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5-HT_{1A} receptor agonist properties of the antipsychotic, nemonapride: comparison with bromerguride and clozapine

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Abstract

5-HT_{1A} receptor agonists are thought to enhance the antipsychotic-like effects of dopamine D_2 receptor antagonists while reducing their potential to produce extrapyramidal side effects. Thus, 5-HT_{1A} receptor agonist properties of mixed 5-HT_{1A} receptor agonists/D_2 receptor antagonists might be of clinical importance. The antipsychotics, clozapine and nemonapride, and the putative antipsychotic, bromerguride, have intermediate to high affinity for 5-HT_{1A} receptors. The present study examined the 5-HT_{1A} receptor agonist activity of nemonapride and bromerguride, in comparison with clozapine, which has partial 5-HT_{1A} receptor agonist properties in vitro. Here, 5-HT_{1A} receptor activation was examined in vitro, by measuring forskolin-stimulated cAMP accumulation in HeLa cells expressing human 5-HT_{1A} receptors, and in vivo, by using microdialysis to measure the extracellular concentration of hippocampal 5-hydroxytryptamine (5-HT) in rats. Nemonapride markedly decreased both forskolin-stimulated cAMP accumulation and the extracellular concentration of 5-HT; both effects were antagonized by the 5-HT_{1A} receptor antagonist, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide (WAY100635). In contrast, clozapine only partially decreased forskolin-stimulated cAMP accumulation and extracellular 5-HT, and only its effects on cAMP accumulation were attenuated by WAY100635. Bromerguride decreased neither forskolin-stimulated cAMP accumulation nor extracellular 5-HT; instead, it antagonized the decrease of cAMP accumulation produced by 5-HT and the decrease of extracellular 5-HT produced by the 5-HT_{1A} agonist (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT). The selective D_2 receptor antagonist, raclopride, affected neither forskolin-stimulated cAMP in vitro nor extracellular 5-HT in vivo. Thus, in contrast with clozapine and bromerguride, only the novel antipsychotic, nemonapride, exhibited marked 5-HT_{1A} receptor agonist properties both in vitro and in vivo; conceivably, these properties may play a role in its preclinical and clinical effects.

Keywords: 5-HT_{1A} receptor; Antipsychotic; cAMP accumulation; HA7 cells; 5-HT (5-hydroxytryptamine, serotonin), extracellular concentration; Microdialysis

1. Introduction

The clinical efficacy of conventional neuroleptic drugs is thought to involve their ability to block dopamine D_2 receptors. Such blockade, however, can also induce extrapyramidal side effects. Some antipsychotic compounds appear to have, if any, only a low propensity to cause extrapyramidal side effects. To account for the reduced extrapyramidal side effects liability of these so-called atypical antipsychotics, a variety of mechanisms has been proposed, involving, for example, 5-HT_2 receptors (e.g. Meltzer et al., 1989), D_3 receptors (e.g. Sokoloff et al., 1990), or D_4 receptors (e.g. Seeman et al., 1997). In addition, agonist activity at 5-HT_{1A} receptors might be involved in the clinical profile of certain atypical antipsychotics. This is because 5-HT_{1A} receptor agonists attenuate neuroleptic-induced catalepsy in rodents, a measure of extrapyramidal side effects liability (for review, see Kapur and Remington, 1996 and Wadenberg, 1996), and enhance some of the antipsychotic-like effects of neuroleptics (e.g. Wadenberg and Ahlenius, 1991; Prinssen et al., 1996). Compatible with these findings, the atypical antipsychotic compound, clozapine, which has less extrapyramidal side effects liability than conventional neuroleptics (see Kulkarni and Verma, 1993), has intermediate affinity for 5-HT_{1A} receptors in rat and human brain tissue (pK_i: 6.3 and 6.5, respectively; Assié et al., 1993; Mason and Reynolds, 1992), and exerts partial agonist activity in vitro at cloned, human 5-HT_{1A} receptors (Newman-Tancredi et al., 1996). Thus, it is conceivable that 5-HT_{1A} receptor agonist prop-

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erties play a role in the clinical effects of clozapine, and, perhaps, of other antipsychotics.

Other antipsychotics have been identified that, like clozapine, have intermediate, or even high affinity, for 5-HT_{1A} receptors. Because of the potential role of 5-HT_{1A} receptor activation in their clinical profile, it is of interest to investigate whether these antipsychotics are able to activate 5-HT_{1A} receptors, and to study this not only in vitro, as was done for clozapine (Newman-Tancredi et al., 1996), but also in vivo. Here, we examined the ability of nemonapride and bromerguride to exert 5-HT_{1A} agonist activity, in comparison with that of clozapine. Nemonapride (YM-09151-2; (±)-*cis*-*N*-(1-benzyl-2-methylpyrrolidin-3-yl)-5-chloro-2-methoxy-4-methylaminobenzamide) is a novel antipsychotic that has been reported to be effective in the treatment of both positive and negative symptoms of schizophrenia, without producing severe extrapyramidal side effects (Tanaka, 1992; Satoh et al., 1997). In addition to its potent dopamine receptor antagonist properties (Usuda et al., 1981), nemonapride has high affinity for 5-HT_{1A} receptors (pK_i values in rat brain tissue: 8.1 and 8.3; Terai et al., 1989; Assié et al., 1993), but it is not known whether it has 5-HT_{1A} receptor agonist or antagonist properties. The putative antipsychotic, bromerguride (2-bromolisuride; 3-(2-bromo-9,10-didehydro-6-methylergolin-8α-yl)-1,1-diethylurea) (Wachtel and Sauer, 1988), a potent dopamine receptor antagonist (Wachtel et al., 1983), has been used for quantitative in vivo imaging of dopamine D_2 receptors (Mazière et al., 1986). Bromerguride has been claimed to bind to 5-HT_{1A} receptors (Löschmann et al., 1992; pK_i value 8.1, unpublished observations from our laboratory) and to have 5-HT agonist properties (Fink et al., 1991).

The present study examined possible 5-HT_{1A} agonist properties of nemonapride, bromerguride and clozapine in vitro and in vivo. HeLa cells expressing high levels (500 fmol/mg protein) of cloned human 5-HT_{1A} receptors (HA7 cells; Fargin et al., 1989) provide a specific and sensitive in vitro system to examine 5-HT_{1A} receptor agonist properties. Indeed, stimulation of cloned human 5-HT_{1A} receptors in HA7 cells, which, like 5-HT_{1A} receptors in rat hippocampus, are negatively coupled to adenylate cyclase, produced 80–90% inhibition of forskolin-stimulated cAMP (Fargin et al., 1989; Pauwels et al., 1993), whereas in rat hippocampal homogenates, 5-HT_{1A} receptor stimulation produced at most 30% inhibition (Liau et al., 1991). Intracerebral microdialysis was used to identify 5-HT_{1A} receptor agonist properties in vivo. 5-HT_{1A} receptor agonists decrease the extracellular concentration of 5-HT, measured by microdialysis, in various brain regions (Sharp and Hjorth, 1992; Kreiss and Lucki, 1994; Bosker et al., 1996) through activation of somatodendritic receptors located in the raphe nuclei. These effects involve 5-HT_{1A} receptors, because they are reversed by the selective 5-HT_{1A} receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexane carboxamide (WAY100635) (Fletcher et al., 1996; Gurling et al., 1994). Further, the extracellular concentration of 5-HT does not appear to be modified by dopamine D_2 receptor antagonists (Sharp et al., 1989; Ferré and Artigas, 1995). Thus, its measurement may allow one to examine in vivo 5-HT_{1A} receptor agonist properties of mixed 5-HT_{1A} receptor agonists/D_2 receptor antagonists unconfounded by their D_2 receptor antagonist effects. To examine further this lack of sensitivity, the effects of the selective D_2 receptor antagonist, raclopride, which has at best weak affinity for 5-HT_{1A} receptors (pK_i: 5.2; Assié et al., 1993), were also studied.

The results confirm that clozapine has 5-HT_{1A} agonist properties in vitro, but failed to provide evidence that clozapine exerts 5-HT_{1A} agonist effects in vivo. Thus, the low propensity of clozapine to produce extrapyramidal side effects does perhaps not involve its interactions with 5-HT_{1A} receptors. This, however, does not rule out a possible role for 5-HT_{1A} receptor activation in the clinical profile of other antipsychotics. Indeed, based on the present finding that the antipsychotic, nemonapride, has marked 5-HT_{1A} agonist properties in vitro and in vivo, it is conceivable that these properties are involved in its preclinical and clinical effects.

2. Materials and methods

2.1. Measurement of cyclic AMP accumulation

2.1.1. Cell culture

The HeLa cell line permanently transfected with the human 5-HT_{1A} receptor gene and permanently expressing the 5-HT_{1A} receptor protein, 500 fmol/mg protein, (HA7) as described by Fargin et al. (1989) was commercially obtained from Duke University, Durham, NC, USA. HA7 cells were grown in Dulbecco's modified Eagle's medium (DMEM) (GIBCO) supplemented with 10% foetal calf serum, gentamicin (100 μg/ml), geneticin (G418) (400 μg/ml) in 5% CO_2 at 37°C in a water-saturated atmosphere. The cells were plated in 6-well culture plates and used for experimentation at a confluency of 80–90%. Culturing medium (DMEM, 10% foetal calf serum, gentamicin 100 μg/ml, G418 400 μl/ml) was replaced by DMEM supplemented with 10% foetal calf serum without antibiotics 24 h before experimentation.

2.1.2. cAMP measurement

Cells were preincubated with DMEM, 10 mM Hepes for 10 min at room temperature. Drugs, at concentrations varying from 0.1 nM to 100 μM, were then added in DMEM, 10 mM Hepes, 100 μM forskolin, and 100 μM 3-isobutyl-1-methylxanthine (IBMX) to the cells. Antagonists were added at the same time as the agonists, as described by Fargin et al. (1989). At the end of the treatment (10 min, room temperature), the reaction was

stopped by aspiration of the medium and addition of 0.1 N HCl. Cellular extract was diluted 1:500 or 1:400 in radioimmunoassay buffer, and cAMP content was measured by radioimmunoassay using a commercially available kit (Dupont NEN: NEK-033). Basal cAMP levels were 10 ± 0.9 pmol/well ($n = 8$).

2.2. Microdialysis

2.2.1. Animals

Male Sprague–Dawley rats [Ico: OFA SD (I.O.P.S. Caw); Iffa Credo, France], weighing 260–340 g, were group-housed (three rats per cage) in the animal-keeping facilities, under controlled conditions (12 h/12 h light/dark cycle: lights on 07.00 a.m.; ambient temperature 21 ± 1°C; humidity 55 ± 5%), with rat food (AO4, UAR, France) and filtered (0.2 μm) tap water available ad libitum. At least 5 days were allowed for adaptation before the rats were used in the experiments. The experimental procedures were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the National Institutes of Health guide for the care and use of Laboratory animals (NIH publication 85-23, revised 1985), and were approved by the Institutional Protocol Review Committee (Protocol 069).

2.2.2. Microdialysis procedure

The method used in the present experiments has been described previously (Assié and Koek, 1996a). Briefly, a probe (CMA/12, 2 mm length, 0.5 mm diameter, CMA, Microdialysis AB) was implanted into the left hippocampus (stereotaxic coordinates: rostral -4.8 mm, lateral $+4.6$ mm, ventral -7.5 mm, from bregma and dura surface according to Paxinos and Watson (1986)) of anaesthetized rats (chloral hydrate, 400–500 mg/kg i.p. and supplementary doses to maintain anaesthesia). The probe was continuously perfused (1.1 μl/min) with artificial cerebrospinal fluid containing 1 μM of the selective 5-HT uptake inhibitor citalopram. Starting approximately 2 h after implantation, perfusates were collected every 20 min and directly analyzed for 5-HT content using high performance liquid chromatography with electrochemical detection (DECADE detector, ANTEC Leiden BV).

Four baseline control samples were collected before administration of the drugs. When two drugs were tested, the second was injected 40 min after the first. Samples were collected for 140 min after administration of the last drug.

At the end of the experiment, the animal was killed by decapitation and the brain was removed, frozen and cut in a cryomicrotome (Jung Frigocut 2800) to verify the placement of the probe.

2.3. Chemicals

5-HT creatinine sulphate and IBMX were purchased from Sigma (Saint Quentin Fallavier, France), chloral hydrate from Acros (Geel, Belgium), clozapine, forskolin and 8-OH-DPAT from RBI (Bioblock, Illkirch, France). Bromerguride was kindly donated by Schering (Berlin, Germany), raclopride tartrate by Astra (Sodertälje, Sweden), and citalopram by Lundbeck (Copenhagen, Denmark). WAY100635 dihydrochloride and nemonapride (YM-09151-2) were provided by the chemistry department of the Centre de Recherche Pierre Fabre (Castres, France). For in vitro experiments, 5-HT and WAY100635 were dissolved in distilled water; all other compounds were dissolved in dimethyl sulfoxide. For in vivo experiments, the doses of compounds were expressed as the base, and the volume of the injection was 10 ml/kg. The compounds were injected s.c., except clozapine and 8-OH-DPAT, which were injected i.p.. Raclopride and 8-OH-DPAT were dissolved in distilled water; all other compounds were dissolved with 10–20 μl lactic acid in distilled water and the pH was adjusted to 4.5–5 by adding NaOH, 1 M.

2.4. Analysis of data

2.4.1. cAMP

Dose–effect relationships were expressed as $-\log$ [M] of the test compound versus the cAMP content expressed as a percentage of forskolin-induced stimulation of cAMP accumulation (forskolin-induced stimulation = 100%). IC_{50} values and maximal effects for compounds were estimated by nonlinear regression using the sigmoidal dose–response model of the GraphPad Prism program (GraphPad Software, San Diego, CA) and were expressed as mean $\pm$ S.E.M. of 4 or 5 determinations.

2.4.2. Microdialysis

The perfusate levels of 5-HT were expressed as percent of the mean amount of 5-HT collected in the four pre-injection control samples (baseline). The percent area under the curve (AUC) for the 140 min period after administration of the compounds was used as a measure of drug effects. Treatment effects on percent AUC values were analyzed by one-way ANOVA followed by Newman–Keuls test. ED_{50} values were estimated by linear interpolation (vehicle control as 0% and maximal effect of the compound as 100%). The antagonist effects were analyzed by Student's *t*-test.

3. Results

3.1. cAMP accumulation

Nemonapride and clozapine, but not bromerguride, inhibited forskolin-stimulated cAMP accumulation (Fig. 1, left panels). Nemonapride, like 5-HT and the 5-HT_{1A} agonist 8-OH-DPAT, inhibited cAMP accumulation ($IC_{50} \pm$ S.E.M.: 159 ± 34 nM, 34 ± 10 nM, 35 ± 13 nM, re-

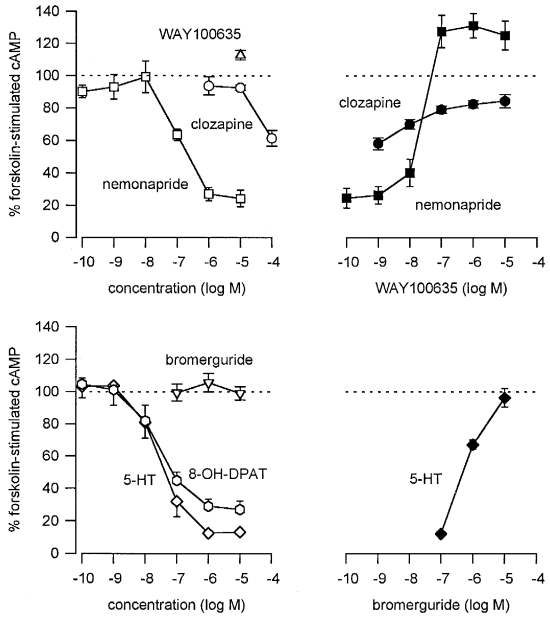

Fig. 1. Effects of the antipsychotics, nemonapride and clozapine, and the putative antipsychotic, bromerguride, on forskolin-stimulated cAMP accumulation in HA7 cells in comparison with those of 5-HT, of the 5-HT_{1A} receptor agonist 8-OH-DPAT, and of the 5-HT_{1A} receptor antagonist, WAY100635. Values are mean ± S.E.M. from 3–5 independent experiments, each performed in triplicate. Left panels: effects of the compounds alone; right panels: antagonism by WAY100635 of 10 μM nemonapride or 100 μM clozapine, and by bromerguride of 1 μM 5-HT.

spectively) and its effects reached a maximum of 20 ± 6% compared to 10 ± 0.4% for 5-HT and 26 ± 4% for 8-OH-DPAT. In contrast, clozapine decreased cAMP accumulation to only 61 ± 5% at the highest concentration tested (100 μM). Like bromerguride and WAY100635, raclopride was ineffective (104 ± 5% at 10 μM; not shown).

WAY100635 antagonized the maximal inhibition produced by 10 μM nemonapride (to 134 ± 9%; IC_{50}: 25 ± 2 nM) and 100 μM clozapine (to 84 ± 4%) (Fig. 1, upper right panel). The inhibition produced by 1 μM 5-HT (i.e. 10%) was antagonized by bromerguride (Fig. 1, lower right panel), but not by 100 μM clozapine (data not shown).

3.2. Microdialysis results

The mean baseline extracellular concentration of 5-HT in the rat ventral hippocampus was 32 ± 1 fmol/20 μl ($n = 100$) in the presence of the uptake inhibitor citalopram (1 μM).

Nemonapride, clozapine and bromerguride significantly affected the extracellular concentration of 5-HT, but did so in a different manner (Fig. 2, left panels). Nemonapride, like the 5-HT_{1A} receptor agonist 8-OH-DPAT, decreased dose-dependently the concentration of 5-HT (ED_{50}: 0.62 mg/kg). Clozapine also decreased the concentration of 5-HT but only at an intermediate dose. Bromerguride, in contrast to nemonapride and clozapine, and unlike the 5-HT_{1A} receptor antagonist WAY100635, significantly increased the concentration of 5-HT. Note that the selective dopamine D_2 receptor antagonist, raclopride, did not significantly affect extracellular 5-HT (mean AUC ± S.E.M.: 96.2 ± 3.5 after 2.5 mg/kg raclopride, and 84.0 ± 4.8 after vehicle control; not shown).

Interaction experiments (Fig. 2, right panels), showed that WAY100635 (0.16 mg/kg), prevented the effects of 2.5 mg/kg nemonapride, but not those of 10 mg/kg clozapine, on extracellular 5-HT. At a dose that did not significantly affect the concentration of 5-HT when given alone (i.e. 0.63 mg/kg), bromerguride appeared to antagonize the effects of 0.31 mg/kg 8-OH-DPAT; this effect of bromerguride, however, just fell short of statistical significance ($P = 0.059$). The ability of a higher dose of bromerguride (i.e. 2.5 mg/kg) to increase the concentration of 5-HT when given alone (mean AUC: 139.0 ± 6.3) was not significantly attenuated by pretreatment with 0.16 mg/kg WAY100635 (mean AUC: 135.0 ± 12.6; not shown).

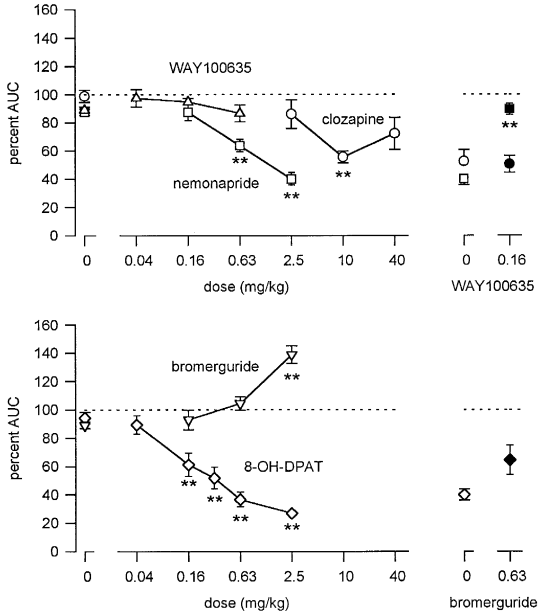

Fig. 2. Effects of the antipsychotics, nemonapride and clozapine, and the putative antipsychotic, bromerguride, on extracellular 5-HT in rat ventral hippocampus, in comparison with those of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, and of the 5-HT_{1A} receptor antagonist, WAY100635. Data are expressed as the mean percent AUC (± S.E.M.; 5 animals per group) for the 140 min period after administration of the compounds. Left panels: dose–response curves for the effects of the compounds alone; right panels: nemonapride 2.5 mg/kg, clozapine 10 mg/kg or 8-OH-DPAT 0.31 mg/kg (same symbols as in left panels) were injected 40 min after vehicle (open symbols), WAY100635 or bromerguride (filled symbols). Significant differences from dose 0 are indicated as $^{**}P < 0.01$, one-way ANOVA followed by Newman–Keuls test (left panels) or Student's *t*-test (right panels). Dose–response curves for 8-OH-DPAT and WAY100635 (except 0.63 mg/kg) are replotted from Assié and Koek, 1996b and Assié and Koek, 1996a, respectively.

4. Discussion

The present study demonstrates that the novel antipsychotic, nemonapride (Tanaka, 1992; Satoh et al., 1997), unlike clozapine and the potential antipsychotic bromerguride (Wachtel and Sauer, 1988), acts as a 5-HT_{1A} receptor agonist not only in vitro, but also in vivo. Clozapine showed only weak intrinsic activity at 5-HT_{1A} receptors in vitro; its in vivo effects examined here, however, do not appear to involve 5-HT1A receptors. Bromerguride exhibited 5-HT_{1A} receptor antagonist rather than agonist properties, both in vitro and in vivo.

This is, to our knowledge, the first report showing nemonapride to have 5-HT_{1A} receptor agonist properties. In vitro, nemonapride was one-fourth as potent as the prototypical 5-HT_{1A} receptor agonist, 8-OH-DPAT, to inhibit forskolin-stimulated cAMP accumulation (IC_{50}: 159 and 35 nM, respectively), but appeared to have similar intrinsic activity (maximal effect: 20 and 26%). Although the affinity of nemonapride for 5-HT_{1A} receptors is in the nanomolar range, it appears to be 38–85 times lower than its affinity for dopamine D_2 receptors (Terai et al., 1989; Assié et al., 1993). In tests predictive of neuroleptic activity (i.e. inhibition of apomorphine- and methamphetamine-induced stereotyped behaviour in rats), Usuda et al. (1981) reported effects of nemonapride at doses (ED_{50} values 0.0076 and 0.0032 mg/kg s.c., respectively) about 100-fold lower than those found here to decrease the hippocampal extracellular 5-HT concentration (ED_{50} 0.62 mg/kg). In the present study, however, the effects of nemonapride alone do not appear to be mediated by D_2 receptor antagonism, because they were not mimicked by the selective dopamine D_2 receptor antagonist, raclopride. The lack of effects of raclopride observed here is in agreement with previous data indicating that systemic administration of the dopamine D_2 receptor antagonists sulpiride or haloperidol did not affect the extracellular concentration of 5-HT in the hippocampus or the nucleus accumbens, respectively (Sharp et al., 1989; Ferré and Artigas, 1995). Nemonapride decreased extracellular 5-HT very likely by activating somatodendritic 5-HT_{1A} receptors, because its effects could be antagonized completely by the selective 5-HT_{1A} receptor antagonist, WAY100635, at a dose (i.e. 0.16 mg/kg) that has been shown previously to completely block the effects of 8-OH-DPAT on hippocampal 5-HT concentration (Assié and Koek, 1996a).

In vitro, clozapine has been shown to stimulate GTPγS binding partially (maximum effect: 49% of the effect induced by 5-HT) in membranes from CHO cells expressing high levels (2–8 pmol/mg of proteins) of human 5-HT_{1A} receptors, and this effect was antagonized by WAY100635 (Newman-Tancredi et al., 1996). The present study confirms and extends these findings by showing clozapine to have weak agonist activity at 5-HT_{1A} receptors in HA7 cells. Clozapine (100 μM) inhibited forskolin-stimulated cAMP to 61% (i.e. 43% of the maximal inhibition produced by 5-HT), and its effects were antagonized by WAY100635. It is unclear what could account for the shallow nature of the antagonist curve; conceivably, the interaction of clozapine with other receptors possibly present in HA7 cells, or other factors, could be involved. At a concentration of 100 μM, clozapine failed to antagonize the maximal inhibition of cAMP produced by 5-HT (1 μM). Clozapine has been shown to decrease extracellular 5-HT in the nucleus accumbens in vivo (Ferré and Artigas, 1995). In agreement with these findings, clozapine significantly decreased extracellular hippocampal 5-HT in the present study; this decrease, however, is unlikely to be mediated by 5-HT_{1A} receptors, because it could not be antagonized by WAY100635. This is consistent with recent findings that clozapine inhibited methylphenidate-induced gnawing and antagonized haloperidol-induced catalepsy, like 5-HT_{1A} receptor agonists, but that its effects could not be antagonized by WAY100635, unlike those of 5-HT_{1A} receptor agonists (Kleven et al., 1996; Bartoszyk et al., 1996). The ability of clozapine to inhibit the firing of 5-HT neurons in the dorsal raphé has been suggested to involve its antagonist properties at α_1-adrenoceptors for which it has high affinity (pK_i 8.2; Lejeune et al., 1994). Indeed, the selective α_1-adrenoceptor antagonist, prazosin, also decreases extracellular 5-HT (Rouquier et al., 1994; Hjorth et al., 1995; Assié and Koek, 1996a). Thus, it is conceivable that the in vivo effects of clozapine reported here involve its α_1-adrenoceptor antagonist properties.

Bromerguride, at low doses (0.03, 0.06 mg/kg), has been claimed to have agonist properties at somatodendritic 5-HT receptors based on its effects on apomorphine-induced hypermotility (Fink et al., 1991), and has been said to bind to 5-HT_{1A} receptors (Löschmann et al., 1992). Consistent with this, bromerguride was found to have high affinity at 5-HT_{1A} receptors (pK_i 8.1, unpublished observations from our laboratory). In vitro, bromerguride, however, appeared to have antagonist instead of agonist properties at these receptors: it did not affect forskolin-stimulated cAMP when given alone at concentrations up to 10 μM, and it antagonized the maximal effect of 5-HT. In vivo, bromerguride appeared to antagonize the effects of 8-OH-DPAT, when given at a dose that did not affect extracellular 5-HT when given alone. At a higher dose, bromerguride increased extracellular 5-HT, unlike the 5-HT_{1A} receptor antagonist, WAY100635, which did not affect extracellular 5-HT when given alone. WAY100635 was unable to antagonize the bromerguride-induced increase of 5-HT, suggesting that this effect does not result from conceivable inverse agonist properties of bromerguride at 5-HT_{1A} receptors. An increase in extracellular 5-HT has been observed with 5-HT_{1B} receptor antagonists (Hjorth and Sharp, 1993; Assié and Koek, 1996b) or with 5-HT uptake inhibitors (see Fuller, 1994). Bromerguride, however, has low affinity for 5-HT_{1B} receptors (pK_i 6.2), and inhibits [^{3}H]5-HT uptake only very weakly ($pIC_{50} < 5$)

(unpublished observations from our laboratory). Thus, it is unlikely that high doses of bromerguride increase extracellular 5-HT through mechanisms involving 5-HT uptake sites, 5-HT_{1B} or 5-HT_{1A} receptors. At lower doses, however, bromerguride has 5-HT_{1A} receptor antagonist-like properties, consistent with its in vitro effects reported here.

Taken together, the results confirmed that clozapine has weak 5-HT_{1A} agonist properties in vitro, but did not provide evidence that it exerts 5-HT_{1A} agonist effects in vivo. Although this makes it less likely that interactions with 5-HT_{1A} receptors underlie the low propensity of clozapine to produce extrapyramidal side effects, it does not rule out a possible role for 5-HT_{1A} receptor activation in the clinical profile of other antipsychotics. In contrast with clozapine, the novel antipsychotic, nemonapride, was found to have 5-HT_{1A} agonist properties not only in vitro, but also in vivo. Nemonapride is the first antipsychotic identified so far to have marked in vivo 5-HT_{1A} agonist properties in addition to its antidopaminergic properties. Thus, it may offer a unique opportunity to test further previously advanced hypotheses about the consequences of such combined properties for the antipsychotic-like, extrapyramidal side effects, and clinical effects of antipsychotics.

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