Psychopharmacology (1993) 112:S60–S67

Psychopharmacology

Electrophysiological, biochemical and behavioral evidence for 5–HT_2 and 5–HT_3 mediated control of dopaminergic function

Michael G. Palfreyman, Christopher J. Schmidt, Stephen M. Sorensen, Mark W. Dudley, John H. Kehne, Paul Moser, Maurice W. Gittos, and Albert A. Carr

Marion Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, OH 45215, USA

Abstract. Several lines of evidence have suggested a link between serotonergic and dopaminergic systems in the brain. The interpretation of much of these early data needs careful reevaluation in light of the recent understanding of the plethora of serotonin receptor subtypes, their distribution in the brain and the new findings with more selective serotonin antagonists. Electrophysiological, biochemical and behavioral evidence obtained using highly selective antagonists of the 5–HT_2 or 5–HT_3 receptor subtypes, MDL 100,907 or MDL 73,147EF, respectively, supports the thesis that serotonin modulates the dopaminergic system. This modulation is most evident when the dopaminergic system has been activated.

Key words: 5-Hydroxytryptamine$_2$ (5–HT_2) receptors – 5-Hydroxytryptamine$_3$ (5–HT_3) receptors – Dopamine – Substantia nigra – Ventral tegmental area – Nucleus accumbens – Striatum – Behavior – Amphetamine

Ascending serotonergic systems which have cell bodies in the median and dorsal raphe innervate areas of the brain rich in dopamine neurons (Dray et al. 1976; Giambalvo and Snodgrass 1978; Herve et al. 1989). The distribution of serotonin receptor subtypes throughout the brain is quite heterogeneous. Although distributed in low density, both 5–HT_2 and 5–HT_3 receptors can be identified in cell body and terminal regions of both the nigro-striatal and meso-limbicocortical dopamine systems (Pazos et al. 1985; Kilpatrick et al. 1987). We have used a highly selective 5–HT_2 receptor antagonist, MDL 100,907 and a highly selective 5–HT_3 antagonist, MDL 73,147EF, in biochemical, behavioral and electrophysiological studies investigating a controlling or modulating role of the serotonin system on the function of the nigro-striatal and ventral tegmental-limbic dopamine systems. The data suggest that such serotonergic modulation is most evident when the dopamine systems are activated.

Correspondence to: M.G. Palfreyman

Materials and methods

Male Sprague Dawley rats or male CD mice from Charles River were used throughout. The selective 5–HT_3 antagonist, MDL 73,147EF ((1H-indole-3-carboxylic acid-trans-octahydro-3-oxo-2,6-methano-2H-quinolizine-8-yl-ester, Anemet; Gittos and Fatmi 1989; Sorensen et al. 1989) and the selective 5–HT_2 antagonist, MDL 100,907 (R(+)-α-(2,3-dimethoxyphenyl)-1[2-(4-fluorophenylethyl)]-4-piperidinemethanol; Carr et al. 1990) were synthesized at the Marion Merrell Dow Research Institute. Clozapine was a generous gift from Sandoz, 3,4-methylene dioxymethamphetamine (MDMA) was obtained from the National Institute on Drug Abuse, and Amperozide was kindly provided by Leo Pharmaceuticals. Haloperidol was purchased from Research Biochemicals Inc. (Natrick, MA). All other agents were purchased from standard commercial sources.

Biochemical studies

MDMA studies. MDMA and MDL 100,907 were administered simultaneously in saline by the subcutaneous route. Animals were sacrificed 1 week later by decapitation and the brains were rapidly removed. The cortex, striatum, and hippocampus were dissected free and immediately frozen on dry ice. Tissue samples were stored at −70° C until assayed. Regional 5–HT concentrations were determined by HPLC–EC as described elsewhere (Schmidt 1987).

Significant differences in 5 HT concentrations among treatment groups were determined by ANOVA with individual differences being determined by the least significant difference test. Statistical analysis was performed using Microstat II software (Ecosoft, Inc., Indianapolis, IN) on an IBM–PC AT.

Microdialysis. Concentric microdialysis probes were made using 26 gauge stainless steel tubing as described elsewhere (Schmidt et al. 1992a, b). A 4-mm dialysis membrane was used for all studies (Spectro/Por hollow fibers, cutoff 6000 MW, Spectrum Medical Industries, Los Angeles, CA). Probes were implanted stereotaxically in the right striatum of Sprague-Dawley rats (250–300 g) under pentobarbital anesthesia. Coordinates relative to bregma were 1, 2.5, and −8.0 mm from the top of the skull.

All experiments were performed 1 day after probe implantation. Each implanted rat was tethered in a circular 12-in Plexiglas container to allow freedom of movement. The containers were themselves placed inside lighted and sound-attenuated environmental chambers. A two-channel liquid swivel was used to connect both inlet and outlet lines to the probe. A Harvard 22 pump (Harvard

Apparatus, Inc., South Natick, MA) was used to perfuse the probe at 2 µl/min with artificial CSF containing (mM): NaCl (147.0), KCl (4.0), $CaCl_2$ (2.3) and $MgCl_2$ (0.9), pH 6.9. The dialysate was collected into tubes containing 10 µl of 0.5 M perchloric acid using a refrigerated microfraction collector (Carnegie Medicine, Stockholm, Sweden). A collection period of 20 min was used for all experiments.

MDMA (20 mg/kg, SC) was administered after 2 h of basal dialysates had been collected. In 7 of 17 animals, MDL 100,907 (1 mg/kg, SC) was administered 20 min prior to MDMA. Dialysis was discontinued 4 h after MDMA administration and samples were frozen at −70° C until assayed. All animals were sacrificed 1 week later and the site of probe implantation was verified visually.

Dopamine, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were determined by high performance liquid chromatography with electrochemical detection as described (Schmidt et al. 1992a, b). No corrections for recovery were made. Dopamine release for each animal was calculated as the percent of basal release using the first 2 h of collection to calculate a mean basal value. The percent basal release at each time point was then averaged for all animals in the MDMA or MDMA plus MDL 100,907 groups. The data are presented as the mean percent basal release ±SEM. Total release over the 3-h period following MDMA administration was calculated from the area under the curve (AUC_{180}) for each animal after subtraction of the basal release. The results were averaged for each group and compared by the two-tailed Student's *t*-test.

Receptor binding. The methods used to determine receptor affinity are all published procedures: 5–HT_{1A}, 8-hydroxy-DPAT (Gozlan et al. 1983); 5–HT_{1C}, [^{3}H]mesulergine (Pazos et al. 1984); 5–HT_{1D}, [^{3}H]5–HT (Waeber et al. 1988); 5–HT_2, [^{3}H]ketanserin (Leysen et al. 1982); α_1, [^{3}H]prazosin (Greengrass and Bremner 1979); α_2, [^{3}H]rauwolscine (Cheung et al. 1985); D_2, [^{3}H]spiroperidol (Creese et al. 1977); muscarinic cholinergic, [^{3}H]L-3-quinuclidenyl benzylate (QNB) (Snyder et al. 1975); H_1, [^{3}H]pyrilamine (Chang et al. 1978); $GABA_A$ receptor complex, GABA site, [^{3}H]muscimol (DeFeudis et al. 1980) and the benzodiazepine site, [^{3}H]flunitrazepam (Braestrup and Squires 1978); Sigma, [^{3}H](+)3–PPP ((+)-3-(3-hydroxyphenyl)-N-1-(propyl)piperidine) (Itzhak 1989); and the strychinine-sensitive glycine receptor, [^{3}H]strychnine (Young and Snyder 1973). K_i values were calculated by the method of Cheng and Prusoff (1973). Protein concentrations were determined by the Bradford dye binding assay (Bradford 1976). All the radioligands for the binding studies were obtained from Dupont NEN (Boston, MA).

Dopamine metabolite measurements in rat brain. The animals were administered MDL 100,907 or MDL 73,147EF and 1 h later sacrificed by decapitation, the brain rapidly removed and briefly chilled in ice-cold 0.32 M sucrose. The neostriata and nucleus accumbens were removed from sections made in a brain block and immediately frozen on dry ice. The tissue samples were stored at −70° C until assay for dopamine, DOPAC (dihydroxyphenylacetic acid), HVA (homovanillic acid), 5–HT (serotonin) and 5–HIAA (5-hydroxyindoleacetic acid) by HPLC with electrochemical detection (Schmidt 1987).

Electrophysiological studies

Extracellular recordings were made from substantia nigra (A9) and ventral tegmental (A10) neurons using techniques that have been previously described (Sorensen et al. 1989, 1992). In the dose-response studies, one dopamine neuron was isolated in the A10 region for each animal in the study and its firing rate recorded. After observing and recording a baseline firing rate for at least 10 min, drugs were administered according to the protocol described below. Firing rate was then recorded for at least 20 min after each drug administration. In expeirments where the number of dopamine neurons was counted, an electrode was passed through six tracks, randomly chosen from nine potential tracks through both the A9 and the A10 brain regions. In each area, the number of spontaneously active dopamine neurons was observed and recorded. For acute experiments in which the number of active dopamine neurons were counted, IP injections of saline (1.0 ml/kg), haloperidol (0.1 or 0.5 mg/kg), clozapine (20 mg/kg), MDL 100,907 (1.0 mg/kg) or MDL 73,14EF (0.5 mg/kg) were administered acutely, 20 min prior to the beginning of the recording session. For chronic experiments animals received daily IP injections of saline (1.0 ml/kg), haloperidol (0.5 mg/kg), clozapine (20 mg/kg), MDL 100,907 (1.0 mg/kg) or MDL 73,147EF (5 mg/kg) for 21 consecutive days. On day 21 of injections for the chronically treated animals, the recording session was begun approximately 60 min after the final injection. Recordings were made from both the A9 and A10 region in each animal. The order of recording was reversed in half of the subjects. The investigator recording from the animal was unaware of the drug treatment schedule.

Firing rates were determined utilizing the inter-spike interval histogram program (Brainwave, Thornton, CO). Data from individual neurons were normalized to basal firing rates so that the averages could be expressed as a percentage of the control firing rate. In the cell count experiments, the average number of cells for each brain region was determined for the five treatment groups. All results were subjected to statistical comparisons using a one-way ANOVA and the Newman-Keuls test (Microstat-II, Ecosoft, Indianapolis IN). Significance was set at $P<0.05$.

Locomoter activity in mice

Opto-Varimax activity monitors (Columbus Instruments, Columbus, Ohio) were used to measure locomoter activity ("distance travelled"). The activity monitors were housed in a closed chamber which was illuminated by white light. Data were analyzed using analysis of variance followed by appropriate post-hoc tests and/or by linear regression.

Two designs were used. (1) To measure the effects of the test compounds alone on spontaneous locomotor activity, mice were injected with the test compound, placed singly in clear Plexiglas boxes (16 × 16 × 8 in), and allowed to acclimatize for 30 min. Thereafter, the boxes were placed in the activity monitors and measurements were taken for 30 min. (2) To measure the effects of various pretreatments on amphetamine-stimulated (2 mg/kg IP) motor activity, mice (four per test chamber) were acclimatized in the Plexiglas boxes for 90 min, injected with the test compounds, and tested for 90 min.

Results

MDL 100,907 was highly selective for the 5–HT_2 receptor with a K_i of 0.36 nM. The separation of activity from the structurally related 5–HT_{1C} receptor was particularly striking (K_i 5–HT_{1C} = 105 nM). MDL 100,907 had very low or negligible affinity for α_1 (K_i = 545 nM), α_2, D_2, H_1, 5–HT_{1A}, 5–HT_{1D}, 5–HT_3, muscarinic, GABA, glycine and benzodiazepine receptors (K_is > 1000 nM).

MDL 73,147EF was highly selective for the 5–HT_3 receptor, with a K_i of 15.8 nM. Of particular importance, the major reduced ketone metabolite of MDL 73,147EF, i.e., MDL 74,156EF, had a K_i of 0.5 nM. In vivo, MDL 73,147EF is rapidly transformed to MDL 74,156 and this potent metabolite has a long half-life consistent with a once-daily dosing regimen. MDL 73,147EF and MDL 74,156EF are essentially devoid of affinity for the other receptors studied.

Acute administration of MDL 100,907 (1–10 mg/kg) or MDL 73,147EF (5 mg/kg) was without effect on steady state tissue concentrations of dopamine, DOPAC or HVA in the striatum and nucleus accumbens. Clozapine (10 mg/kg IP) was similarly without effect (this report and Palfreyman et al. 1991). This contrasts with the substantial (150–200%) increases in DOPAC and HVA seen in these brain regions following haloperidol (1 mg/kg IP).

Chronic administration of haloperidol (1 mg/kg × 21 days) produced the expected increase in the numbers of dopamine D_2 receptors in the striatum from 981 ± 21 to 1400 ± 33 fmol · mg^{-1} protein, whereas MDL 100,907 (1 mg/kg × 21 days), MDL 73,147EF (5 mg/kg × 21 days) and clozapine (10 mg/kg × 21 days) were without effect.

MDMA experiments

The characteristic reduction in cortical 5–HT concentrations seen 1 week after the administration of MDMA (30 mg/kg, SC) is shown in Fig. 1. Simultaneous administration of MDL 100,907 (Fig. 1) dose-dependently reversed the effect of the amphetamine analogue. Significant antagonism of the effect of MDMA was observed at doses of MDL 100,907 as low as 0.03 mg/kg. Schmidt et al. (1989, 1991a, b) have shown that the decreases in serotonin concentrations following MDMA administration are consequent on the release of dopamine. Although the neurotoxic effects of MDMA are blocked by 5–HT_2 antagonists, these antagonistic effects are attenuated by co-administration of L-dopa, suggesting that 5–HT_2 receptor blockade interferes with the availability of newly synthesized dopamine for release.

Microdialysis experiments

The effect of the 5–HT_2 antagonist MDL 100,907 on the MDMA-induced increase in striatal concentrations of extracellular dopamine in the awake, freely moving rat are shown in Fig. 2. Dopamine concentrations began to increase within 40 min of MDMA (20 mg/kg SC) administration, peaking at approximately 1300% of basal values at 100 min and then slowly declining over the remainder of the experiment. Extracellular dopamine was still at 400% of the basal level 4 h after MDMA administration. A somewhat blunted pattern of release was observed when MDL 100,907 (1 mg/kg SC) was administered 20 min prior to MDMA. Although the onset of release was similar to that observed with MDMA alone, the peak response in the presence of MDL 100,907 reached only 600% of control. This level of release then gradually declined such that by the end of the experiment, extracellular dopamine concentrations were similar to that of the MDMA alone group.

The mean AUC_{180} for MDMA-treated animals was 7268 ± 1365. In the presence of MDL 100,907 this value was reduced by 46% to 3940 ± 578 ($P < 0.05$).

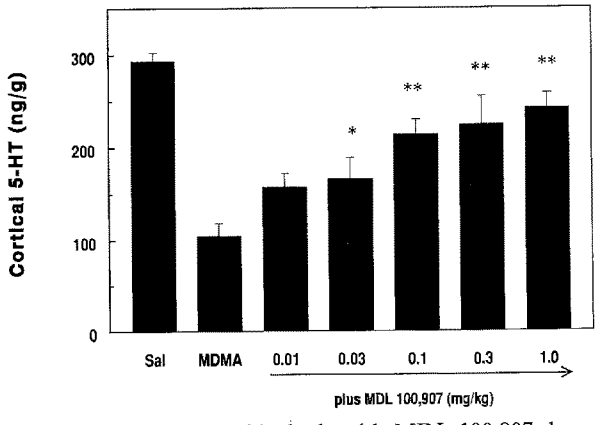

Fig. 1. 5–HT_2 receptor blockade with MDL 100,907 dose-dependently prevents the dopamine-dependent depletion of cortical 5–HT concentrations produced by MDMA. Rats received MDMA (30 mg/kg, SC) and MDL 100,907 (SC) simultaneously 1 week prior to sacrifice. *, ** $P < 0.05$, 0.01 vs MDMA alone by ANOVA with LSD

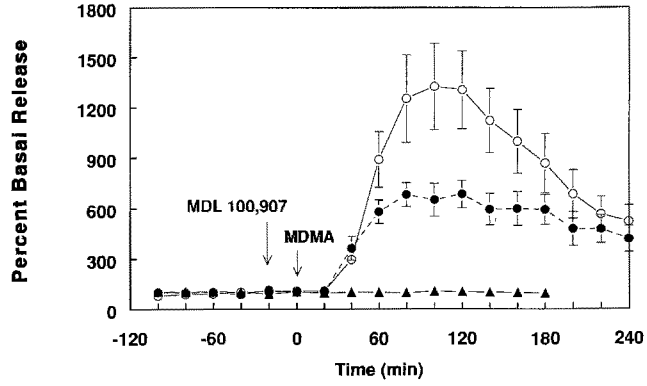

Fig. 2. MDL 100,907 attenuates the MDMA-induced increase in extracellular dopamine concentrations in the rat striatum as assessed by in vivo microdialysis. Both MDL 100,907 and MDMA were given SC at the times and the doses indicated. AUC calculations indicate a significant reduction in MDMA-induced release in the presence of the antagonist. —○— MDMA (20 mg/kg); ---●--- MDMA + MDL 100,907; —▲— MDL 100,907 (1 mg/kg)

Acute electrophysiology of A9 and A10 neurons

On average, the number of active dopamine neurons per electrode track was 0.80 in the A9 region and 0.90 in the A10 region in rats treated acutely with saline (Fig. 3). Acute treatment with haloperidol (0.5 mg/kg IP) caused a marked increase in the number of active neurons per track in the A9 (1.44 cells/track; 180% of control) and a smaller increase in A10 (1.27 cells/track; 141% of control; Fig. 3). Acute treatment with clozapine (20 mg/kg IP) produced smaller changes in A9 (1.11 cells/track; 139% of control) and in A10 (1.08 cells/track; 120% of control; Fig. 3). In animals treated with MDL 100,907 the results were even less than those seen with clozapine, with only small changes in the number of active cells encountered in A9 (1.07 cells/track; 134% of control) and in A10 (0.97 cells/tack; 108% of control; Fig. 3). Acute administration of MDL 73,147EF (0.5 mg/kg) had no significant effect in either brain region (Fig. 4).

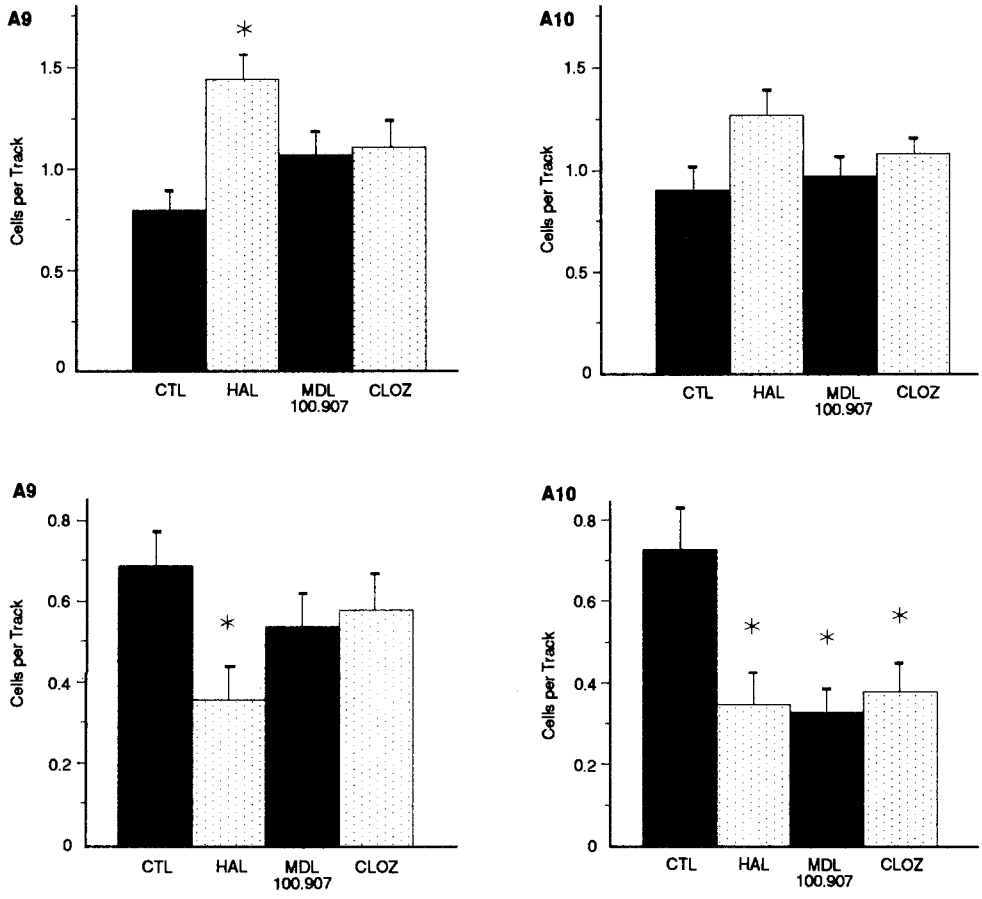

Fig. 3. The effect of haloperidol (0.5 mg/kg IP), MDL 100,907 (1.0 mg/kg IP) or clozapine (20 mg/kg IP) on the number of spontaneously active dopamine neurons. The *upper graphs* represent acute drug treatment; the *lower graphs* represent chronic (21 days) drug treatment. The number of neurons from the A9 brain region is illustrated on the left; the number of neurons from the A10 brain region on the right. The *control bar* represents the number of neurons observed with no drug treatment (saline 1 mg/kg IP). All *bars* illustrate the means plus the standard error for cells seen per track in either the A9 or A10 region. *Asterisks* indicate results significantly different from control ($P<0.05$)

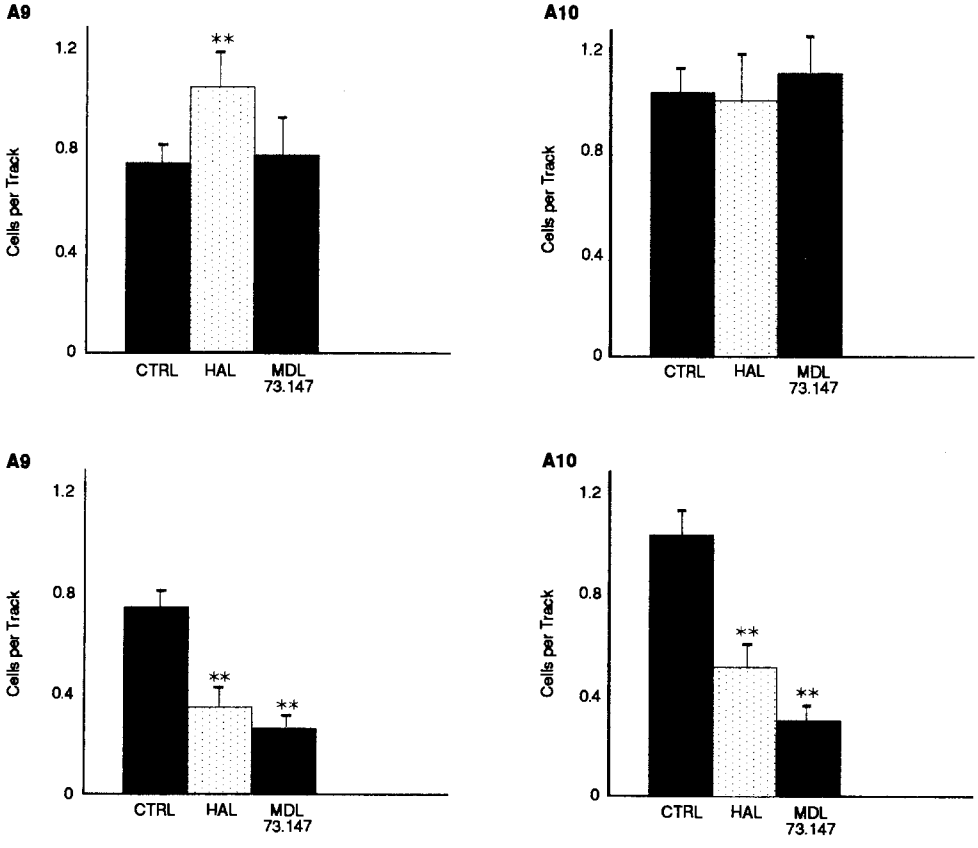

Fig. 4. The effect of haloperidol or MDL 73,147EF on the number of spontaneously active dopamine neurons. The *upper graphs* represent acute drug treatment (either 0.1 mg/kg haloperidol IV or 0.5 mg/kg MDL 73,147EF IV); the *lower graphs* represent chronic (21 days) drug treatment (either 0.5 mg/kg haloperidol IP or 5.0 mg/kg MDL 73,147EF IP). The number of neurons from the A9 brain region are illustrated on the left; the number of neurons from the A10 brain region on the right. The *control bar* represents the number of neurons observed with no drug treatment (saline 1 ml/kg). All *bars* illustrate the means plus the standard error for cells seen per track in either the A9 or A10 region. *Asterisks* indicate results significantly different from control ($P<0.01$)

Table 1. Amfonelic acid test for extra-pyramidal liability

	% Vehicle control values					
	DOPAC			HVA		
	Drug	AFA	Drug+AFA	Drug	AFA	Drug+AFA
Haloperidol	198±24	71±4	382±42	221±43	101± 5	404±30
Clozapine	138± 6	71±4	80± 7	180± 9	101± 5	136±13
MDL 100, 907	95± 4	86±4	74± 5	127±12	107±10	122± 9

Rats were administered amfonelic acid (2.5 mg/kg, SC) or vehicle 5 min prior to haloperidol (1 mg/kg, IP), clozapine (25 mg/kg IP) or MDL 100907 (25 mg/kg, IP) Animals were killed 90 min later and striatum removed. Values are means±SEM expressed as percent of control, $n=4$–6 animals

Chronic electrophysiology of A9 and A10 neurons

Following chronic saline treatment there were 0.69 cells/track in the A9 brain region and 0.73 cells/track in the A10 brain region (Fig. 3). These are not statistically different from the results obtained with acute saline.

Chronic treatment with haloperidol (0.5 mg/kg IP × 21 days) produced a marked, significant decline in the number of cells/track seen in the A9 (0.36 cells/track; 52% of control) and in the A10 (0.35 cells/tack; 48% of control) brain regions. In contrast, chronic treatment with clozapine produced a marked and significant decline only in the A10 region (0.38 cells/track; 52% of control) with no significant change in the A9 region (0.58 cells/track; 84% of control). Chronic treatment with MDL 100,907 produced results similar to those seen with clozapine; a significant decline in the A10 region (0.33 cells/track; 45% of control) and a small, non-significant decline in the A9 region (0.54 cells/track; 78% of control). Chronic treatment with MDL 73,147EF (5 mg/kg) produced a highly significant decrease in the number of active cells per track in the A10 region of the brain (Fig. 4). This effect was substantially larger for MDL 73,147EF than for haloperidol. Both compounds also produced a significant decrease in the number of active DA neurons in the A9 region (Fig. 4).

Test for extrapyramidal liability

A neurochemical model believed to be predictive of extrapyramidal liability is based upon the observation that typical antipsychotics such as haloperidol will synergize with the dopamine uptake inhibitor amfonelic acid to produce massive increases in dopamine turnover. However, antipsychotics with an atypical profile such as clozapine and potentially MDL 100,907 should not show this interaction.

Amfonelic acid had no effect on DOPAC or HVA levels by itself; coadministration of the uptake blocker with haloperidol resulted in a massive increase in dopamine turnover (Table 1). In contrast, the addition of amfonelic acid to clozapine reduces its already minimal effect on the two metabolites. MDL 100,907 appears to have less effect on striatal concentrations of DOPAC and HVA than does clozapine but, like clozapine, produces

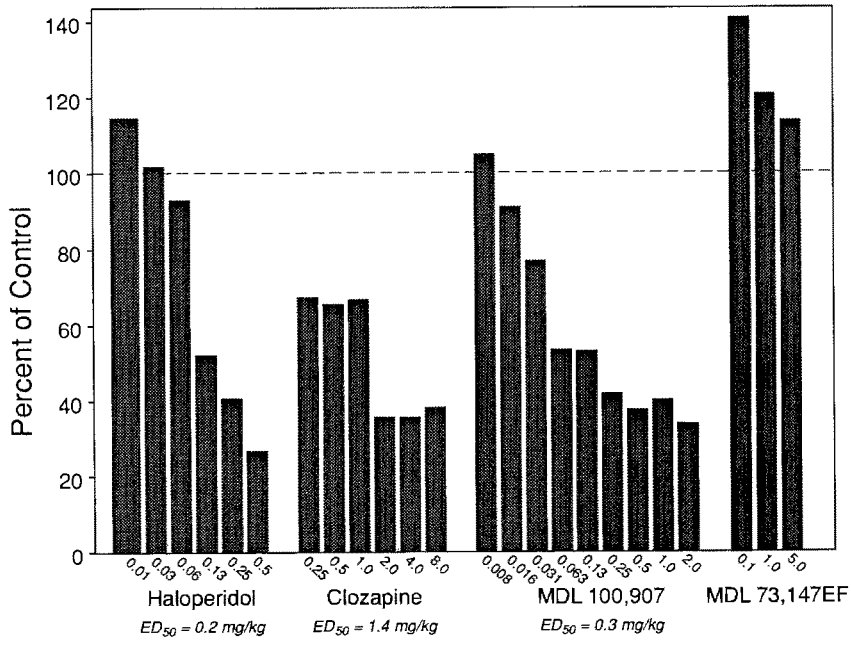

Fig. 5. The effects of various doses (IP, 30 min pretreat) of haloperidal (0.01–0.5 mg/kg), clozapine (0.25–8.0 mg/kg), MDL 100,907 (0.008–2.0 mg/kg), or MDL 73,147EF (0.1–5.0 mg/kg) on the stimulation of locomotor activity produced by 2.0 mg/kg *d*-amphetamine in mice. Each *bar* represents the mean (90 min test session) for the data calculated as percent of amphetamine-treated controls. Doses of antagonists used, and ED_{50}s (where calculable) for suppression of amphetamine-stimulated activity, are listed below the graph

no interaction with the uptake blocker. None of the drug treatments altered dopamine concentrations in either brain region. The most robust changes in dopamine turnover were observed in the striatum but the results in the nucleus accumbens were qualitatively similar.

Locomotor activity in mice

MDL 100,907 given alone did not significantly depress locomotor activity in mice ($ED_{50} > 8$ mg/kg). In contrast, locomotor activity was significantly reduced by haloperidol ($ED_{50} = 0.1$ mg/kg), or clozapine ($ED_{50} = 4.8$ mg/kg). As seen in Fig. 5, *d*-amphetamine (2.0 mg/kg)-stimulated locomotor activity was antagonized by MDL 100,907 ($ED_{50} = 0.3$ mg/kg), haloperidol ($ED_{50} = 0.2$ mg/kg), or cozapine ($ED_{50} = 1.4$ mg/kg). MDL 73,147EF did not produce a decrease in amphetamine-stimulated locomotor activity, consistent with the lack of effect of ondansetron on systemically administered amphetamine (Costal et al. 1979). Lower doses (0.1, 1 mg/kg) exhibited a non-significant trend to increase amphetamine-stimulated activity.

Discussion

Examination of the anatomical connectivity of the serotonergic and dopaminergic systems in the brain indicates several levels at which modulation of dopaminergic function by the serotonergic system could occur. Moreover, the distribution of the serotonin receptor subtypes in different dopamine-rich brain regions further implicates specific receptor mediated functional control. 5–HT_2 receptors are found in moderate density in the striatum and nucleus accumbens and in high density in the limbic cortical areas, suggesting that axo-axonic modulation can occur (Pazos et al. 1985). 5–HT_2 receptors also occur in both the A_9 and A_{10} dopamine cell body areas, albeit in low density, suggesting the possibility of axo-dendritic and axo-somatic modulation of dopaminergic function.

The density of 5–HT_3 receptors in dopamine-rich regions of the brain is also low (Kilpatrick et al. 1987). However, measurable binding of the high affinity ligand [^{3}H]GR65630 does indicate that 5–HT_3 receptors are present in the nucleus accumbens, striatum and entorhinal cortex.

From a large number of biochemical, electrophysiological and behavioral experiments it is apparent that antagonists of 5–HT_2 or 5–HT_3 receptors in the CNS are singularly devoid of obvious effects on the basal activity of the nigro-striatal or mesolimbic/cortical dopaminergic pathways, at least following acute treatment. The situation is clearly different in the case of chronic treatment with the selective 5–HT antagonists. Their impact on the tuberoinfundibular dopamine system has not been extensively explored. Thus, the potent and selective 5–HT_2 antagonist MDL 100,907 does not alter dopamine, DOPAC or HVA levels; does not alter the basal release of dopamine in vivo as measured by microdialysis; does not alter the firing rate of A_9 or A_{10} dopamine cells; and at selective 5–HT_2 inhibiting doses is devoid of acute behavioral effects suggestive of blockade (or activation) of striatal or mesolimbic dopaminergic systems. In marked contrast, MDL 100,907 clearly attenuates the activation of the dopaminergic system produced by amphetamine or 3,4-methylene dioxymethamphetamine (MDMA), measured biochemically and behaviorally. When MDL 100,907 was given once daily for 21 days, the number of active dopamine cells counted in the substantia nigra (A_9 area) was not decreased. This contrasts with the significant decrease in A_9 dopamine cell firing seen following chronic administration of haloperidol but is similar to the lack of effect of chronic clozapine administration in this same brain region. Chronic administration of MDL 100,907, like clozapine and haloperidol, produced a significant attenuation of firing in the ventral tegmental area (A_{10} area) of the ascending dopaminergic limbic system. The basis of this regional difference remains to be explored.

Evidence which also supports MDL 100,907 having atypical antipsychotic activity is the lack of synergism with the DA uptake inhibitor amfonelic acid. Neither MDL 100,907 nor clozapine increased DOPAC or HVA levels when co-administered with amfonelic acid. Haloperidol in combination with amfonelic acid significantly increased DOPAC and HVA levels in the striatum and nucleus accumbens above levels seen with haloperidol alone.

Antagonism of 5–HT_3 receptors by the potent and very selective compound MDL 73,147EF produced a spectrum of dopamine-related biochemical, behavioral and electrophysiological effects that had some similarities but several differences from those seen following blockade of 5–HT_2 receptors. Acute administration of MDL 73,147EF was without effect on basal activity of dopaminergic systems as determined by measurement of dopamine, DOPAC and HVA levels in the striatum or nucleus accumbens; was without acute behavioral effect consistent with activation or inhibition of the dopamine systems of the nigro-striatal or mesolimbic systems; and was without effect on the firing rate of A_9 or A_{10} neurons.

Chronic administration of MDL 73,147EF suggested modulation of both the nigro-striatal and mesolimbic dopamine systems. The interaction at the biochemical level was complex. There were minimal effects on steady state dopamine, DOPAC or HVA concentrations or on the number of dopamine receptors. However, rats treated with MDL 73,147EF for 21 days and then challenged on day 22 with a low dose of haloperidol demonstrated increases in DOPAC and HVA concentrations in the striatum and nucleus accumbens that were greater than those seen in rats treated chronically with saline. A similar response was seen in rats treated chronically with clozapine. In contrast, chronic administration of haloperidol significantly attenuated the DOPAC and HVA elevations provoked by a challenge dose of haloperidol on day 22. These changes were seen in both the striatum and nucleus accumbens (Palfreyman et al. 1991). Chronic administration of MDL 73,147EF produced a decrease in the number of active dopamine

cells in both the A_9 and A_{10} dopamine cell body regions (Sorensen et al. 1989). However, preliminary studies using apomorphine indicate this decrease in firing is not due to depolarization-induced blockade. This contrasts with the situation following chronic haloperidol administration where a similar decrease in A_9 and A_{10} dopamine cell firing can be reversed by apomorphine, consistent with depolarization-induced blockade.

From a number of studies conducted with highly selective 5–HT_2 and 5–HT_3 antagonists it is apparent that these compounds have little influence on resting or basal dopamine activity following acute administration (this report and Goldstein et al. 1989; Ashby et al. 1991, 1992). However, if the dopamine system is activated, then the role of serotonergic control becomes more apparent. From biochemical, behavioral and electrophysiological studies it is clear that activation of the dopamine system by amphetamine or substituted amphetamines such as MDMA can be attenuated by 5–HT_2 antagonists. This attenuation may involve control of the rate-limiting step of dopamine biosynthesis, tyrosine hydroxylase, as the response to 5–HT_2 antagonists can be overcome by administration of L-dopa (Schmidt et al. 1991a, 1992b; Sorensen et al. 1992). Blockade of 5–HT_3 receptors, on the other hand, produces more subtle modulation of activated dopamine systems. If amphetamine is administered systemically, 5–HT_3 receptor blockade is without a statistically significant effect (this report and Costall et al. 1987). However, Costall et al. (1987) have demonstrated that the hypelocomotor effects of intra-accumbens injections of amphetamine (and dopamine) can be attenuated by low doses of the selective 5–HT_3 antagonist ondansetron given systemically or directly into the accumbens. Many of the attenuating effects of certain 5–HT_3 antagonists on activated dopamine systems, including those of ondansetron described by Costall et al. (1987), are marked by bell-shaped dose-response curves. This is particularly apparent with granisetron and LY277359 in electrophysiological experiments measuring A_9 and A_{10} dopamine cell firing (Ashby et al. 1991, 1992; see also Palfreyman et al. 1992). In behavioral experiments MDL 73,147EF appears to have a monophasic dose-response curve (Moser, unpublished data).

Chronic administration of the highly selective 5–HT_2 antagonist, MDL 100,907, produces electrophysiological changes which are very similar to those produced by clozapine, i.e., a decrease in firing of mesolimbic A_{10} dopamine neurons with no effect on A_9 cell firing. This differs from the non-selective depression of firing produced by classic neuroleptics such as haloperidol. Clozapine is a potent 5–HT_2 antagonist and Meltzer and coworkers (Meltzer 1989; Meltzer et al. 1989) have argued cogently for an important functional role for 5–HT_2 antagonism in the unique spectrum of clinical benefits seen with clozapine. Because MDL 100,907 produces a similar profile to clozapine, it too may have unique antipsychotic activity.

The situation with the 5–HT_3 antagonists is somewhat more complex. In studies conducted by Sorensen et al. (1989), the very selective 5–HT_3 antagonist MDL 73,147EF decreased the firing rate of both A_9 and A_{10} neurons, albeit with greater effect on A_{10} neurons. Subsequent studies by Ashby et al. with BRL 43694 (Ashby et al. 1990, 1991, 1992) have demonstrated that preferential decreases in A_{10} dopamine firing can occur following chronic administration of these potent and selective 5–HT_3 antagonists. As noted previously, marked bell-shaped dose-response curves were obtained. These data would suggest that 5–HT_3 antagonists have some potential in treating schizophrenia, although dose-response issues with certain compounds may limit their usefulness. In this regard, it is important to note that clozapine has a significant affinity for the 5–HT_3 receptor (47 nM, Palfreyman et al. 1992) and some speculation has been forwarded on this action contributing to the unique profile of this novel antipsychotic (Palfreyman et al. 1991, 1992).

A large number of studies have demonstrated that 5–HT_3 antagonists attenuate the activation of the mesolimbic dopamine pathway produced by drugs of abuse such as nicotine, alcohol and morphine (see Palfreyman et al. 1991 for a review). This dopamine attenuating effect may have therapeutic relevance.

In summary, serotonergic pathways clearly modulate dopaminergic neurons and this modulation is particularly evident when dopamine neurons are activated or when serotonergic pathways are blocked chronically. In this context, both 5–HT_2 and 5–HT_3 receptors appear to play a permissive role, allowing the full expression of dopaminergic activation. If excessive activation of the dopaminergic system plays a role in any pathological processes, the selective antagonists of 5–HT_2 and 5–HT_3 receptors may have an important place in the therapeutic armamentarium.

Acknowledgements. The authors would like to thank Teresa Humphries, Bert Ketteler, Vicki Ecker, Debbie McCarty, Mary Bickers and Margaret Racke for their valuable technical contributions and Debbie Wagner for her help in the preparation of the manuscript.

References

Bradford MM (1976) A rapid and sensitive model for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Braestrup C, Squires RF (1978) Pharmacological characterization of benzodiazepine receptors in the brain. Eur J Pharmacol 48:263–270

Carr AA, Hay DA, Dudley MW, Kehne JH, Nieduzak TR (1990) Derivatives of MDL 11,939 as highly potent and selective inhibitors of serotonin 5–HT_2 receptors. Presented at the Second IUPHAR Serotonin Satellite Meeting, Basel, Switzerland, July 11–13

Chang RSL, Tran VT, Snyder SH (1978) Histamine H_1 receptors in brain labelled with [^{3}H]mepyranine. Eur J Pharmacol 48:463–464

Cheng YC, Prusoff WH (1973) Relationship between inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (I_{50}) of an enzyme reaction. Biochem Pharmacol 22:3099–3108

Cheung Y, Bennett DB, Nahorski SR (1985) [^{3}H]Rauwolscine and [^{3}H]yohimbine to rat cerebral and human platelet membranes: possible heterogeneity of α_2-adrenoceptors. Eur J Pharmacol 84:79–85

Creese I, Schneider R, Snyder SH (1977) [^{3}H]-Spiroperidol labels dopamine receptors in the pituitary and brain. Eur J Pharmacol 46:377–381

DeFeudis FS, Ossola L, Schmitt G, Wolff P, Mandel P (1980) Na^+-Independent binding of [^{3}H]GABA and [^{3}H]muscimol to subcellular particles of neuronal primary cultures and whole brains. J Neurochem 34:216–218

Dray A, Gonye TJ, Oakley NR, Tanner T (1976) Evidence for the existence of a raphe projection to the substantia nigra in rat. Brain Res 113:45–57

Giambalvo CT, Snodgrass SR (1978) Biochemical and behavioral effects of serotonin neurotoxins on the nigrostriatal dopamine system: comparison of injection sites. Brain Res 152:555–566

Goldstein JM, Litwin LC, Sutton EB, Malick JB (1989) Effects of ICI169,369, a selective serotonin antagonist, in electrophysiological tests predictive of antipsychotic activity. J Pharmacol Ther 249:673–680

Gozlan H, El Mestikawy S, Pichat L, Glowinski J, Hamon M (1983) Identification of presynaptic serotonin autoreceptors using a new ligand: ^{3}H–PAT. Nature 305:140–142

Greengrass P, Bremner R (1979) Binding characteristics of [^{3}H]prazosin to rat brain α-adrenergic receptors. Eur J Pharmacol 55:322–326

Herve DJ, Pickal UM, Joh TH, Beaudet A (1987) Serotonin axon terminals in the ventral tagmental area of the rat: same structure and synoptic input to dopaminergic neurons. Brain Res 435:71–83

Itzhak Y (1989) Multiple affinity binding slotes of the sigma receptor: effect of GTP-binding protein modifying agents. Mol Pharmacol 36:512–517

Kilpatrick GJ, Jones BJ, Tyers MB (1987) Identification and distribution of 5–HT_3 receptors in rat brain using radioligand binding. Nature 330:746–748

Leysen JE, Niemegeurs CJR, van Heuten JM, Laduron PM (1982) [^{3}H]Ketanserin (R41468), a selective ^{3}H-ligand for serotonin$_2$ receptor binding sites. Mol Pharmacol 21:310–314

Meltzer HY (1989) Clinical studies on the mechanism of action of clozapine: the dopamine-serotonin hypothesis of schizophrenia. Psychopharmacology 99:S18–S27

Meltzer HY, Matsubara S, Lee JC (1989) Classification of typical and atypical antipsychotic drugs on the basis of dopamine D–1, D–2 and serotonin2 pK_i values. J Pharmacol Exp Ther 251:238–246

Minabe Y, Ashby CR, Wang RY (1991) The effect of acute and chronic LY 277359, a selective 5–HT_3 receptor antagonist, on the number of spontaneously active midbrain dopamine neurons. Eur J Pharmacol 209:151–156

Minabe Y, Ashby CR, Wang RY (1992) Effects produced by acute and chronic treatment with granisetron alone or in combination with haloperidol on mid brain dopamine neurons. Eur Neuropsychopharmacol (in press)

Palfreyman MG, Sorensen SM, Carr AA, Cheng HC, Dudley MW (1991) 5–HT_3 receptor antagonists and their potential in psychiatric disorders. In: Sandler M, Coppen A, Harnett S (eds) 5-Hydroxytryptamine in psychiatry: a spectrum of ideas. Oxford University Press, Oxford, pp 324–330

Palfreyman MG, Sorensen SM, Baron BM, Humphreys TM, Moser P, Dudley MW (1992) Antipsychotic potential of 5–HT_3 antagonists. In: Meltzer HY (ed) Novel antipsychotic drugs. Raven Press, New York, pp 211–223

Pazos A, Hoyer D, Palacios JM (1984) The binding of serotonergic ligands to the porcine choroid plexus: characterization of a new type of serotonin recognition site. Eur J Pharmacol 106:546–589

Pazos A, Cortes R, Palacios J (1985) Autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. Brain Res 346:231–249

Schmidt CJ (1987) Neurotoxicity of the psychedelic amphetamine, methylenedioxy-methamphetamine. J Pharmacol Exp Ther 240:1–7

Schmidt CJ, Black CK, Taylor VL (1990b) Antagonism of the neurotoxicity due to a single administration of methylenedioxy/methamphetamine. Eur J Pharmacol 181:59–70

Schmidt CJ, Taylor VL, Abbate GM, Nieduzak TR (1991a) 5–HT_2 antagonists stereoselectively prevent the neurotoxicity of 3,4-methylenedioxy-methamphetamine by blocking the acute stimulation of dopamine synthesis: reversal by L-dopa. J Pharmacol Exp Ther 256:230–235

Schmidt CJ, Black CK, Taylor VL (1991b) L-Dopa-potentiation of the serotonergic deficits due to a single administration of 3,4 methylene dioxymethamphetamine, *p*-chloroamphetamine or methamphetamine to rats. Eur J Pharmacol 203:41–49

Schmidt CJ, Fadayel GM, Black CK, Taylor VL (1992a) 5–HT_2 receptors exert a state-dependent regulation of dopaminergic function: studies with MDL 100,907 and the amphetamine analogue, 3,4-methylenedioxymethamphetamine.

Schmidt CJ, Black CK, Taylor VL, Fadayel GM, Humphreys TM, Nieduzak TR, Sorensen SM (1992b) The 5–HT_2 antagonist, MDL 28,133A disrupts the serotonergic-dopaminergic interaction mediating the long-term neurochemical effects of 3,4-methylenedioxymethamphetamine.

Snyder SH, Chang KJ, Keehar MJ, Yamamura HI (1975) Biochemical identification of the mammalian muscarinic cholinergic receptor. Fed Proc 34:1915–1921

Sorensen SM, Humphreys TM, Palfreyman MG (1989) Effect of acute and chronic MDL 73,147EF, a $5HT_3$ antagonist, on A9 and A10 dopamine neurons. Eur J Pharmacol 163:115–118

Sorensen SM, Humphreys TM, Taylor VL, Schmidt CJ (1992) 5–HT_2 antagonists reverse amphetamine-induced slowing of dopaminergic neurons by interfering with stimulated dopamine synthesis. J Pharmacol Exp Ther 260:872–878

Waeber C, Schoeffler P, Palacios JM, Hoyer D (1988) Molecular pharmacology of 5–HT_{1D} recognition sites: radioligand binding studies in human, pig and calf brain membranes Naunyn-Schmiedeberg's Arch Pharmacol 337:595–601

Young AB, Snyder SH (1973) Strychnine binding associated with glycine receptors of the central nervous system. Proc Natl Acad Sci USA 70:2832–2836