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BRIEF REPORT

The Selective Serotonin$_{2A}$ Receptor Antagonist, MDL100,907, Elicits a Specific Interoceptive Cue in Rats

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Employing a two-lever, food-reinforced, Fixed Ratio 10 drug discrimination procedure, rats were trained to recognize the highly-selective serotonin (5-HT)$_{2A}$ receptor antagonist, MDL100,907 (0.16 mg/kg, i.p.). They attained criterion after a mean ± S.E.M. of 70 ± 11 sessions. MDL100,907 fully generalized with an Effective Dose (ED)$_{50}$ of 0.005 mg/kg, s.c.. A further selective 5-HT$_{2A}$ antagonist, SR46349, similarly generalized with an ED$_{50}$ of 0.04 mg/kg, s.c. In distinction, the selective 5-HT$_{2B}$ antagonist, SB204,741 (0.63 and 10.0 mg/kg), the 5-HT$_{2B/2C}$ antagonist, SB206,553 (0.16 and 2.5 mg/kg) and the selective 5-HT$_{2C}$ antagonists, SB242,084 (2.5 and 10.0 mg/kg,) and RS102221 (2.5 and 10.0 mg/kg), did not significantly generalize. In conclusion, selective blockade of 5-HT$_{2A}$ receptors by MDL100,907 elicits a discriminative stimulus in rats which appears to be specifically mediated via 5-HT$_{2A}$ as compared with 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors.
[Neuropsychopharmacology 26:552–556, 2002]

KEY WORDS: *Drug discrimination; Interoceptive; 5-HT$_{2A}$ receptors*

Drug discrimination procedures have been extensively used in the characterization of psychoactive agents, including drugs interacting with 5-HT reuptake sites (Millan et al. 1999b) and agonists at 5-HT$_{1A}$ (Schreiber et al. 1995b) and 5-HT$_3$ (Glennon et al. 1992) receptors. Although it has proven difficult to differentiate the roles of closely-related 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors (Glennon 1991), it was suggested that discriminative stimulus (DS) properties of several 5-HT$_2$ agonists and hallucinogens, such as mescaline (Appel and Callahan 1989), lysergic acid diethylamide (LSD) (Fiorella et al. 1995) and quipazine (Friedman et al. 1984), are mediated by 5-HT$_{2A}$ receptors. Further, use of the highly selective 5-HT$_{2A}$ receptor antagonist, MDL100,907 (Kehne et al. 1996), demonstrated that 5-HT$_{2A}$ receptors mediate DS properties of 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI), a further hallucinogen (Schreiber et al. 1994). In contrast, DS properties of the 5-HT$_2$ ligand, m-chlorophenylpiperazine (mCPP), appear to be mediated by 5-HT$_{2C}$ receptors (Callahan and Cunningham 1994; see Gommans et al. 1998), and employing the 5-HT$_{2B/2C}$ antagonist, SB206,553, and the selective 5-HT$_{2C}$ antagonist, SB242,084, it was shown that 5-HT$_{2C}$ receptors likewise mediate DS properties of the novel, mixed 5-HT$_{2C/2B}$ agonist, RO60,0175 ((S)-2-(6-chloro-5-fluoroindol-1-yl)-1-methylethylamine; Dekeyne et al. 1999).

Interest in 5-HT$_{2A}$ receptors has been reinforced by evidence that their blockade may contribute to the distinctive functional profile of the "atypical" antipsychotic, clozapine, and, possibly, other novel agents employed

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Received April 5, 2001; revised August 14, 2001; accepted September 28, 2001.

Online publication: 10/12/01 at www.acnp.org/citations/Npp 101201185.

Published by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010

0893-133X/02/$–see front matter
PII S0893-133X(01)00389-X

for management of psychotic states (Roth and Meltzer 1995). Further, clozapine and several other antipsychotics block DS properties of 5-HT_{2A} agonists such as DOI (Palumbo and Winter 1994; Schreiber et al. 1994), while 5-HT_{2A} receptors are, at least partially, involved in the DS properties of clozapine itself (Millan et al. 1999c).

It would, thus, be of considerable interest to establish whether discrete *blockade* of 5-HT_{2A} receptors generates a specific DS in rats. In view of its pronounced selectivity for 5-HT_{2A} receptors, the potential antipsychotic agent, MDL100,907, appeared an optimal ligand to address this issue (Kehne et al. 1996; Millan et al. 1999c). In the present study, we evaluated whether MDL100, 907 elicits a reliable DS in rats. We also characterized the role of 5-HT_{2A} as compared with 5-HT_{2B} and 5-HT_{2C} receptors in the mediation of its potential DS properties. Generalization testing was conducted with a further selective and potent antagonist at 5-HT_{2A} receptors, SR46349 (Rinaldi-Carmona et al. 1992), the 5-$HT_{2B/2C}$ antagonist, SB206,553 (Kennett et al. 1996), the selective 5-HT_{2B} antagonist, SB204,741 (Forbes et al. 1995), and the novel selective 5-HT_{2C} antagonists, RS102221 and SB242,084 (Bonhaus et al. 1997; Kennett et al. 1997).

METHODS

All animal use procedures conformed to international European ethical standards (86/609-CEE) and the French National Committee (décret 87/848) for the care and use of laboratory animals. As described previously (Millan et al. 1999b), male Wistar rats (180–200 g upon arrival, Iffa-Credo, L'Arbresle, France) were trained to discriminate MDL100,907 (0.16 mg/kg, i.p.) from saline in operant conditioning chambers equipped with two levers. The training dose was selected in light of the ability of MDL100,907 to abolish actions mediated by 5-HT_{2A} receptors in other paradigms without exerting significant effects at other receptors (Schreiber et al. 1994, 1995a; Millan et al. 1999a; Gobert et al. 2000).

The animals were reinforced with food according to a Fixed Ratio 10 schedule of reinforcement. Each 15-min daily session (five days/week) started 15 min after injection. "MDL100,907" or "saline" sessions alternated randomly. Correct responding was defined as no more than 13 presses on both levers to obtain the first reinforcement. The discrimination criterion was ten consecutive sessions with correct responding. Thereafter, generalization tests were conducted every Wednesday and Friday, whereas training sessions were continued on the other days. Rats were tested only if they showed correct responding on the two preceding training sessions. Test drugs were administered instead of MDL100,907, 15 min before the session. During testing, responding on the selected lever, i.e., the lever for which ten responses were recorded first, was reinforced for the remainder of the 15-min session.

All drug doses are in terms of the base. Test drugs were dissolved in sterile water and administered s.c., except SB204,741 and SB242,084, which were administered i.p. as suspensions in water with a few drops of Tween 80. In order to avoid potential cutaneous toxicity, RS102221 and SB206,553 were also administered i.p. at the highest dose tested. Drug structures, salts and sources were as follows. RS102221 (8-[5-(2,4-dimethoxy -5-(4-trifluoromethylphenyl sulphonamino)phenyl-5-oxopentyl]-1,3,8-triazaspiro[4.5]decane-2,4-dione) HCl was purchased from Tocris Cookson (Bristol, UK), SB206,553 (5 methyl-1-(3-pyridil-carbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-f] indole) HCL was purchased from Sigma (Chesnes, France) and SR46349B (1(Z)-[2-(dimethylamino)ethoxyimino]-1-(2-fluorophenyl)-3-(4-hydroxyphenyl)-2(E)-propene) hemifumarate was a generous gift of Sanofi Winthrop (Montpellier, France). MDL100, 907 (*R*(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol), SB204,741 (1-(1-methylindol-5-yl)-3-(3-methylisothiazol-5-yl)urea) and SB242, 084 (6-chloro-5-methyl-1-[6-(2-methylpyridin-3-yloxy) pyridin-3-yl carbamoyl) indoline HCl were synthetized by Servier chemists (G. Lavielle and J.-L. Péglion).

RESULTS

The mean ± S.E.M. number of sessions required to reach the discrimination criterion was 70 ± 11 (n = 7). Administered s.c., MDL100,907 displayed dose-dependent, significant and full generalization (0.16 mg/kg, s.c., 100%, $n = 7$, $p < .05$ in Fisher's Exact Probability Test as compared with control training session), an action mimicked by a further selective 5-HT_{2A} antagonist, SR46349 (0.16 mg/kg, s.c., 80%, $n = 5$, $p < .05$) (Figure 1). In distinction, the selective 5-HT_{2B} antagonist, SB204,741 (0.63 mg/kg, i.p., 0%, $n = 5$ and 10 mg/kg, i.p., 0%, n = 5), the 5-$HT_{2B/2C}$ antagonist, SB206,553 (0.16 mg/kg, s.c., 40%, $n = 5$; 2.5 mg/kg, s.c., 20%, $n = 5$ and 10 mg/kg, i.p., 40%, $n = 5$), and the selective 5-HT_{2C} antagonists, SB242,084 (2.5 mg/kg, i.p., 20%, $n = 5$ and 10 mg/kg, i.p., 20%, $n = 5$) and RS102221 (2.5 mg/kg, s.c., 0%, $n = 5$ and 10 mg/kg, i.p., 40%, $n = 5$), did not show significant generalization. None of these antagonists decreased response rates (not shown), with the exception of SB204,741 (10 mg/kg, i.p.) for which a decrease of 5% as compared with the preceding saline training session was observed.

DISCUSSION

The present study demonstrates that MDL100,907 elicits a specific and stable DS in rats. Although the 5-HT_2

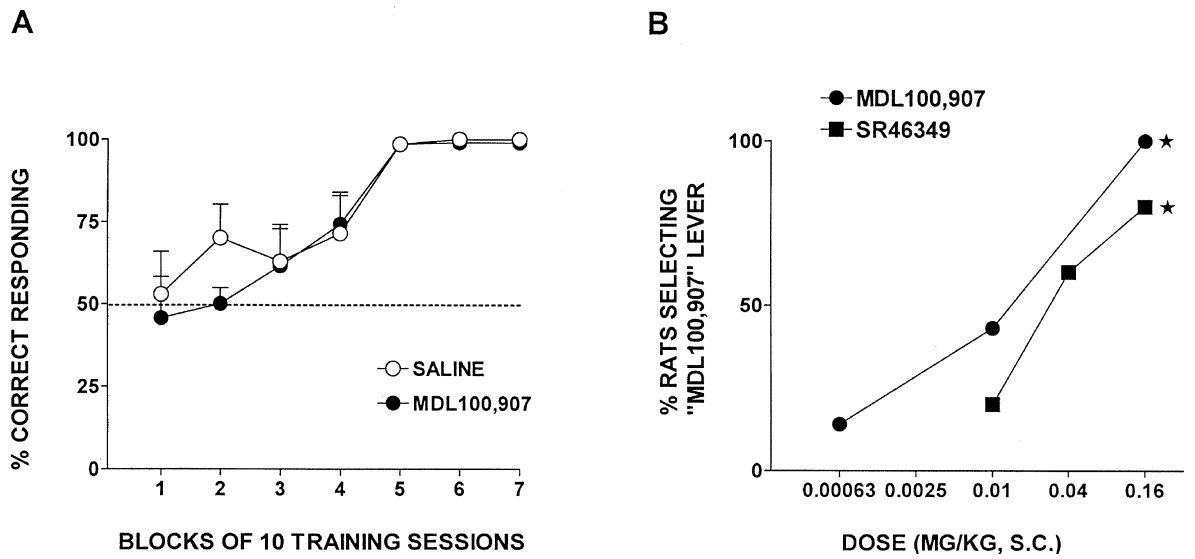

Figure 1. Discriminative stimulus properties of the selective 5-HT_{2A} antagonist, MDL100,907 (0.16 mg/kg, i.p.), in rats. Panel A: Acquisition. Panel B: Generalization of MDL100,907 and SR46349. Data in Panel A are means ± S.E.M. and are shown separately for saline and MDL100,907 sessions. In Panel B, data are percentage of animals generalizing. The Effective $Dose_{50}$s (95% Confidence Limits) for generalization of MDL100,907 and SR46349 were respectively 0.005 (0.001–0.018) and 0.04 (0.01–0.11) mg/kg, s.c.. Asterisks in Panels B indicate significance of differences to control sessions in Fisher's Exact Probability Test. * $p < .05$.

antagonist, pizotifen, has been shown to generate a DS in rats, it does not differentiate 5-HT_{2A} from 5-HT_{2B} and 5-HT_{2C} sites (Minnema et al. 1984). Further, several 5-HT_2 antagonists failed to generalize to pizotifen, which interacts with many other sites, including histaminergic receptors, which play a major role in its DS properties (Minnema et al. 1984). Thus, the present study constitutes the first demonstration, to our knowledge, that *selective* blockade of 5-HT_{2A} receptors can sustain a DS.

Support for a specific role of 5-HT_{2A} receptors in the DS properties of MDL100,907 is provided by several lines of evidence. First, MDL100,907 is >200-fold selective for native, tissue and cloned, human 5-HT_{2A} versus 5-HT_{2B} and 5-HT_{2C} receptors (Table 1). Second, the training dose of MDL100,907, as well as the Effective $Dose_{50}$ for its "auto-generalization", were low. These doses correspond well to actions of MDL100,907 in other models of 5-HT_{2A} receptor-mediated responses: notably, inhibition of a DOI-induced DS, blockade of DOI-induced head-twitches and antagonism of hyperlocomotion elicited by phencyclidine (Schreiber et al. 1994, 1995a; Millan et al. 1999a). At these doses, MDL100,907 does not exert significant action in functional models of 5-HT_{2C} receptor-mediated activity (Millan et al. 1997; Dekeyne et al. 1999). Third, a further, highly-selective and potent antagonist at 5-HT_{2A} receptors, SR46349, similarly generalized to MDL100,907 at low doses corresponding to those active in the above-mentioned models (Rinaldi-Carmona et al. 1992; Millan et al. 1999c). Fourth, at doses producing robust increases of extracellular norepinephrine and dopamine levels in the frontal cortex of freely-moving rats, and which abolish 5-HT_{2C} receptor-mediated DS and penile erections, both SB206,553 and SB242,084 (Kennett et al. 1997; Millan et al. 1997; Dekeyne et al. 1999; Gobert et al. 2000), did not generalize to MDL100,907. A further, novel 5-HT_{2C} antagonist, RS102221 (Bonhaus et al. 1997) also did not generalize. Fifth, SB206,553 is a potent antagonist at 5-HT_{2B} as well as 5-HT_{2C} receptors (Kennett et al. 1996), and a further (selective) antagonist at this site, SB204,741 (Forbes et al. 1995; Dekeyne et al. 1999), similarly did not generalize to MDL100,907.

These observations provide compelling evidence that selective blockade of 5-HT_{2A} receptors mediates the DS properties of MDL100,907, although the possibility that

Table 1. Affinities of Compounds used in the Present Study at Native, Tissue and Cloned, Human 5-HT_2 Receptor Subtypes

Drug	h5-HT_{2A}	h5-HT_{2B}	h5-HT_{2C}	r5-HT_{2A}	p5-HT_{2C}
MDL100,907	9.9	6.6	7.7	9.2	7.0
SR46349	10.3	< 6.0	8.8	8.9	7.5
SB204,741	< 5.0	7.3	5.7	5.1	5.9
SB206,553	6.1	7.9	8.6	6.7	7.9
SB242,084	6.5	7.3	9.3	6.3	9.3
RS102221	6.6	6.7	8.5	6.4	6.6

Affinities are expressed as pK_is. r = rat; p = porcine, h = human (CHO-transfected). Data are from this laboratory (Gobert et al. 2000; Newman-Tancredi A and Cussac D, unpub. obs.).

other receptor types are (indirectly) involved in their expression would be of interest to evaluate further. It will be of interest to characterize generalization patterns of clozapine and other antipsychotic agents, as well as other classes of psychoactive drug known to interact with 5-HT_{2A} receptors, such as antidepressant agents (Frazer 1997).

In conclusion, the present study shows that MDL100, 907 elicits a stable DS in rats that appears to be mediated by blockade of 5-HT_{2A} receptors. Further characterization of the interoceptive properties of MDL100,907 may provide important insights into the actions of antipsychotic agents and other drug classes which interact with 5-HT_{2A} receptors.

REFERENCES

Appel JB, Callahan PM (1989): Involvement of 5-HT receptor subtypes in the discriminative stimulus properties of mescaline. Eur J Pharmacol 159:41–46

Bonhaus DW, Weinhardt KK, Taylor M, Desouza A, McNeeley PM, Szczepanski K, Fontan DJ, Trinh J, Rocha CL, Dawson MW, Flippin LA, Eglen RM (1997): RS-102221: A novel high affinity and selective, 5-HT_{2C} receptor antagonist. Neuropharmacology 36:621–629.

Callahan PM, Cunningham KA (1994): Involvement of 5-HT_{2C} receptors in mediating the discriminative stimulus properties of m-chlorophenylpiperazine (mCPP). Eur J Pharmacol 257:27–38

Dekeyne A, Girardon S, Millan MJ (1999): Discriminative stimulus properties of the novel serotonin $(5\text{-HT})_{2C}$ receptor agonist, RO60–0175: a pharmacological analysis. Neuropharmacology 38:415–423

Fiorella D, Rabin RA, Winter JC (1995): The role of 5-HT_{2A} and 5-HT_{2C} receptors in the stimulus effects of hallucinogenic drugs. I: Antagonist correlation analysis. Psychopharmacology 121:347–356

Forbes IT, Jones GE, Murphy OE, Holland V, Baxter GS (1995): N-(1-Methyl-5-indolyl)-N′-3-methyl-5-isothiazolyl)urea: a novel, high-affinity 5-HT_{2B} receptor antagonist. J Med Chem 38:855–857

Frazer A (1997): Antidepressants. J Clin Psychiatry 58:9–25

Friedman RL, Barrett RJ, Sanders-Bush E (1984): Discriminative stimulus properties of quipazine: mediation by serotonin_2 binding sites. J Pharmacol Exp Ther 228:628–635

Glennon RA (1991): Discriminative stimulus properties of hallucinogens and related designer drugs. In Glennon RA, Järbe TUC, Frankenheim J (eds), Drug Discrimination: Application to Drug Abuse Research. Rockville, Department of Health and Human Services Publication, pp 25–44

Glennon RA, Young R, Dukat M (1992): 5-HT_3 agonist, 2-methylserotonin, as a training drug in drug discrimination studies. Pharmacol Biochem Behav 41:361–364

Gobert A, Rivet J-M, Lejeune F, Newman-Tancredi A, Adhumeau-Auclair A, Nicolas J-P, Cistarelli L, Melon C, Millan MJ (2000): Serotonin_{2C} receptors tonically suppress the activity of mesocortical dopaminergic and adrenergic, but not serotonergic, pathways: a combined dialysis and electrophysiological analysis in the rat. Synapse 36:205–221

Gommans J, Hijzen TH, Maes RAA, Olivier B (1998): Discriminative stimulus properties of mCPP: evidence for a 5-HT_{2C} receptor mode of action. Psychopharmacology 137:292–302

Kehne JH, Baron BM, Carr AA, Chaney SF, Elands J, Feldman DJ, Franck RA, Van Giersbergen PLM, McCloskey TC, Johnson MP, McCarty DR, Poirot M, Senyah Y, Siegel BW, Widmaier C (1996): Preclinical characterization of the potential of the putative atypical antipsychotic MDL 100907 as a potent 5-HT_{2A} antagonist with a favorable CNS safety profile. J Pharmacol Exp Ther 277:968–981

Kennett GA, Wood MD, Bright F, Cilia J, Piper DC, Gager T, Thomas D, Baxter GS, Forbes IT, Ham P, Blackburn TP (1996): In vitro and in vivo profile of SB 206553, a potent 5-HT_{2C}/5-HT_{2B} receptor antagonist with anxiolytic-like properties. Br J Pharmacol 117:427–434

Kennett GA, Wood MD, Bright F, Trail B, Riley G, Holland V, Avenell KY, Stean T, Upton N, Bromidge S, Forbes IT, Brown AM, Midlemiss DN, Blackburn TP (1997): SB 242084, a selective and brain penetrant 5-HT_{2C} receptor antagonist. Neuropharmacology 36:609–620

Millan MJ, Brocco M, Gobert A, Joly F, Bervoets K, Rivet J-M, Newman-Tancredi A, Audinot V, Morel S (1999a): Contrasting mechanisms of action and sensitivity to antipsychotics of phencyclidine versus amphetamine: importance of nucleus accumbens 5-HT_{2A} sites for PCP-induced locomotion in the rat. Eur J Neurosci 11:4419–4432

Millan MJ, Gobert A, Girardon S, Dekeyne A (1999b): Citalopram elicits a discriminative stimulus in rats at a dose selectively increasing extracellular levels of serotonin vs. dopamine and noradrenaline. Eur J Pharmacol 364: 147–150

Millan MJ, Peglion J-L, Lavielle G, Perrin-Monneyron S (1997): 5-HT_{2C} receptors mediate penile erections in rats: actions of novel and selective agonists and antagonists. Eur J Pharmacol 325:9–12

Millan MJ, Schreiber R, Monneyron S, Denorme B, Melon C, Queriaux S, Dekeyne A (1999c): S-16924, a novel, potential antipsychotic with marked serotonin_{1A} agonist properties. IV. A drug discrimination comparison with clozapine. J Pharmacol Exp Ther 289:427–436

Minnema DJ, Hendry JS, Rosecrans JA (1984): Discriminative stimulus properties of pizotifen maleate (BC105): a putative serotonin antagonist. Psychopharmacology 83:200–204

Palumbo PA, Winter JC (1994): Interactions of clozapine with the stimulus effects of DOM and LSD. Pharmacol Biochem Behav 49:115–120

Rinaldi-Carmona M, Congy C, Santucci V, Simiand J, Gautret B, Nelait G, Labeeuw B, Le Fur G, Soubrié P, Brelière JC (1992): Biochemical and pharmacological properties of SR46349B, a new potent and selective $\text{5-hydroxytryptamine}_2$ receptor antagonist. J Pharmacol Exp Ther 262:759–768

Roth BL, Meltzer HY (1995): The role of serotonin in schizophrenia. In Bloom FE, Kupfer DJ (eds), Psychopharmacology: The Fourth Generation of Progress. New York, Raven Press, pp 1215–1228

Schreiber R, Brocco M, Audinot V, Gobert A, Veiga S, Millan

MJ (1995a): [1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane]-induced head-twitches in the rat are mediated by 5-hydroxytryptamine (5-HT)$_{2A}$ receptors: modulation by novel 5-HT$_{2A/2C}$ antagonists, D$_1$ antagonists and 5-HT$_{1A}$ agonists. J Pharmacol Exp Ther 273:101–112

Schreiber R, Brocco M, Lefèbvre de Ladonchamps B, Monneyron S, Millan MJ (1995b): A drug discrimination analysis of the actions of novel serotonin$_{1A}$ receptor ligands in the rat using the 5-HT$_{1A}$ receptor agonist, 8-hydroxy-2-(di-n-propylamino)tetralin. J Pharmacol Exp Ther 275:822–831

Schreiber R, Brocco M, Millan MJ (1994): Blockade of the discriminative stimulus effects of DOI by MDL 100,907 and the 'atypical' antipsychotics, clozapine and risperidone. Eur J Pharmacol 264:99–102