Effect of Several 5-Hydroxytryptamine$_{1A}$ Receptor Ligands on the Micturition Reflex in Rats: Comparison with WAY 100635$^1$

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ABSTRACT

Several novel N-aryl)piperazine derivatives were synthesized and tested for their 1) affinity and functional activity on 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) receptors in vitro; 2) activity in models predictive of antagonism at somatodendritic and postsynaptic 5-HT$_{1A}$ receptors; and 3) effects on the micturition reflex in anesthetized and conscious rats. These studies also included 1-[2-(methoxyphenyl)]-4-[2-phthalimido]butyl] piperazine hydrobromide (NAN 190), 8-[2-[4-(2-methoxyphenyl)]-1-piperazinyl]-ethyl]-8-azaspiro[4,5]decane-7,9-dione dihydrochloride (BMY 7378), and N-[2-[4-(2-methoxyphenyl)]-1-piperazinyl]ethyl-N-(2-pyridinyl)cyclohexanecarboxamide (WAY 100635). Almost all compounds were found to be potent and selective for the human recombinant 5-HT$_{1A}$ receptor, with $K_i$ values in the nanomolar range. $[^{35}S]$GTP$^\gamma$S binding in HeLa cells expressing the recombinant human 5-HT$_{1A}$ receptor allowed classification of the compounds into neutral antagonists and partial agonists. Almost all neutral antagonists were active in blocking 8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT)-induced forepaw treading in rats (postsynaptic model) and hypothermia in mice (somatodendritic model) with the same potency, whereas compounds showing partial agonistic activity were active in the postsynaptic model but were inactive, or poorly active, in the somatodendritic model. Neutral antagonists potently inhibited volume-induced bladder-voiding contractions in anesthetized rats. Contractions were completely blocked, and the disappearance of bladder contractions lasted 7 to 13 min after the highest doses tested. Furthermore, neutral antagonists increased bladder volume capacity in conscious rats during continuous transvesical cystometry, whereas micturition pressure was only slightly, and not dose-dependently, reduced. Partial agonists were inactive or poorly active, inducing a disappearance time of bladder contractions that did not exceed 6 min in anesthetized rats, and failing to increase bladder volume capacity in conscious rats. These findings indicate that only neutral 5-HT$_{1A}$ receptor antagonists are endowed with inhibitory effects on the bladder.

Normal bladder function requires coordinated detrusor relaxation and urethral sphincter contraction during the filling phase and the converse during micturition. This is achieved by the integration of excitatory, inhibitory, and sensory nerve activity in control centers in the spinal cord, pons, and forebrain (de Groat et al., 1993). The descending bulbospinal pathway to the urinary bladder is essentially an inhibitory circuit (de Groat et al., 1993), although bladder contraction may occur through the stimulation of locus coeruleus in the micturition center and consequent release of noradrenaline in the spinal cord (Yoshimura et al., 1988).

Several neurotransmitters have been identified as inhibitory transmitters in the micturition reflex pathways at both spinal and supraspinal sites, including 5-hydroxytryptamine (5-HT), $\gamma$-aminobutyric acid, and glycine, dopamine, acetylcholine, enkephalins, and other peptides (de Groat et al., 1993). Electrical stimulation of 5-HT-containing neurons in the caudal raphe and activation of postsynaptic 5-HT receptors in the spinal cord of cats, via the release of 5-HT, inhibits bladder contractions (Morrison and Spillane, 1986).

Multiple 5-HT receptors have been characterized in mammalian species and divided into seven subfamilies (from 5-HT$_1$ to 5-HT$_7$) based on their affinity for different 5-HT agonists and antagonists and/or gene structure (Gerhardt and Heerikhuizen, 1997). Some 5-HT receptor subfamilies have been further subdivided. The largest subclass of 5-HT receptors, 5-HT$_1$ receptors, consists of five subtypes (5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$, and 5-HT$_{1F}$; all G protein-coupled receptors), with the 5-HT$_{1A}$ receptor being the most extensively investigated and characterized.

The 5-HT$_{1A}$ receptor is located both on the cell body and terminals of nerves (somatodendritic, presynaptic receptor), where it modulates neuronal cell firing, and at the postsynaptic level, where it mediates different functions (Saxena, 1995).

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$^1$ Some of these data have already been presented in abstract form (Testa et al., 1998a; Guarneri et al., 1998).

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); CHO, Chinese hamster ovary; NRM, nucleus raphe magnum; VIVC, volume-induced bladder-voiding contractions; DT, disappearance time; BVC, bladder volume capacity; MP, micturition pressure.
A number of nonselective antagonists of the 5-HT₁₄ receptor have been reported [spiperone and (S)-UH-301], and over the years, other compounds were initially described as selective 5-HT₁₄ antagonists [8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]l]ethyl]-8-azaspiro[4,5]decane-7,9-dione dihydrochloride (BMY 7378) and 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl] piperazine hydrobromide (NAN 190)]. Although these compounds demonstrated antagonist-like activity in several functional measures of postsynaptic 5-HT₁₄ receptor activation, they showed agonist-like activity when examined at the somatodendritic 5-HT₁₄ autoreceptors (Hjorth and Sharp, 1990; Sharp et al., 1990). Recently, a phenylpiperazine derivative, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexane-carboxamide (WAY 100635), was shown to be an antagonist at both the pre- and postsynaptic level (Fletcher et al., 1996).

Pharmacological studies have shown that 5-HT₁₄ receptor agonists and antagonists influence central control of lower urinary tract function. The selective agonist 8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT), injected i.v., intrathecal, or i.c.v., activates the micturition reflex by inducing an increase in the frequency of volume-induced bladder-voiding contractions (VIVC) in anesthetized rats. On the contrary, the postsynaptic 5-HT₁₄ antagonist NAN 190 blocked the contractions (Lecci et al., 1992), suggesting that 5-HT₁₄ receptors are involved in the control of the micturition reflex, although an α₁-adrenergic mechanism cannot be excluded for this compound.

To better define the effect of 5-HT₁₄ Receptor ligands on the micturition reflex and bladder function, we have synthesized several new, highly selective 5-HT₁₄ compounds and examined their activity on the bladder in comparison to WAY 100635, NAN 190, and BMY 7378. Biochemical and pharmacological experiments to define the nature of the interaction of the compounds with somatodendritic and postsynaptic 5-HT₁₄ receptors are also described.

Materials and Methods

Male and female Sprague-Dawley rats (Crl:CD(SD)BR, 200–300 g b.wt.) and male mice (Crl:CD-1(ICR)BR, 25–35 g b.wt.) from Charles River, Calco, Italy were used in these experiments. Animals were housed with free access to food and water and maintained on a forced 12-h light/dark cycle at 22–24°C for at least 1 week before the experiments were carried out. The animals were handled according to internationally accepted principles for care of laboratory animals (European Economic Community Council Directive 86/609, O. J. no. L358, December 18, 1986).

Binding Studies

Radioligand-Binding Assay at Human Recombinant 5-HT₁₄ Receptors and α₁-Adrenoceptor Subtypes. Human cell line (HeLa) stably transfected with genomic clone G-21 coding for the human 5-HT₁₄-serotoninergic receptor was used. HeLa cells were grown as monolayers in Dulbecco’s modified Eagle’s medium, supplemented with 10% fetal calf serum and gentamicin (100 μg/ml) under 5% CO₂ at 37°C. Cells were detached from the growth flask at 95% confluence by a cell scraper and were lysed in 5 mM ice-cold Tris and 5 mM EDTA buffer (pH 7.4). Homogenates were centrifuged for 20 min at 40,000g, and pellets were resuspended in a small volume of ice-cold 5 mM Tris and 5 mM EDTA buffer (pH 7.4) and immediately frozen and stored at −70°C until use. On the experimental day, cell membranes were resuspended in binding buffer (50 mM Tris, 2.5 mM MgCl₂, and 10 μM pargeline, pH 7.4). Membranes were incubated in a final volume of 1 ml for 30 min at 30°C with 1 nM [³H]8-OH-DPAT, in the absence or presence of various concentrations of the competing drugs (1 pM to 10 μM); each experimental condition was performed in triplicate. Nonspecific binding was determined in the presence of 10 μM 5-HT.

Binding to recombinant human α₁-adrenoceptor subtypes was performed in membranes from Chinese hamster ovary (CHO) cells transfected by electroporation with DNA expressing the gene encoding each α₁-adrenoceptor subtype. Cloning and stable expression of the human α₁-adrenoceptor genes was performed as described (Testa et al., 1995). CHO cell membranes were incubated in 50 mM Tris (pH 7.4) with 0.2 nM [³H]prazosin, in a final volume of 1.02 ml for 30 min at 25°C, in the absence or presence of competing drugs (1 pM to 10 μM). Nonspecific binding was determined in the presence of 10 μM phentolamine. The incubation was stopped by addition of ice-cold Tris buffer and rapid filtration through 0.25% polyethyleneimine pre-treated Schleicher & Schuell (Tecnovetro, Monza, Italy) GF/F filters.

Radioligand-Binding Assay at Different Native Receptors. Binding studies on native α₁-adrenergic receptors, 5-HT₂A serotoninergic receptors, and D₂-dopaminergic receptors were carried out in membranes of rat cerebral cortex (α₁- and 5-HT₂A) and striatum (D₂) as reported (Leonardi et al., 1994).

[³S]GTPγS Binding. The effects of the different compounds tested on [³S]GTPγS binding in HeLa cells expressing the recombinant human 5-HT₁₄ receptor were evaluated according to the method of Stanton and Beer (1997) with minor modifications.

Stimulation Experiments. On the experimental day, cell membranes were resuspended in buffer containing 20 mM HEPES, 3 mM MgSO₄, and 120 mM NaCl (pH 7.4). The membranes were incubated with 30 μM GDP and different concentrations (from 0.1 nM to 100 μM) of test drugs or 5-HT (reference curve) for 20 min at 30°C in a final volume of 0.5 ml. Samples were transferred to ice, [³S]GTPγS (200 pM) was added, and samples were incubated for another 30 min at 30°C.

Antagonism Experiments. On the experimental day, cell membranes were resuspended in buffer containing 20 mM HEPES, 3 mM MgSO₄, and 120 mM NaCl (pH 7.4). The membranes were incubated with 30 μM GDP and various concentrations of 5-HT (from 0.1 nM to 100 μM) in the absence or presence of antagonist, or with 30 μM GDP and different concentrations (from 0.1 nM to 100 μM) of test drugs in the presence of 5-HT (1 μM) for 20 min at 30°C in a final volume of 0.5 ml. Samples were transferred to ice, [³S]GTPγS (200 pM) was added, and samples were incubated for a further 30 min at 30°C. The preincubation with both agonist and antagonist, before initiating the [³S]GTPγS binding, ensures that agonist and antagonist were at equilibrium.

For both procedures, nonspecific binding was determined in the presence of 10 μM GTPγS. Incubation was stopped by the addition of ice-cold HEPES buffer and rapid filtration on Schleicher & Schuell GF/F filters using a Brandel (GDV, Roma, Italy) cell harvester. The filters were washed with ice-cold buffer, and the radioactivity retained on the filters was counted by liquid scintillation spectrometry at efficiency >90%.

In Vivo Antagonistic Activity at Somatodendritic 5-HT₁₄ Receptors

The in vivo antagonistic activity on somatodendritic 5-HT₁₄ receptors was evaluated as antagonism of hypothermia induced in mice by 8-OH-DPAT. On the day of the experiment, mice were placed singly in clear plastic boxes and maintained at a controlled temperature (21 ± 2°C). Body temperature was measured by the use of a temperature probe connected to an electronic thermometer, inserted 2 cm into the rectum. Test compounds or vehicle was administered i.v. to groups of six animals per treatment 5 min before the s.c. injection of standard challenge dose of 8-OH-DPAT (0.5 mg/kg). Temperatures were measured immediately before vehicle or compound administration (basal temperature) and 30 min after injection of 8-OH-DPAT.
In vivo Antagonistic Activity at Postsynaptic 5-HT1A Receptors

The in vivo antagonistic activity on postsynaptic 5-HT1A receptors was evaluated as inhibition of 8-OH-DPAT-induced forepaw treading in rats. On the day of the experiment, rats were placed singly in clear plastic boxes, 10 to 15 min before i.v. injection of test compounds or saline. Five minutes after administration of the compounds, the rats were treated with a s.c. injection of 8-OH-DPAT in a dose of 1 mg/kg. Groups of four to eight rats per dose of test compounds were used. Only the major component, i.e., forepaw treading, of the 5-HT1A syndrome was evaluated by an observer “blind” to drug pretreatments. Observation sessions of 30 s began 3 min after 8-OH-DPAT treatment and were repeated every 3 min over a period of 15 min (five observation sessions). The appearance of forepaw treading was noted, and its intensity was scored using the following ranked intensity scale: 0, absent; 1, equivocal; 2, present; 3, intense. The maximum cumulative score attainable was 15/rat.

Activity on Isovolumic VIVC in Anesthetized Rats

Effect of Tested Compounds Alone. Female rats were anesthetized with s.c. injection of urethane 1.25 g/kg (5 ml/kg), and the urinary bladder was catheterized via the urethra by use of polyethylene tubing (0.58 mm i.d., 0.96 mm o.d.) filled with physiological saline. The catheter was tied in place with a ligature around the external urethral orifice, and intravesical pressure was measured by a pressure transducer and displayed continuously on a chart recorder. The bladder was filled via the recording catheter by incremental volumes of warmed (37°C) saline until spontaneous bladder contractions occurred (usually 0.8–1.5 ml) as a result of central activity. The activity of the tested compounds was assessed after i.v. administration (through a polyethylene cannula inserted into the jugular vein) in individual animals (at least four to six rats per group per dose). Because the compounds tested produced an effect that was relatively rapid in onset and led to complete cessation of bladder contractions, the activity was conveniently estimated by measuring the duration of bladder quiescence [disappearance time (DT) of contractions] in minutes. The effects on amplitude of bladder contraction were estimated comparing them (when contractions restarted) with those previously recorded for 15 min after the i.v. administration in the same animals of vehicle alone.

Interaction Experiments. Experiments were undertaken to test whether 5-HT release and its interaction with serotonergic receptors, other than the 5-HT1A subtype, were involved in the inhibitory effect of compounds on VIVC. Interaction experiments were performed in rats pretreated with citalopram, a selective 5-HT uptake effect of compounds on VIVC. Interaction experiments were performed in clear plastic boxes, 10 to 15 min before i.v. injection of test compounds or saline. The rats were pretreated with 8-OH-DPAT under evaluation in normal rats (pretreatment with vehicle) (Van Wijngaarden et al., 1990). To compare the effect of the compounds under evaluation in normal rats (pretreatment with vehicle) with drug pretreated animals, matched experiments were performed (five to eight rats per dose per group). An interval of 15 min was maintained between the injection of vehicle, citalopram, or mesergine, and the administration of the test compound.

Cystometrographic Recordings in Conscious Rats

Male rats, anesthetized with i.p. administration of 3 ml/kg of equetinsin [1% pentobarbital and 4% (v/v) chloral hydrate] solution, were placed in a supine position, and an ~10-mm long midline incision was made in the shaved and cleaned abdominal wall. The urinary bladder was gently freed from adhering tissues, emptied, and cannulated, via an incision in the dome, with a polyethylene cannula (0.58 mm i.d., 0.96 mm o.d.), which was permanently sutured with silk thread. For i.v. bolus injection, a similar polyethylene tubing filled with physiological saline was inserted into the jugular vein. The cannulas were exteriorized through a s.c. tunnel in the retrocapsular area, where they were connected with a plastic adapter, to avoid the risk of removal by the animal.

For drug testing, rats were used 1 day after implantation. On the day of the experiment, the rats were placed in Bolman’s cages; after a stabilization period of about 20 min, the free tip of the cannula was connected through a T-shaped tube to a pressure transducer and to a peristaltic pump for a continuous infusion of warmed saline solution (37°C) into the urinary bladder at the constant rate of 0.1 ml/min. Intraluminal pressure signal during infusion of saline into the bladder was continuously recorded on polygraph, and two urodynamic parameters for cystometrogram were evaluated: bladder volume capacity (BVC) and micturition pressure (MP). BVC (in ml) is defined as the volume of saline infused in the bladder necessary to induce detrusor contractions followed by micturition. MP (in mm Hg) is defined as the maximal intravesical pressure determined by the contraction of detrusor during micturition. Basal BVC and MP were evaluated as mean of the values observed in the first two complete and reproducible recorded cystometrograms. At this point, drugs were administered i.v. by bolus injection under continuous filling of the bladder. Changes in BVC and MP were evaluated as average of the values recorded in the second and third cystometrogram after treatment.

Drugs and Chemicals

All of the compounds coded as Rec and WAY 100635 (Tables 1 and 2) were synthesized in the Recordati chemical department according to the methods described in the following patents or papers: Rec 0/0260, WO 95/33743; Rec 0/0263, GB 2263110A; Rec 0/0250, GB 2262093A; Rec 0/0241, WO 94/15928; Rec 0/0243, EP 574313A1; Rec 0/0249, J.P. 60169470; Rec 0/0259, WO 95/33743; Rec 0/0277, WO 94/1610; Rec 0/0252, Mokrosz et al. (1994); WAY 100635, GB 2255337A.

Mesulergine HCl, NAN 190 HBr, BM 7378 2HCl, and 8-OH-DPAT HBr were obtained from RBI (Research Biochemicals International, Natick, MA). Oxbytutin HCl, phentolamine, GMP, and GTP·S were purchased from Sigma-Aldrich (Milan, Italy). 5-HT was obtained from Merck (Milan, Italy), and citalopram HBr was purchased from Recordati (Milan, Italy). [3H]Prazosin, [3H]rauwolscine, [3H]piperoxane, [3H]8-OH-DPAT, and [35S]GTP·S were obtained from NEN Life Science Products (Milan, Italy). All of the other substances were from commercial sources.

For receptor-binding studies, the compounds were dissolved in absolute alcohol or deionized water according to their solubility. For i.v. administration in rats and mice, WAY 100635, BM 7378, and Rec 0/0260 were dissolved in saline solution; NAN 190, Rec 0/0263, Rec 0/0250, and Rec 0/0252 were dissolved in deionized water; Rec 0/0241, Rec 0/0243, Rec 0/0249, Rec 0/0259, and Rec 0/0277 were dissolved using N,N-dimethylformamide (4% v/v) and Tween 80 (8% v/v) in deionized water. All of the reported doses were those of the corresponding salts or bases.

Statistical Analysis

The inhibition curves of the tested compounds for the different binding sites examined were analyzed by nonlinear curve fitting of the logistic equation according to the method reported by De Lean et al. (1978), using the ALLFIT program (from National Institutes of Health, Bethesda, MD). The IC50 values and pseudo-Hill slope coefficients were estimated by the program. The value for the inhibition constant, Kp, was calculated by using the equation of Cheng and Prusoff (1975).

Stimulation of [35S]GTP·S binding induced by the compounds tested was expressed as the percent increase in binding above basal value, with the maximal stimulation observed with 5-HT taken as 100%. The concentration-response curves of the agonistic activity were analyzed by ALLFIT as reported above. The maximum percentage of stimulation of [35S]GTP·S binding (Emax) achieved for each drug, and the concentration required to obtain 50% of Emax (pD2 = −log10 EC50 value), were evaluated. The agonistic activity was quantified by evaluating the shift to the right of the 5-HT concen-
tration-response curve in presence of different concentrations of tested compounds. The apparent \( pK_B \) value was evaluated according to the equation \( pK_B = \log_{10}(\frac{[B]}{dr^2 - 1}) \), where \([B]\) is the antagonist concentration and the dose-ratio (dr) is the ratio between the concentrations of agonist required to produce half-maximal response in the presence and the absence of the antagonist.

In the VIVC model, the maximum time (min) of bladder quiescence (DT of rhythmic contractions: DT \( \pm \) S.E.) observed after injection of the different doses tested was recorded. The sigmoidal dose-response curves were analyzed by the nonlinear curve fitting of the logistic equation as reported above. The maximal extrapolated DT (\( E_{max} \) in min) and the dose (in \( \mu g/kg \)) corresponding to 50% \( E_{max} \) were also evaluated. In interaction experiments, the statistical significance of the differences was evaluated by Student's \( t \) test for independent data. Statistical significance of the differences in urodynamic parameter values in conscious rats, before and after the treatments (data on II and III cystometrogram), was evaluated by Student's \( t \) test for paired data.

Results

The chemical structures of the compounds used in this study are shown in Tables 1 and 2. These molecules were synthesized in our laboratory according to the methods described in the literature (see Materials and Methods), and their purity and structures were checked by chromatographic methods and NMR spectra.

Radioreceptor-Binding Profile

Most of the compounds potently inhibited \(^{3}H\)8-OH-DPAT binding to human recombinant 5-HT\(_{1A}\) receptors, with \( K_i \) values close to or lower than 1 nM (Tables 1 and 2). However, two compounds, Rec 0/0252 and 0/0260, had a \( K_i \) value close to 10 nM.

Although the synthesized compounds showed similar affinity at the cloned human 5-HT\(_{1A}\) receptor, they differed in their ability to stimulate its receptor-mediated G protein activation, as measured by \(^{35}S\)GTP\(\gamma\)S binding. In this

TABLE 1

<table>
<thead>
<tr>
<th>Compounds, salt and M.W.</th>
<th>General Formula:</th>
<th>Recombinant Receptors</th>
<th>Native Receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5-HT(_{1A})</td>
<td>( \alpha_{1A} )</td>
</tr>
<tr>
<td>WAY 100635</td>
<td>( R_1 - A - N - R_2 )</td>
<td>(CH(_2))(_2)</td>
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<td>Rec 0/0263</td>
<td>(CH(_2))(_2)</td>
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<td>27.1</td>
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<tr>
<td>1.9 HCl 0.6 H(_2)O; 501.673</td>
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<td></td>
</tr>
<tr>
<td>Rec 0/0277</td>
<td>(CH(_2))(_2)</td>
<td>0.17</td>
<td>97.4</td>
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<td>Base; 421.59</td>
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<td>Rec 0/0249</td>
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<td>98.2</td>
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<td></td>
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<tr>
<td>BMY 7378</td>
<td>(CH(_2))(_2)</td>
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<td>378</td>
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<td>2HCl 458.43</td>
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<tr>
<td>Rec 0/0252</td>
<td>(CH(_2))(_3)</td>
<td>14.7</td>
<td>4.6</td>
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model, in fact, the full agonists 5-HT (Fig. 1) and 8-OH-DPAT (data not shown) induced a concentration-dependent increase in $[^{35}S]$GTP$^{gS}$ binding with $pD_2$ values of 7.31 and 7.90, respectively.

Compounds like Rec 0/0243 and NAN 190 behaved as partial agonists with an effectiveness ($E_{max}$) value of about 30% of the maximal 5-HT effect. WAY 100635 and Rec 0/0259, tested at concentrations up to 100 $\mu M$, did not stimulate $[^{35}S]$GTP$^{gS}$ binding (Fig. 1).

The results obtained in this assay with all of the compounds tested are shown in Table 3.

The compounds endowed with partial agonistic activity dose-dependently antagonized the stimulation of $[^{35}S]$GTP$^{gS}$

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**TABLE 2**

<table>
<thead>
<tr>
<th>Compounds, salt and M.W.</th>
<th>General Formula: $R_1-A-N-N-R_2$</th>
<th>Recombinant Receptors</th>
<th>Native Receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5-HT$_{1A}$</td>
<td>$\alpha_{1a}$</td>
</tr>
<tr>
<td><strong>NAN 190</strong> HBr, 483.4</td>
<td>$\text{[Image]}$</td>
<td>0.98</td>
<td>0.40</td>
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<tr>
<td><strong>Rec 0/0241</strong> 2HCl, 423.424</td>
<td>$\text{[Image]}$</td>
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<tr>
<td><strong>Rec 0/0243</strong> HCl, 410.948</td>
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<td>0.17</td>
<td>0.66</td>
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<tr>
<td><strong>Rec 0/0250</strong> 0.8HCl 0.75H$_2$O; 506.312</td>
<td>$\text{[Image]}$</td>
<td>0.22</td>
<td>117</td>
</tr>
<tr>
<td><strong>Rec 0/0260</strong> 2.9HCl 2H$_2$O; 463.196</td>
<td>$\text{[Image]}$</td>
<td>11.6</td>
<td>13.3</td>
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<tr>
<td><strong>Rec 0/0259</strong> Base, 431.585</td>
<td>$\text{[Image]}$</td>
<td>0.19</td>
<td>96.7</td>
</tr>
</tbody>
</table>

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**Fig. 1.** Stimulation of $[^{35}S]$GTP$^{gS}$ binding at recombinant human 5HT$_{1A}$ receptors stably expressed in HeLa cells by 5-HT and selected compounds. Values are expressed as a percentage of the maximum response given by 5-HT (10 $\mu M$). Points shown are means of triplicate determinations from representative experiments.
In Vivo Antagonistic Activity on Somatodendritic and Postsynaptic 5-HT₁₅ Receptors

Several models have been proposed for measuring responses evoked by the activation of somatodendritic and postsynaptic 5-HT₁₅ receptors in vivo (for review, see Fletcher et al., 1993). We used in vivo models in which the response of a full agonist was antagonized by the compounds administered, namely, the inhibition of 8-OH-DPAT-induced hypothermia in mice (somatodendritic) and the inhibition of 8-OH-DPAT-induced forepaw treading in rats (postsynaptic).

Somatodendritic Model. When mice were injected with increasing doses of 8-OH-DPAT (0.1, 0.3, 1, and 3 mg/kg s.c.), a decrease in temperature was observed, which was dose-dependent for the lowest dosages used (data not shown). The maximum hypothermic effect (ranging from −2.5 to −3.3°C) was observed 30 min after 8-OH-DPAT administration, and 0.3, 1, and 3 mg/kg of the agonist gave approximately the same decrease. The effects of the compounds tested on 8-OH-DPAT-induced hypothermia were evaluated using 0.5 mg/kg of 8-OH-DPAT, because this dose has been widely used in the literature (Martin et al., 1992).

Almost all compounds behaving as neutral antagonists in the [³⁵S]GTPγS-binding assay, administered i.v. 5 min before 8-OH-DPAT, potently and dose-dependently (Fig. 3) inhibited 8-OH-DPAT-induced hypothermia, with the exception of Rec 0/0260. Table 4 lists the ID₅₀ values (dose inhibiting 50% the hypothermia induced by the agonist) of all compounds studied.

As distinct from neutral antagonists, the partial agonists (as defined in the [³⁵S]GTPγS-binding assay), poorly inhibited 8-OH-DPAT-induced hypothermia or exacerbated it (Table 4). For instance, NAN 190 showed no obvious antagonism of the effect of the 8-OH-DPAT at low doses (1 and 10 μg/kg), and, when administered at 100 μg/kg, it induced an increase in the hypothermia produced by 8-OH-DPAT (Fig. 3). After administration of the lowest dose of Rec 0/0241 (100 μg/kg), a hypothermic effect stronger than that induced by 8-OH-DPAT alone was measured, whereas at higher doses there was a small dose-dependent reduction of hypothermia (Fig. 3).

Postsynaptic Model. The s.c. injection of 8-OH-DPAT in rats at a dose of 1 mg/kg induces, usually within 1 to 2 min, increased locomotion, forepaw treading, head weaving, and flat body posture. This “5-HT syndrome” is induced in rats by treatments that increase synaptic levels of 5-HT or by compounds that directly stimulate 5-HT₁₅ receptors and is mediated by postsynaptic 5-HT₁₅ receptors. Among the symptoms evoked by 5-HT₁₅ agonist, forepaw treading appears to be the most closely associated with activation of 5-HT₁₅ postsynaptic receptors (Tricklebank et al., 1984).

Any compounds tested (both neutral antagonists and partial agonists) induced some of the 5-HT syndrome symptoms in the period before 8-OH-DPAT injection. In contrast, all compounds tested dose-dependently antagonized the 8-OH-DPAT-induced forepaw treading (examples are given in Fig. 3). The inhibitory potencies of the compounds tested, expressed in terms of ID₅₀ value (dose inhibiting forepaw treading by 50%) are reported in Table 4.

In Vivo Antagonistic Activity on Somatodendritic and Postsynaptic 5-HT₁₅ Receptors

Several models have been proposed for measuring responses evoked by the activation of somatodendritic and postsynaptic 5-HT₁₅ receptors in vivo (for review, see Fletcher et al., 1993). We used in vivo models in which the response of a full agonist was antagonized by the compounds administered, namely, the inhibition of 8-OH-DPAT-induced hypothermia in mice (somatodendritic) and the inhibition of 8-OH-DPAT-induced forepaw treading in rats (postsynaptic).

Somatodendritic Model. When mice were injected with increasing doses of 8-OH-DPAT (0.1, 0.3, 1, and 3 mg/kg s.c.), a decrease in temperature was observed, which was dose-dependent for the lowest dosages used (data not shown). The maximum hypothermic effect (ranging from −2.5 to −3.3°C) was observed 30 min after 8-OH-DPAT administration, and 0.3, 1, and 3 mg/kg of the agonist gave approximately the same decrease. The effects of the compounds tested on 8-OH-DPAT-induced hypothermia were evaluated using 0.5 mg/kg of 8-OH-DPAT, because this dose has been widely used in the literature (Martin et al., 1992).

Almost all compounds behaving as neutral antagonists in the [³⁵S]GTPγS-binding assay, administered i.v. 5 min before 8-OH-DPAT, potently and dose-dependently (Fig. 3) inhibited 8-OH-DPAT-induced hypothermia, with the exception of Rec 0/0260. Table 4 lists the ID₅₀ values (dose inhibiting 50% the hypothermia induced by the agonist) of all compounds studied.

As distinct from neutral antagonists, the partial agonists (as defined in the [³⁵S]GTPγS-binding assay), poorly inhibited 8-OH-DPAT-induced hypothermia or exacerbated it (Table 4). For instance, NAN 190 showed no obvious antagonism of the effect of the 8-OH-DPAT at low doses (1 and 10 μg/kg), and, when administered at 100 μg/kg, it induced an increase in the hypothermia produced by 8-OH-DPAT (Fig. 3). After administration of the lowest dose of Rec 0/0241 (100 μg/kg), a hypothermic effect stronger than that induced by 8-OH-DPAT alone was measured, whereas at higher doses there was a small dose-dependent reduction of hypothermia (Fig. 3).

Postsynaptic Model. The s.c. injection of 8-OH-DPAT in rats at a dose of 1 mg/kg induces, usually within 1 to 2 min, increased locomotion, forepaw treading, head weaving, and flat body posture. This “5-HT syndrome” is induced in rats by treatments that increase synaptic levels of 5-HT or by compounds that directly stimulate 5-HT₁₅ receptors and is mediated by postsynaptic 5-HT₁₅ receptors. Among the symptoms evoked by 5-HT₁₅ agonist, forepaw treading appears to be the most closely associated with activation of 5-HT₁₅ postsynaptic receptors (Tricklebank et al., 1984).

Any compounds tested (both neutral antagonists and partial agonists) induced some of the 5-HT syndrome symptoms in the period before 8-OH-DPAT injection. In contrast, all compounds tested dose-dependently antagonized the 8-OH-DPAT-induced forepaw treading (examples are given in Fig. 3). The inhibitory potencies of the compounds tested, expressed in terms of ID₅₀ value (dose inhibiting forepaw treading by 50%) are reported in Table 4.
Activity on VIVC in Anesthetized Rats

Effect of Tested Compounds Alone. In urethane-anesthetized rats, distension of the urinary bladder provoked by filling with saline via a transurethral catheter, produces a series of rhythmic isovolumetric bladder voiding contractions, which are fairly reproducible and repetitive due to the inability of the animal to micturate. We generally observed about 6 to 20 contractions/15 min for basal frequency with a peak amplitude of 10 to 40 mm Hg.

Under our experimental conditions, no consistent changes in the frequency and amplitude of the voiding contractions were observed by pretreating the animals with the different vehicles used for compound solubilization.

In general, the compounds used in the present study induced a complete but transient disappearance of the isovolumetric voiding contractions, rather than a true reduction in the frequency of evoked contractions (Fig. 4). The i.v. administration of very low doses of the compounds behaving as neutral antagonists at the 5-HT1A receptors dose-dependently induced an increasing block of the rhythmic bladder-voiding contractions. At higher doses, the inhibitory effect of the compounds generally reached a maximum, showing a plateau effect ranging from 7 to 13 min in duration (Table 5).

All compounds showing partial agonistic activity in the [35S]GTPγS-binding assay were poorly active on VIVC. These compounds induced at the highest dose tested (1000 μg/kg) a comparatively small DT which, with the exception of NAN 190, did not exceed 6 min (Table 5).

With regard to the amplitude of bladder contractions, all compounds tested did not markedly alter this parameter (no more than 20%, and not dose-dependently), indicating no direct effect on bladder contractility (example in Fig. 4).

Interaction Experiments. After administration of citalopram (300 μg/kg i.v.), baseline VIVC were not inhibited, and only a slight increase in frequency was observed (data not shown). The effects of all neutral antagonists were potentiated by pretreatment with citalopram (Table 6). In contrast, neither the effects of NAN 190 nor Rec 0/0243, two

### Table 4

Antagonistic activity of tested compounds at somatodendritic and postsynaptic 5-HT1A receptors.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Somatodendritic</th>
<th>Postsynaptic</th>
</tr>
</thead>
<tbody>
<tr>
<td>WAY 100635</td>
<td>2.3 (1.4–3.6)</td>
<td>2.4 (1.8–3.2)</td>
</tr>
<tr>
<td>Rec 0/0259</td>
<td>5.1 (1.7–15)</td>
<td>6.9 (5.9–8.2)</td>
</tr>
<tr>
<td>Rec 0/0260</td>
<td>&gt;1000</td>
<td>316 (283–354)</td>
</tr>
<tr>
<td>Rec 0/0263</td>
<td>221 (147–333)</td>
<td>350 (280–430)</td>
</tr>
<tr>
<td>Rec 0/0277</td>
<td>16 (9.7–25)</td>
<td>37 (33–41)</td>
</tr>
<tr>
<td>Rec 0/0250</td>
<td>53 (11–251)</td>
<td>66 (45–96)</td>
</tr>
<tr>
<td>Rec 0/0249</td>
<td>&gt;1000</td>
<td>397 (310–505)</td>
</tr>
<tr>
<td>Rec 0/0252</td>
<td>n.a.</td>
<td>2220 (1974–2496)</td>
</tr>
<tr>
<td>Rec 0/0243</td>
<td>&gt;1000</td>
<td>648 (434–967)</td>
</tr>
<tr>
<td>Rec 0/0252</td>
<td>n.a.</td>
<td>350 (280–430)</td>
</tr>
<tr>
<td>Rec 0/0241</td>
<td>n.a.</td>
<td>337 (277–410)</td>
</tr>
<tr>
<td>BMY 7378</td>
<td>n.a.</td>
<td>271 (57–1281)</td>
</tr>
</tbody>
</table>

n.a., not active. Compounds were classified as not active when an hypothermic effect stronger than that induced by 8-OH-DPAT alone was observed.
Partial agonists, were changed by the 5-HT uptake inhibitor (Table 6).

Administration of 100 μg/kg i.v. of mesulergine did not block VIVC, and in general, a tendency toward a slight increase in the frequency of the contractions was observed (data not shown). However, mesulergine (100 μg/kg i.v.) virtually blocked completely the effect of neutral antagonists on DT, whereas the effect of high doses of partial agonists were unaffected (Table 6).

**Cystometrographic Recordings in Conscious Rats**

In control animals, administration of vehicle did not induce significant changes in BVC or MP when evaluated as the mean of the second and third cystometrogram after administration (changes did not exceed ± 10%; data not shown).

WAY 100635 (Fig. 5) induced significant increases of BVC at all doses. Its effect was dose-dependent between 0.1 and 0.3 mg/kg, and the increase in BVC remained similar (about 30%) with doses up to 3.0 mg/kg. The neutral antagonists, Rec 0/0259, Rec 0/0260, and Rec 0/0263, also dose-dependently increased BVC (Fig. 5). The effects on BVC of Rec 0/0277 and Rec 0/0250 (the less potent neutral antagonists in the VIVC model) were low and poorly dose-dependent (data not shown).

With regard to MP, neutral antagonists induced a decrease of this parameter that was often significant but generally not related to the dose. However, the extent of the inhibitory effect of neutral antagonists on MP was generally only minor compared to the marked and dose-dependent effect of oxybutynin (Fig. 5).

The partial agonists BMY 7378 and NAN 190 (Fig. 6) were practically inactive in the cystometry model, because both urodynamic parameters (BVC and MP) were not modified by these compounds.

Rec 0/0249, the most potent partial agonist at the 5HT1A receptor (Table 3), greatly reduced the BVC (57% after 0.3 mg/kg), with a trend toward an increase the MP at the lowest dose tested. Its behavior was similar to that of the full agonist 8-OH-DPAT (Fig. 6), although its potency was less.

**Discussion**

In the present study, several compounds recently synthesized in our laboratories and previously described in published patents were evaluated for affinity at human recombinant 5-HT1A receptor and other selected G protein-coupled receptors. The compounds showed high affinity for the 5-HT1A site with different degrees of selectivity, Rec 0/0250
and Rec 0/0259 being the most selective (about 100-fold) for 5-HT₁A receptors.

When tested in a functional model of receptor-mediated G protein activation (stimulation of [³⁵S]GTPγS binding in HeLa cells stably expressing the cloned human 5-HT₁A receptor), some compounds behaved as partial agonists, e.g., NAN 190 as previously shown by Stanton and Beer (1997), whereas others did not modify the basal binding of the labeled guanine nucleotide, and behaved as neutral antagonists, as shown by Newman-Tancredi et al. (1996) for WAY 100635.

In an attempt to correlate the chemical structure of the

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (µg)</th>
<th>Pretreatment</th>
<th>Citalopram, 300 µg/kg</th>
<th>P</th>
<th>Pretreatment</th>
<th>Mesulergine, 100 µg/kg</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>WAY 100635</td>
<td>100</td>
<td>Vehicle</td>
<td>27.50 ± 3.60</td>
<td>&lt;0.01</td>
<td>Vehicle</td>
<td>3.22 ± 1.00</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Rec 0/0259</td>
<td>10</td>
<td>Vehicle</td>
<td>33.18 ± 4.40</td>
<td>&lt;0.01</td>
<td>Vehicle</td>
<td>5.00 ± 1.46</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Rec 0/0263</td>
<td>100</td>
<td>Vehicle</td>
<td>30.30 ± 5.29</td>
<td>&lt;0.01</td>
<td>Vehicle</td>
<td>3.64 ± 0.94</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>NAN 190</td>
<td>100</td>
<td>Vehicle</td>
<td>8.87 ± 0.77</td>
<td>NS</td>
<td>Vehicle</td>
<td>4.11 ± 0.66</td>
<td>NS</td>
</tr>
<tr>
<td>Rec 0/0243</td>
<td>1000</td>
<td>Vehicle</td>
<td>5.44 ± 0.93</td>
<td>NS</td>
<td>Vehicle</td>
<td>3.60 ± 1.94</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, no significant difference between groups.

Fig. 5. Effects of different i.v. doses of some neutral antagonists, in comparison to oxybutynin, on cystometrographic parameters in conscious rats. Data represent the percentage of change in BVC and MP, evaluated as mean of the second and third cystometrogram after injection, in comparison to the basal values. Statistical significance (inside bars) was evaluated on BVC and MP values.
investigated compounds with their pharmacological profile, it can be observed that all compounds are N-substituted alkyl-N-arylpiperazines. The nature of the aryl group seems not to affect the pharmacological activity, whereas the nature of substituents at the alkyl chain can be considered relevant. In fact, all of the partial agonists, irrespective of the nature (linear or cyclic) and length of the alkyl chain, bear a single bulky group. On the contrary, all neutral antagonists, with the exception of Rec 0/0260, which is also the less potent, are characterized by the presence of an ethylene bridge bearing a group containing two bulky substituents, namely, an alicyclic carbonyl and an aryl moiety bound to a nitrogen atom or to a methine group.

All compounds tested in this study antagonized 8-OH-DPAT-induced forepaw treading, a model considered representative of activity at postsynaptic 5-HT\textsubscript{1A} receptors (Fletcher et al., 1993), indicating that all compounds can be considered postsynaptic antagonists. Although no direct correlation between the affinity for the 5-HT\textsubscript{1A} receptor and the potency in the postsynaptic model was found (probably due to different pharmacokinetic properties of the compounds), partial agonists were, as expected, generally less potent at antagonising 8-OH-DPAT than neutral antagonists.

At somatodendritic 5-HT\textsubscript{1A} sites, which display high density and receptor reserve, the efficacy of the partial agonists may be amplified, and indeed, they can exert their partial agonistic activity. Accordingly, whereas neutral antagonists were generally as potent as in the postsynaptic model, the compounds behaving as partial agonists (including NAN 190 and BMY 7378) were poorly or practically inactive in inhibiting 8-OH-DPAT-induced hypothermia in mice, an in vivo model depending on activation of the somatodendritic 5-HT\textsubscript{1A} receptor (Fletcher et al., 1993), and some of them also increased the hypothermic effects of the selective 5-HT\textsubscript{1A} agonist.

With regard to the effects of the compounds tested on the micturition reflex, our findings confirm that 5-HT\textsubscript{1A} receptor is implicated in the control of micturition in anesthetized and conscious rats, as previously reported (Lecci et al., 1992; Leonardi and Testa, 1997). The selective 5-HT\textsubscript{1A} agonist 8-OH-DPAT was shown to stimulate the micturition reflex in anesthetized rats, as well as the partial agonist buspirone (Lecci et al., 1992), and also in conscious rats (Testa et al., 1998b). Furthermore, it has been recently reported that i.v., intrathecal, and i.c.v. administration of WAY 100635 dose-dependently abolished VIVC for periods of 3 to 15 min (Leo-
agonists and partial agonists at somatodendritic 5-HT1A receptors, Fornal et al. (1994) in cats demonstrated that serotonin activity through a local negative-feedback mechanism (Agha-nardi and Testa, 1997; Kakizaki et al., 1998). This compound was shown to inhibit the descending limb of the micturition reflex pathway through actions on the lumbosacral spinal cord as well as pontine micturition center, but not at the peripheral level. The amplitude of bladder contraction evoked by electrical stimulation of the distal stump of pelvic nerve, in fact, was not changed by i.v. injection of this compound (Kakizaki et al., 1998).

The central pathways controlling lower urinary tract function are organized as a simple on-off switching circuit (de Groat et al., 1993). During urine storage, a low level of afferent activity activates efferent input to the urethral sphincter. A high level of afferent activity induced by bladder distension activates the switching circuit producing firing in the efferent pathways to the bladder, inhibition of efferent outflow to the sphincter, and urine elimination.

Numerous studies have implicated serotoninergic systems in the descending inhibitory control of lower urinary tract function. In the central nervous system, several independent serotoninergic cell clusters located in the raphe nuclei have been identified, possessing differential projection patterns. Serotonin within the dorsal horn of spinal cord arises primarily from neurons in the pontomedullary nucleus raphe magnus (NRM) (Bowker et al., 1981).

Electrical stimulation of 5-HT-containing neurons of NRM and activation of postsynaptic 5-HT receptors in the spinal cord of cats inhibit bladder contractions and reflex firing in the sacral efferent pathways to the bladder (Morrison and Spillane, 1986; Sugaya et al., 1998). Stimulation of the NRM also inhibits the firing of spinal dorsal horn neurons activated by afferents in the pelvic nerve (Lumb, 1986). Thus, it would appear that descending raphe-spinal pathways modulate bladder sensory mechanisms in the spinal cord, and this in turn may account for the inhibitory effect of raphe stimulation on bladder reflexes. Raphe neurons are activated by bladder distension (Lumb, 1986; Oh et al., 1986). This raises the possibility that afferent input from the bladder may trigger a reflex loop, resulting in activation of a raphe-spinal inhibitory pathway that could suppress the micturition reflex.

Serotonin released within the raphe region from dendrites and possibly axon terminals (Chazal and Ralston, 1987) acts on somatodendritic 5-HT1A autoreceptors to inhibit neuronal activity through a local negative-feedback mechanism (Aghanian and Vander-Maelen, 1986). Gobert et al. (1995) in rats and Fornal et al. (1994) in cats demonstrated that serotonin agonists and partial agonists at somatodendritic 5-HT1A receptors (including NAN 190 and BMY 7378) inhibited the firing of raphe neurons, leading to an inhibition of 5-HT turnover in different brain areas and in the spinal cord. On the contrary, the neutral antagonist WAY 100635 increased the firing rate of raphe nuclei cells of rats in vitro (Corradetti et al., 1996) as well as in cats and guinea pigs in vivo (Fornal et al., 1996; Mundey et al., 1996).

It could be hypothesized, therefore, that neutral antagonists, by increasing the firing of NRM, lead to an increase of spinal 5-HT, thus inhibiting the micturition reflex. The potentiating action of citalopram on the inhibition of VIVC by neutral antagonists confirms this suggestion. On the contrary, compounds acting as full agonists (e.g., 8-OH-DPAT) or strong partial agonists (e.g., Rec 0/0249) inhibit the firing of NRM, thus removing the endogenous inhibitory mechanism and leading to the observed increase of the frequency of the voiding contractions (Lecci et al., 1992; Testa et al., 1998b) and decrease of bladder capacity (present data and Testa et al., 1998b).

Thus, for a desirable action on the lower urinary tract, antagonism at presynaptic 5-HT1A receptors appears to be required. However, the 5-HT receptor subtype(s) activated by released 5-HT, probably in the spinal cord (Kakizaki et al., 1998), remains to be defined. Due to the facilitatory effect of 8-OH-DPAT on bladder activity, a role of the 5-HT1A subtype at this level can be excluded.

The reversal of the effects of neutral antagonists induced by a low dose of mesulergine suggests an involvement of 5-HT2 receptors, possibly a 5-HT2 receptor, for which mesulergine has high affinity. This assumption is also supported by interaction studies with WAY 100635 in rats pretreated with ketanserin (data not shown), in which this partially selective 5-HT2A antagonist was devoid of inhibitory effects. Furthermore, 1-(3-chlorophenyl)piperazine (mCPP), a compound having a wide spectrum of pharmacological actions, some of which have been attributed to agonism at the 5-HT2C subtype, blocked the bladder-voiding contractions in rats, and its effect is completely reversed by mesulergine (Guarnieri et al., 1996).

Most of the compounds tested also showed affinity for the α1-adrenoceptor subtypes. The influence of central norepinephrine systems on bladder is controversial. In anesthetized rats, i.v. prazosin antagonized the volume-induced rhythmic bladder contractions (Testa et al., 1998b). In conscious cats and rats, however, no effect of α1-adrenoceptor antagonists on micturition has been found (Durant et al., 1988; Downie et al., 1991; Espey et al., 1992; Testa et al., 1998b).

Our findings show that NAN 190 was the only 5-HT1A partial agonist active in anesthetized rats, and this could well be due to its antagonistic effect and high affinity for the α1-adrenoceptor subtypes, similar to those of prazosin. On the other hand, no effects of NAN 190 on cystometrographic parameters in conscious animals were observed, confirming the above findings. Among the neutral antagonists, Rec 0/0259 showed high efficacy in blocking micturition reflexes both in anesthetized and in conscious animals. Taking into account that this compound shows the lowest affinity for the α1-adrenoceptor subtypes, it could be concluded that requirement for α1-adrenoceptors is ruled out in the activity of 5-HT1A neutral antagonists at the bladder level.

In conclusion, our findings demonstrate that only neutral pre- and postsynaptic 5-HT1A antagonists are endowed with favorable effects at bladder level. These compounds, in fact, induced a marked block of VIVC in anesthetized rats and induced increase of bladder capacity without consistently impairing bladder contractility in conscious animals. On the contrary, compounds showing partial agonistic activity were poorly or not active in blocking voiding contractions in anesthetized rats and were inactive or decreased bladder capacity in conscious rats in analogy with the full agonist 8-OH-DPAT.

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Neutral 5-HT\textsubscript{1A} Antagonists and Micturition Reflex in Rats


Guarnieri I, Ibbà M, Testa R and Leonardi A (1997) The effects of MCP on bladder voiding contractions in rats are mediated by the 5-HT\textsubscript{1A}/5-HT\textsubscript{1B} receptors. Neuroul Urodyn 16:316–317.


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