or 3 mg/kg in a volume of 2 mL/kg. Haloperidol, diluted in NaCl .9%, was administered subcutaneously at .01 or .1 mg/kg in a volume of 1 ml/kg. The 5-HT_{2C} inverse agonist SB 206553 was diluted in a 99:1 vol/vol mixture of apyrogenic water and lactic acid; the selective 5-HT_{2C} antagonists SB 243213 and SB 242084 were dissolved in a mixture of NaCl .9% containing hydroxypropyl-β-cyclodextrin (8% by weight) plus citric acid (25 mmol/L). SB 206553 (5 mg/kg), SB 243213 (1 mg/kg), and SB 242084 (.3 and 1 mg/kg) were injected intraperitoneal (IP) in a volume of 2 mL/kg, 15 minutes (SB 206553 and SB 242084) or 30 minutes (SB 243213) before APDs or appropriate vehicles. The 5-HT_{2C} agonist Ro 60-0175 was dissolved in NaCl .9% and administered intraperitoneally at 3 mg/kg in a volume of 1 ml/kg, 30 minutes before 1 mg/kg clozapine or appropriate vehicle. The doses of the different 5-HT compounds used were chosen on the basis of previous studies to keep both selectivity and efficiency toward the targeted sites (De Deurwaerdère et al 2004; Kennett et al 1996, 1997). Also, the administration time of 5-HT agents was chosen on the basis of their pharmacokinetic properties such that they were at their pharmacodynamic maximums when the effect of APDs on DA release was maximal (De Deurwaerdère et al 2004; Navailles, unpublished results, 2005). All drug doses were calculated as the free base. In each experimental group, animals received either drugs or their appropriate vehicle.

Statistical Analysis

Dopamine contents in each sample were 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. Drug overall effect was calculated as the average of DA content from dialysates collected after their administration. The statistical analysis of the effect of the APDs haloperidol and clozapine on basal DA outflow was assessed by a one-way analysis of variance (ANOVA) (using group as the main factor) with time as repeated measures, performed for the eight samples that followed drug administration. When significant ($p < .05$), the one-way ANOVA was followed by the Fisher's protected least significance difference test (PLSD) to allow adequate multiple comparisons between groups. The interaction between 5-HT_{2C} compounds and APDs was studied by a two-way ANOVA