**ABSTRACT**

N-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-nitrophenyl)cyclohexanecarboxamide (Rec 15/3079) was synthesized with the aim of obtaining a novel compound with 5-hydroxytryptamine (5-HT) antagonistic properties and activity in controlling bladder function at the level of the central nervous system. Rec 15/3079 showed a selective high affinity for the 5-HT, receptor (Kᵢ = 0.2 nM). At the human recombinant 5-HT receptor, Rec 15/3079 acted as a competitive, neutral antagonist in that it did not modify basal [³⁵S]guanosine-5′-O-(3-thio)triphosphate binding to HeLa cell membranes but shifted the activation isotherm to 5-HT to the right, in a parallel manner, with a pKᵢ value of 10.5. Accordingly, Rec 15/3079 (i.v.) potently antagonized 8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT)-induced hypothermia in mice (ID₅₀ = 20 µg/kg) and 8-OH-DPAT-induced forepaw treading in rats (ID₅₀ = 36 µg/kg). In vitro Rec 15/3079 was poorly active in antagonizing carbachol-induced bladder (pD₂ = 5.03) and norepinephrine-induced urethral (apparent pKᵢ = 6) contractions. However, in anesthetized rats, Rec 15/3079 (10–100 µg/kg i.v.) blocked isovolumic bladder contractions with no effect on their amplitude. In conscious rats and guinea pigs with bladders filled with saline, Rec 15/3079 (300–1000 µg/kg i.v.) increased bladder volume capacity (BVC) without affecting bladder contractility. In conscious rats with bladders filled with dilute acetic acid, Rec 15/3079 (300 µg/kg i.v.) reversed the decrease of BVC induced by the acid. To evaluate apparent selective effect on lower urinary tract reflexes, Rec 15/3079 was tested in experimental models for sedative, analgesic, anxiolytic, and antidepressant activity. Rec 15/3079 showed only a slight decrease in the duration of immobility in the behavioral despair test (antidepressant activity) at 1 mg/kg i.v. No anxiolytic activity was observed at 10 mg/kg i.v. No effect was observed in the hot plate test, but Rec 15/3079 increased tail-flick latencies after 3 to 10 mg/kg i.v. In conclusion, these studies demonstrate that Rec 15/3079 is endowed with favorable effects on bladder function, and it is devoid of unwanted side effects at the level of central nervous system at doses at least 10-fold higher than those active on the bladder.

Disorders of the bladder, in particular incontinence, are extremely common, and improved medical treatment for an aging population is required urgently. Most drugs currently used to treat incontinence are believed to act peripherally and may be classified as drugs whose major action is to reduce detrusor contractility and drugs that affect sensory nerves (Ferguson and Christopher, 1996). Antimuscarinic (e.g., tolterodine) or anticholinergic plus calcium antagonist (e.g., oxybutynin) medication remains the most commonly prescribed treatment, at least in the U.S. (Rovner and Wein, 2000), although drugs endowed with other mechanisms are efficacious, including capsaicin, a drug affecting sensory nerves and blocking the afferent limb of the micturition reflex (Andersson, 1998), at least in patients with neurogenic 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 (deGroat et al., 1993). It is possible that much of bladder pathology is related to derangement in the vesical ganglia, sensory reflex loops, and central control of micturition. This field has not been investigated extensively so far and may prove fruitful.

The descending bulbospinal pathway to the urinary blad-

**ABBREVIATIONS:** 5-HT, 5-hydroxytryptamine; GTP·γS, guanosine-5′-O-(3-thio)triphosphate; 8-OH-DPAT, 8-hydroxy-2-dipropylaminotetralin; BVC, bladder volume capacity; bw, body weight; MP, micturition pressure; Rec 15/3079, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-nitrophenyl)cyclohexanecarboxamide; WAY 100635, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridyl)cyclohexanecarboxamide; DT, disappearance time.
Multiple 5-HT receptors have been characterized in mammalian species and divided into different families (5-HT₁-7) based upon structural diversity, transduction mechanisms, and pharmacology (Gerhardt and van Heerikhuizen, 1997). In addition, 5-HT receptor subtypes exist (e.g., 5-HT₁ receptor was further subdivided into 5-HT₁A, 5-HT₁B, 5-HT₁D, 5-HT₁E, and 5-HT₁F subtypes), all of which exhibit distinct functions in anesthetized rats. These early findings implicated 5-HT₁A receptors in the control of the micturition reflex at supraspinal level and prompted us to test in animal models for micturition other known 5-HT₁A ligands (Testa et al., 1999). 5-HT₁A receptors act as somatodendritic and presynaptic receptors on nerve cells, thus modulating neural firing, and at the postsynaptic level where they mediate inhibitory functions.

It has been demonstrated (Lecci et al., 1992) that the selective 5-HT₁A agonist 8-OH-DPAT, i.e. or intracerebroventricularly, activates the micturition reflex inducing an increase in the frequency of isovolumic bladder contractions in anesthetized rats. These early findings implicated 5-HT₁A receptors in the control of the micturition reflex at supraspinal level and prompted us to test in animal models for micturition other known 5-HT₁A ligands (Testa et al., 1999), in particular neutral 5-HT₁A antagonists, including WAY 100635, the first pre- and postsynaptic 5-HT₁A antagonist described (Schechter and Kelly, 1997; Corradetti et al., 1998). These latter compounds injected i.v. behave oppositely to 8-OH-DPAT, inducing a block of isovolumic bladder contractions and an increase in bladder capacity. In contrast, 5-HT₁A partial agonists or compounds classed as presynaptic agonists and postsynaptic antagonists failed to exhibit this profile (Testa et al., 1999). These data have been confirmed by others (Conley et al., 2001).

Therefore, we synthesized several new chemical entities with the aim of obtaining novel compounds endowed with pre- and postsynaptic 5-HT₁A antagonistic properties, particularly at the structures cited above as controlling bladder function. Among the compounds synthesized, Rec 15/3079 (Leonardi et al., 1998; Fig. 1) was selected for further development.

Fig. 1. Rec 15/3079 structure: N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-nitrophenyl)cyclohexanecarboxamide.

Because a number of therapeutic targets relative to central nervous system diseases have been proposed for 5-HT₁A receptor antagonists, including anxiety, depression, schizophrenia, and Alzheimer’s disease (Fletcher et al., 1993; Schechter and Kelly, 1997), Rec 15/3079 was also tested in several different experimental models to detect sedative, analgesic, anxiolytic, or antidepressant activity to evaluate apparent selective effect on lower urinary tract reflexes. Some of these data have been presented in abstract form (Guarneri et al., 2000).

Materials and Methods

Male and female Sprague-Dawley rats (200–300 g bw), and male Crl:CD-1(ICR)BR mice (25–35 g bw) were supplied from Charles River Italia (Calco, Italy). Male NMRI mice (21–26 g bw) and male Wistar rats (181–225 g bw) were from the Centre d’Elevage Roger Janvier (Le Genest St. Isle, France). Female guinea pigs of Hartley strain (350–400 g bw) were from Rodentia (Torre Pallavicina, Italy), and male New Zealand White rabbits (2–3 kg bw) from Conelli (Arona, Italy). Animals were housed with free access to food and water and maintained on a forced 12-h light/dark cycle at 22 to 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 (E.E.C. Council Directive 86/609, O. J. no L358, 18/12/86).

Effects on 5-HT₁A Receptors

Radio-ligand Binding at Native and Human Recombinant 5-HT₁A Receptors and General Binding Profile. The affinity of Rec 15/3079 for human recombinant 5-HT₁A receptors was evaluated as previously reported (Testa et al., 1999). Likewise, binding affinity for native 5-HT₁A receptors (rat hippocampus) and for human recombinant α₁-adrenoceptor subtypes was evaluated as described previously (Leonardi et al., 1994; Testa et al., 1999).

The general receptor binding profile of Rec 15/3079 was evaluated at Contract Research Organizations, namely MDS Panlabs (Panlabs-Taiwan, Ltd, Taipei, Taiwan), by testing the compound in the SPEC-TRUMSCREEN package, and CEREP (Cell e’ Evescault, Poitiers, France). The activity of Rec 15/3079 in displacing approximately 70 different native or recombinant binding sites was studied at the concentration of 100 nM (in triplicate). The following receptors (and their most common subtypes) were investigated: adenosine, α₁- and β-adrenergic, angiotensin, bombesin, bradykinin, calcium channels, cannabinoind, cholecystokinin, dopamine, endothelin, estrogen, γ-aminobutyric acid, glucocorticoid, glutamate, glycine, histamine, interleukin, leukotriene, muscarinic, neurokinin Y, nicotinic, opioid, potassium channel, 5-HT, sigma, testosterone, thromboxane, tumor necrosis factor, vasoactive intestinal peptide, and vasopressin.

[³⁵S]GTPγS Binding. The effects of Rec 15/3079 on [³⁵S]GTPγS binding in HeLa cells stably expressing the cloned human 5-HT₁A receptor were evaluated as previously described (Testa et al., 1999).

In Vivo Antagonistic Activity on Pre- and Postsynaptic 5-HT₁A Receptors. The in vivo antagonistic activity on pre- and postsynaptic 5-HT₁A receptors was evaluated as antagonism of hypothermia induced in mice by 8-OH-DPAT and inhibition of 8-OH-DPAT-induced forepaw treading in rats, respectively, according to methods previously described (Testa et al., 1999).

Effects on Lower Urinary Tract

In Vitro Activity on Rat Bladder Strips Contracted by Carbachol. Detrusor muscle tissue (bladder dome) was cut into two strip preparations measuring approximately 2 × 20 mm. The strips were suspended in a 10-ml organ bath containing Tyrode’s solution at 37°C, gassed with O₂ (95%) and CO₂ (5%), and loaded with 1 g under isometric conditions. After 60 min of stabilization, tissue con-
tractions were obtained by addition of carbachol (10 μM, final concentration in the bath), then bladder strips were washed with fresh solution, and after about 45 min, the contraction by carbachol was repeated. After about 60 min, a carbachol concentration-response curve was constructed twice (every 45 min) by adding increasing amounts of the agonist (from 10^{-8} M to 10^{-4} M). To evaluate the agonistic activity of Rec 15/3079, increasing concentrations of the compound were added to the bath. In other experiments, the antagonistic activity was estimated by incubating single concentrations of the compound for 30 min before constructing a third carbachol concentration-response curve. Responses to carbachol after incubation with Rec 15/3079 were expressed as a percentage of the maximum response recorded in the second carbachol concentration-response curve.

In Vitro Activity on Rabbit Urethra Strips Contracted by Norepinephrine. Rabbit prostatic urethra (specimens 10–20 mm long, starting from the trigone) was removed, and two strips were prepared and suspended in a 20-ml organ bath under isotonic conditions. After 60 min of stabilization, tissue contractions were obtained by addition of noradrenaline (10 μM, final concentration in the bath). The strips were washed with fresh solution, and after about 45 min, a norepinephrine concentration-response curve was constructed twice (every 45 min) by adding increasing amounts of the agonist (from 10^{-8} M to 10^{-4} M). To evaluate the agonistic activity of Rec 15/3079, increasing concentrations of the compound were added to the bath. In other experiments, antagonistic activity was estimated by incubating single concentrations of the compound for 30 min before constructing a third norepinephrine concentration-response curve. Responses to norepinephrine after incubation with Rec 15/3079 were expressed as a percentage of the maximum response recorded in the second norepinephrine concentration-response curve.

Activity on Isovolumic Bladder-Voiding Contractions in Anesthetized Rats. The effect of compounds on volume-induced rhythmic voiding contractions of the bladder after i.v. administration was examined in female rats using methods previously reported (Testa et al., 1999). Activity was estimated by measuring the duration of bladder quiescence (disappearance time of contractions) in minutes. The effect on amplitude of bladder contraction was estimated by comparing contractions (when contractions restarted) with those previously recorded for 15 min after i.v. administration of vehicle in the same animals as used for compound testing.

Interaction Studies. The effect of pretreatment with citalopram and naloxone on responses to Rec 15/3079 in the rhythmic bladder-voiding model was studied. To compare the effect of the compound in normal rats (considered as control and pretreated with vehicle) and in animals pretreated with citalopram or naloxone, matched experiments were performed comparing the effects in both conditions, using groups of at least five to eight rats per dose. An interval of 15 min was maintained between the injection of vehicle or antagonist, and the administration of the dose of compound selected for the interaction study.

Cystometrographic Recordings in Conscious Rats and Guinea Pigs (Bolus Injection). Cystometrographic studies after i.v. administration of the test drugs were performed in male rats and guinea pigs according to the procedure previously reported (Testa et al., 1999), with some modifications.

Male rats were anaesthetized with intraperitoneal administration of 3 ml/kg of Equitensin solution, whereas female guinea pigs were anaesthetized with 1.7 ml/kg of the same solutions, and to induce local anesthesia, meipivacaine hydrochloride (6.5 mg/kg) was injected subcutaneously in both ventral neck and abdominal areas subjected to operation. Animals were placed in a supine position and an approximately 10-mm-long midline incision was made in the shaved and cleaned abdominal wall. The urinary bladder was freed gently from adhering tissues, emptied, and then cannulated via an incision in the dome with a polyethylene cannula (0.85 mm i.d.; 0.96 mm o.d.), which was sutured permanently with silk thread. For intravenous bolus injection a similar polyethylene tubing filled with heparinized saline (40 IU/ml) was inserted into the jugular vein. The cannulae were exteriorized through a subcutaneous tunnel in the retroscapular area, where they were connected with a plastic adapter, to avoid the risk of removal by the animal.

For compound testing, animals were used 1 day after implantation. On the day of the experiment, the animals 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 for rats and 0.2 ml/min for guinea pigs. Intraluminal pressure signal during infusion of saline into the bladder was recorded continuously on a polygraph, and two urodynamic parameters for cystometrogram were evaluated: bladder volume capacity (BVC) and micturition pressure (MP). BVC (in milliliters) 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 cystometograms. At this point, drugs were administered intravenously by bolus injection in conscious filling of the bladder. Changes in BVC and MP were evaluated as an average of the values recorded in the second and third cystometrogram after treatment.

Cystometrographic Recordings in Conscious Rats (i.v. Infusion). Procedures for animal surgery and the basal recording of BVC and MP values were similar to those described above. Another urodynamic parameter was evaluated simultaneously, namely micturition volume (in milliliters). This was done by placing the Bolman’s cages over a plastic funnel to collect and measure the urinary volume of each micturition. Basal micturition volume corresponds to the mean of volumes collected in the cystometograms reported above. Under continuous saline infusion into the bladder, animals were treated with test compounds or their corresponding vehicles by 30 min of intravenous infusion (rate of infusion, 6 ml/kg/h). Changes in cystometrographic parameters were evaluated after 15 and 30 min during drug infusion.

Cystometrographic Recordings in Conscious Rats with Irritated Bladder. Procedures for animal surgery were similar to those described above. Basal BVC and MP values were evaluated under continuous infusion of the bladder with saline as the mean of two complete and reproducible cystometograms (basal I). At this point, the infusion was switched from saline to 0.2% acetic acid solution to obtain chemical irritation of bladder as reported by Yoshiyama et al. (1990). Cystometrograms were recorded continuously during the infusion for a 1-h period. The last two cystometrograms of this period were meditated to obtain the BVC and MP values after bladder irritation and before treatment (basal II). Then the animals were treated intravenously with the test compound under continuous infusion of the bladder with the irritant solution, and changes in BVC and MP were evaluated for 60 min.

Effects on Central Nervous System

The studies concerning the psychotropic profile of Rec 15/3079 were conducted by the Contract Research Organization (I.T.E.M. LABO, Le Genest-St-Ise, France).

Four Plates Test in Mouse. The method, which detects anxiolytic activity, follows that described by Aron et al. (1971). Animals were placed individually in a white plastic enclosure containing a floor consisting of four metal plates (8 × 11 cm) and were left to explore freely for 15 s. Then, every time the animal crossed from one plate to another, it received a weak electric shock. The number of punished crossings was counted during a 1-min test. Rec 15/3079 was administered at 1, 5, and 10 mg/kg i.v. 15 min before the test. Clobazam (16 mg/kg i.v.) was used as a positive control.
Vogel Conflict Test in Rat. The test, which detects anxiolytic activity, was performed according to that described by Vogel et al. (1971). Rats were deprived of water for 48 h and then placed individually into a transparent Plexiglas enclosure (31 × 18 × 34 cm) with a floor consisting of stainless steel bars (0.4 cm) spaced 1 cm apart. The back wall of the enclosure is made of Plexiglas thereby concealing the observer from the experimental animal. In the center of the opposite wall, 5 cm above the floor, a metal water spout protrudes into the cage and is connected to one pole of a shock generator (model 011346; Aplex). The other pole of the shock generator is connected to the metal grid floor. The rat was left to explore until it found the water spout and then, for 5 min, it received, at each drink, a slight electric shock (1 s; 1.7 mA) 2 s after lapping. Rec 15/3079 was administered as reported above. Clobazam (8 mg/kg i.v.) was used as a positive control.

Tail Suspension Test in Mouse. The method, which detects anxiolytic and antidepressant activity, follows that described by Stëru et al. (1987). The behavior of the animal was recorded automatically for 6 min using a computerized device (Itemat-6TST; 3). Six mice were studied simultaneously, and two parameters were recorded: duration of immobility (antidepressants decrease the duration of immobility, whereas anxious agents increase the duration of immobility) and power of movements (this parameter, based on the energy expended by the animal, is independent of the duration of activity, and a decrease reflects anxiolytic activity). Rec 15/3079 was administered as reported above. Imipramine (8 mg/kg) administered i.v. 30 min before the test and diazepam (2 mg/kg) administered i.v. 15 min before the test were used as reference substances.

Behavioral Despair Test in Mouse. The method, which detects antidepressant activity, follows that described by Porsołt et al. (1977). Mice forced to swim in a situation from which they cannot escape rapidly become immobile. Antidepressants decrease the duration of immobility. Mice were placed individually in a cylinder (height, 24 cm; diameter, 13 cm) containing 13.5 cm of water (22°C) for 6 min and the duration of immobility during the last 4 min was measured. Rec 15/3079 was administered as reported above. Imipramine (8 mg/kg) administered i.v. 30 min before the test was used as reference substance.

Hot Plate Test in Rat. The method, which detects analgesic activity, follows that described by Eddy and Leimbach (1953). Rats were placed onto a hot metal plate maintained at 52°C surrounded by a Plexiglas cylinder (height, 26 cm; diameter, 19 cm; model DS37; Aplex). The latencies to the first foot lick and to the first jump were measured. If no reaction was noted, the test was terminated after 120 s. To reduce the variance, the maximum score for foot licking was 30 s. Ten rats per group were studied, and the test was performed blind. Rec 15/3079 was administered as reported above 15 min before the test. Morphine (4 mg/kg), administered in the same experimental conditions, was used as reference substance.

Tail-Flick Test in Rat. The method, which detects analgesic activity, follows that described by D’Amour and Smith (1941). The rat’s tail is heated by means of a thermal light source (13 V; model DS20; Aplex). The latency before the animal withdraws its tail is measured (maximum, 30 s). Ten rats were studied per group, and the test was performed blind. Rec 15/3079 and morphine (as reference compound) were administered as reported in the hot plate test.

Rotarod Test in Rat. The method, which detects neurological deficits, follows that described by Dunham and Miyra (1957). Rats were placed on a rod (diameter, 7 cm) rotating at a speed of 12 turns per minute. The number of animals that drop off the rod before 3 min was counted, and the drop-off times were recorded (maximum, 3 min). Rec 15/3079 was administered as reported above. Diazepam (2 mg/kg i.v.), administered in the same experimental conditions, was used as reference substance.

Compounds
Rec 15/3079 (N-[2-(1-piperazinyl)ethyl]-N-(2-pyridyl)cyclohexanecarboxamide), and WAY 106635 (N-[2-(1-piperazinyl)[2-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridyl)cyclohexanecarboxamide) were synthesized at Recordati. [3H]Prazosin, [3H]8-OH-DPAT, and [35S]Guanosine 5’-γ-thio)triphosphate were obtained from NEN Life Science Products (Milan, Italy). All other compounds were from commercial sources.

For receptor binding studies Rec 15/3079 was dissolved in absolute alcohol. For i.v. administration in rats and mice, WAY 106635, indomethacin, morphine, cilopram, naloxone, flurazepam, and oxybutynin were dissolved in saline solution; Diazepam and imipramine were dissolved in demineralized water; Rec 15/3079 was dissolved in 0.05 M methanesulphonic acid (0.45% v/v) and demineralized water; and clobazam was dissolved in hydroxypropylmethylcellulose 0.2% in saline solution.

Statistical Analysis
The displacement curves of Rec 15/3079 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 the 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, Ki, was calculated by using the Cheng and Prusoff (1973) equation.

The concentration-response curves of 5-HT-induced stimulation of [35S]GTPγS binding, expressed as percent increase in binding above basal value (with the maximal stimulation observed with 5-HT taken as 100%), were analyzed by ALLFIT as reported above. The antagonistic activity was quantified by evaluating the shift to the right of the 5-HT-concentration-response curve in presence of different concentrations of Rec 15/3079, and the pKia value was evaluated on the Scild plot by linear regression analysis.

In the isolated tissue experiments, at least three tissue preparations for each agonistic or antagonistic activity evaluated were used. The concentration-response curves of the agonistic activity were analyzed by nonlinear fitting program (ALLFIT), and the EC50 values were calculated according to the logistic equation as reported by De Lean et al. (1978). For the antagonistic activity, a pKia value was calculated with the formula: pKia = log[B]/(dose ratio − 1), where [B] is the antagonist concentration and the dose ratio is the ratio between the concentrations of agonist required to produce half-maximal response in the presence and in the absence of the antagonist. When a noncompetitive shift was observed, a pD2 value was calculated by linear regression analysis. pD2 is defined as the concentration of antagonist that reduces the maximal response of the agonist by 50%.

To compare the potency of test compounds at inhibiting the rhythmic bladder-voiding contractions, equieffective doses producing 10 min of disappearance time (ED10 min) were computed by means of least square linear regression procedure.

Statistical significance of the differences in urodynamic parameters measured in conscious rats and guinea pigs before and after the treatments was evaluated by Student’s t test for paired data. Time course changes of BVC and MP values were evaluated by S.A.S./STAT software, version 6.12 using general linear model procedure—repeated measures analysis of variance-analysis of variance of contrast variables.

Statistical significance of the differences between means of controls and treated animals in the different models used to evaluate effects on central nervous system was tested by analysis of variance and Dunnett’s t test.

Results
Effects on 5-HT1A Receptors
Receptor Binding Profile. Rec 15/3079 showed high affinity for the 5-HT1A receptor with Ki values of 0.7 and 0.2
nM on native (rat hippocampus) and recombinant (human, in HeLa cells) receptors, respectively.

The compound also showed an affinity for α₁-adrenoceptor subtypes but was at least 25-fold less potent at these sites than at the recombinant 5-HT₁₅ receptor (Kᵢ values were 81, 105, and 5.7 nM for α₁₅⁺, α₁₉⁺, and α₁₁₉-subtypes, respectively). At a concentration of 100 nM, a concentration which is 100- to 500-fold higher than that active at the 5-HT₁₅ receptors, no relevant displacing activity was seen at the other selected binding sites. In particular, no displacing activity was detected for the serotoninergic subtypes 5-HT₁₅, 5-HT₁₆, 5-HT₂₅, 5-HT₃, and 5-HT₄, and for the 5-HT uptake binding sites labeled by [³H]paroxetine.

**Effects on [³S]GTPγS Binding at 5-HT₁₅ Receptors.**

When tested in a functional model of receptor-mediated G-protein activation (stimulation of [³S]GTPγS binding in HeLa cells stably expressing the cloned human 5-HT₁₅ receptor), Rec 15/3079 did not modify the basal [³S]GTPγS binding at concentrations up to 100,000 nM (data not shown), in contrast to 5-HT, 8-OH-DPAT, buspirone, and NAN 190, behaving as agonists or partial agonists (Cilia et al., 2001). However, it shifted the activation isotherm of 5-HT to the right in a parallel manner with a pKᵦ value of 10.5 indicating that it can be considered a “silent” or neutral antagonist at this receptor (Fig. 2), as far as WAY 100635 (Testa et al., 1999).

**Antagonistic Activity at Pre- and Postsynaptic 5-HT₁₅ Receptors in Vivo.**

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 inhibition of 8-OH-DPAT-induced hypothermia in mice (presynaptic) and inhibition of 8-OH-DPAT-induced forepaw treading in rats (postsynaptic). After i.v. administration, Rec 15/3079 potently and dose-dependently antagonized the effects of 8-OH-DPAT and the ID₅₀ (dose inhibiting the agonist-induced effects by 50%) values (and their 95% confidence limits) were 20 (12–32) and 36 (29–45) μg/kg at pre- and postsynaptic levels, respectively.

**Effects on Lower Urinary Tract**

**In Vitro Effects on Isolated Bladder and Urethra.** Rec 15/3079, up to 3 × 10⁻⁵ M, did not induce contraction of rat bladder strips (data not shown). When tested as an antagonist of carbachol-induced contractions, Rec 15/3079 behaved as an insurmountable antagonist, inducing a progressive decrease in maximal tension. The concentration of the compound required to reduce the maximum response of the carbachol concentration-effect curve by 50% is expressed as pD² value and was 5.03 (Fig. 3). Similarly, the compound did not induce contraction of rabbit urethral strips up to 3 × 10⁻⁶ M (data not shown), but when tested as an antagonist of noradrenaline-induced contractions, it behaved as competitive antagonist, at least when tested at 3 × 10⁻⁶ M concentration (Fig. 3). At this concentration, an apparent pKᵦ value of about 6.0 was estimated. Upon increasing the concentration of Rec 15/3079, an insurmountable antagonism to noradrenaline was observed.

**Effects on Isovolumic Bladder-Voiding Contractions in Anesthetized Rats.** In this experimental model, the bladder of anesthetized rats was cannulated via the external urethra and filled with saline until repetitive and reproducible contractions (isovolumic) were obtained. The effect of test compounds was evaluated in terms of disappearance time (in minutes) of the rhythmic voiding contractions and of decrease in the amplitude of the contractions. Rec 15/3079 was extremely potent in blocking the isovolumic bladder contraction, inducing a complete but transient dose-dependent disappearance of contractions. Its DT₁₀ min (extrapolated dose inducing 10 min of bladder silence) was 60 μg/kg i.v., which is similar in potency to morphine (DT₁₀ min, 50 μg/kg i.v.) and markedly more potent than flavoxate (Fig. 4). No consistent effects on the amplitude of contractions were seen after the i.v. administration of these drugs (data not shown). Oxybutynin at doses up to 100 μg/kg i.v. slightly increased the frequency and decreased the amplitude of the contractions (Guarneri et al., 1993) and did not block contractions consistently up to 1000 μg/kg (Fig. 4).

**Interaction Studies on Isovolumic Bladder-Voiding Contractions in Anesthetized Rats.** To evaluate whether 5-HT release or interaction with opioid receptors was involved in the effect of Rec 15/3079, mechanistic experiments, using the voiding contraction model, were performed (Fig. 5).
Citalopram, a selective 5-HT uptake inhibitor, was devoid per se of effects on isovolumic bladder-voiding contractions in anesthetized rats (Testa et al., 1999). On the other hand, citalopram (at 300 μg/kg i.v.) potentiated the effect of Rec 15/3079, suggesting that a release of 5-HT is involved in the mechanism of action of Rec 15/3079. In contrast to morphine, the effect of Rec 15/3079 was not modified significantly by naloxone pretreatment (100 μg/kg i.v.), indicating that opiate receptors are not involved in its mechanism of action.

Effects on Cystometrographic Parameters in Conscious Rats and Guinea Pigs (Bolus Injection). In conscious rats and guinea pigs, i.v. injection of vehicle did not change significantly cystometrographic parameters (Table 1). The i.v. administration of 0.3 and 1.0 mg/kg of Rec 15/3079 in rats and 0.1 and 0.3 mg/kg in guinea pigs significantly increased BVC. Oxybutynin, when injected at the same doses, did not modify BVC in either rats or guinea pigs (Table 1). Rec 15/3079 induced significant decreases of MP that were not greater than 11% in both animal species. Oxybutynin, on the contrary, induced a dose-dependent decrease in MP, reaching 70% or more after administration of 0.3 and 1.0 mg/kg in guinea pigs and rats, respectively (Table 1).

Effects on Cystometrographic Parameters in Conscious Rats (i.v. Infusion). The effects of Rec 15/3079 on cystometrographic parameters were evaluated also after continuous i.v. infusion (30 min) of the compound, in comparison with WAY 100635, a selective 5-HT1A antagonists active on the bladder (Testa et al., 1999), and oxybutynin. After infusion of 0.01 and 0.03 mg/kg/min of Rec 15/3079, a marked increase of BVC (Table 2) with no consistent effects on MP (data not shown) was observed. Taking into account the total dose administered during the infusion period (0.3 and 0.9
mg/kg), the results obtained may be considered consistent with those obtained after bolus injection (see Table 1). It is important to note that the increase of BVC observed after continuous infusion of Rec 15/3079 is paralleled by an increase of the urinated volume (data not shown), thus residual volume was not increased.

Similar results were obtained after continuous i.v. infusion of 0.03 and 0.1 mg/kg/min of WAY 100635 (Table 2), suggesting that the potency of Rec 15/3079 on the bladder is similar to that of WAY 100635, in agreement with the data previously obtained with this compound in anesthetized and in conscious rats (Testa et al., 1999). Oxybutynin, infused at the same doses as Rec 15/3079, did not change BVC (data not shown), and dose-dependently reduced MP (Table 3).

**Effects on Cystometrographic Parameters in Conscious Rats with Irritated Bladder.** The effects of Rec 15/3079 and WAY 100635 were also evaluated in conscious rats in which distension of the bladder was achieved by infusion of diluted acetic acid (0.2%), instead of saline. Because of the nature of the model, indomethacin, a well-known anti-inflammatory drug, was used as reference compound (Table 4).

After 1 h of infusion with acetic acid, BVC of rats was reduced by 50 to 60% of the initial value. In control animals, the continuous infusion of the irritant for another 1-h period further decreased the BVC by 30 to 40%. The i.v. administration of Rec 15/3079 and WAY 100635 (both at 0.3 mg/kg) counteracted the effect of the acetic acid, inducing an increase of BVC for about 30 min. Indomethacin administered at the same dose was less active than Rec 15/3079, contrasting only the decrease of BVC induced by the irritant in the 2nd h of infusion (data not shown). A clear and marked effect of indomethacin was observed only after administration of 1 mg/kg.

**Effects on Central Nervous System**

**Anxiolytic Activity** (Fig. 6). In the four plates test in the mouse, Rec 15/3079 (1, 3, and 10 mg/kg i.v.) had no clear effects on the number of punished responses, although a nonsignificant tendency toward an increase was apparent at

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**TABLE 1**

Effects of i.v. administration of Rec 15/3079 and oxybutynin on BVC (in milliliters) and MP (in mmHg) in conscious rats and guinea pigs (10–20 animals/group).

Data represent the mean values ± S.E. of BVC and MP and the percent changes versus basal values.

<table>
<thead>
<tr>
<th>Treatments (Dose mg/kg i.v.)</th>
<th>Rats</th>
<th>Guinea Pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>BVC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.70 ± 0.06</td>
<td>0.66 ± 0.07</td>
</tr>
<tr>
<td>Rec 15/3079 (0.1)</td>
<td>0.79 ± 0.09</td>
<td>0.81 ± 0.10</td>
</tr>
<tr>
<td>Rec 15/3079 (0.3)</td>
<td>0.75 ± 0.04</td>
<td>0.93 ± 0.06**</td>
</tr>
<tr>
<td>Rec 15/3079 (1.0)</td>
<td>0.67 ± 0.05</td>
<td>0.79 ± 0.06**</td>
</tr>
<tr>
<td>Oxybutynin (0.1)</td>
<td>0.72 ± 0.11</td>
<td>0.79 ± 0.13</td>
</tr>
<tr>
<td>Oxybutynin (0.3)</td>
<td>0.74 ± 0.08</td>
<td>0.70 ± 0.08</td>
</tr>
<tr>
<td>Oxybutynin (1.0)</td>
<td>0.94 ± 0.19</td>
<td>1.00 ± 0.18</td>
</tr>
<tr>
<td>MP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>97 ± 6</td>
<td>92 ± 6</td>
</tr>
<tr>
<td>Rec 15/3079 (0.1)</td>
<td>103 ± 10</td>
<td>97 ± 12</td>
</tr>
<tr>
<td>Rec 15/3079 (0.3)</td>
<td>99 ± 6</td>
<td>88 ± 7**</td>
</tr>
<tr>
<td>Rec 15/3079 (1.0)</td>
<td>106 ± 10</td>
<td>94 ± 8**</td>
</tr>
<tr>
<td>Oxybutynin (0.1)</td>
<td>105 ± 8</td>
<td>84 ± 8**</td>
</tr>
<tr>
<td>Oxybutynin (0.3)</td>
<td>92 ± 7</td>
<td>51 ± 5**</td>
</tr>
<tr>
<td>Oxybutynin (1.0)</td>
<td>111 ± 10</td>
<td>30 ± 6**</td>
</tr>
</tbody>
</table>

*p < 0.05 and **p < 0.01 versus basal values (Student’s t test for paired data).

**TABLE 2**

Time course of the effects of i.v. infusion of Rec 15/3079 and WAY 100635 on BVC (in milliliters) in conscious rats.

Data represent the mean values ± S.E. of BVC before and at 15 and 30 min during i.v. infusion of saline (0.1 ml/kg/min) or compounds.

<table>
<thead>
<tr>
<th>Treatment (mg/kg/min)</th>
<th>No. of Rats</th>
<th>0 min (basal)</th>
<th>15 min</th>
<th>30 min</th>
<th>15 min</th>
<th>30 min</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>9</td>
<td>0.58 ± 0.07</td>
<td>0.54 ± 0.06</td>
<td>0.59 ± 0.07</td>
<td>−8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Rec 15/3079 (0.01)</td>
<td>9</td>
<td>0.63 ± 0.11</td>
<td>0.80 ± 0.16*</td>
<td>0.84 ± 0.16**</td>
<td>27</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>8</td>
<td>0.67 ± 0.10</td>
<td>0.59 ± 0.06</td>
<td>0.63 ± 0.06</td>
<td>−11</td>
<td>−6</td>
<td></td>
</tr>
<tr>
<td>Rec 15/3079 (0.03)</td>
<td>8</td>
<td>0.67 ± 0.10</td>
<td>0.86 ± 0.15*</td>
<td>0.97 ± 0.14**</td>
<td>28</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>11</td>
<td>0.64 ± 0.05</td>
<td>0.61 ± 0.05</td>
<td>0.60 ± 0.06</td>
<td>−4</td>
<td>−6</td>
<td></td>
</tr>
<tr>
<td>WAY 100635 (0.03)</td>
<td>11</td>
<td>0.71 ± 0.07</td>
<td>0.92 ± 0.08**</td>
<td>0.86 ± 0.08**</td>
<td>30</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>11</td>
<td>0.65 ± 0.09</td>
<td>0.58 ± 0.07</td>
<td>0.63 ± 0.08</td>
<td>−11</td>
<td>−4</td>
<td></td>
</tr>
<tr>
<td>WAY 100635 (0.10)</td>
<td>11</td>
<td>0.60 ± 0.06</td>
<td>0.81 ± 0.09**</td>
<td>0.88 ± 0.11**</td>
<td>34</td>
<td>42</td>
<td></td>
</tr>
</tbody>
</table>

N.S., not significant.

*p < 0.05 and **p < 0.01 versus basal values (within groups).

*p between treatments* represents, at each time, the significance of the difference between the trend (basal to time) observed in the saline and compound groups (analysis of variance of contrast variables).
1 mg/kg. In the same experimental conditions, clobazam (16 mg/kg i.v.) increased significantly the number of punished crossings. Similarly, in the Vogel conflict test in rat, Rec 15/3079 did not affect significantly the number of shocks received, although a slight tendency toward an increase was apparent over the dose-range tested, whereas clobazam (8 mg/kg i.v.) significantly increased the number of shocks received. Furthermore, Rec 15/3079 had no significant effects on the power of the movements in the tail suspension test in the mouse. In contrast, diazepam (2 mg/kg i.v.) induced a marked decrease of the power of the movements.

**Antidepressant Activity (Fig. 7).** In the tail suspension test in mouse, Rec 15/3079 (1, 3, and 10 mg/kg i.v.) had no significant effects on the duration of immobility, whereas imipramine (8 mg/kg i.v.) significantly decreased the duration of immobility (−69%). Rec 15/3079 slightly but significantly decreased the duration of immobility in the behavioral despair test in mouse only at 1 mg/kg. It had no effects at higher doses. In contrast, imipramine (8 mg/kg i.v.) significantly decreased the duration of immobility.

**Analgesic and Neurotoxic Activity (Fig. 8).** In the hot plate test in the rat, Rec 15/3079 (1, 3 and 10 mg/kg i.v.) had no significant effects on foot licking up to the highest dose tested, whereas in tail-flick test in the rat the compound significantly but non-dose-dependently increased the tail-flick latency. Morphine (4 mg/kg i.v.) markedly increased the latency to aversive reaction in both models.

Rec 15/3079 tended to decrease rotarod performance, reaching significance only at the highest dose tested. In the same experimental conditions, diazepam (2 mg/kg i.v.) markedly decreased rotarod performance.

**Discussion**

Rec 15/3079 is a novel derivative of 2-methoxyphenylpiperazine structurally similar to WAY 100635, carrying a 2-nitrophenyl instead of 2-pyridyl moiety. The in vitro and in vivo data presented herein, however, indicate that Rec 15/3079 behaves as a neutral antagonist, shows antagonistic activity on pre- and postsynaptic 5-HT\(_{1A}\) receptors, and is endowed with a distinct pharmacological profile on the lower urinary tract, similar to that previously reported for WAY 100635 (Leonardi and Testa, 1997; Kakizaki et al., 1998; Testa et al., 1999).

Rec 15/3079 exhibited high affinity (subnanomolar) for native and human recombinant 5-HT\(_{1A}\) receptors and showed selectivity versus several other receptors, including 5-HT\(_{1B}\), 5-HT\(_{2A}\), 5-HT\(_{2C}\), 5-HT\(_{3}\), and 5-HT\(_{4}\), and the \(\alpha_{1}\)-adrenoceptor subtypes. In this regard Rec 15/3079 is similar in pharmacological profile to WAY 100635. When tested in a functional model of receptor-mediated G-protein activation (stimulation of \(^{35}\)S)GTP\(\gamma\)S binding in HEK293 cells stably expressing the cloned human 5-HT\(_{1A}\) receptor), Rec 15/3079 did not modify the basal binding of the labeled guanine nucleo-

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**TABLE 3**

<table>
<thead>
<tr>
<th>Treatment (mg/kg/min)</th>
<th>No. of rats</th>
<th>0 min (basal)</th>
<th>15 min</th>
<th>30 min</th>
<th>15 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>5</td>
<td>103 ± 10</td>
<td>96 ± 15</td>
<td>98 ± 15</td>
<td>−6</td>
<td>−5</td>
</tr>
<tr>
<td>Oxybutynin (0.01)</td>
<td>8</td>
<td>102 ± 11</td>
<td>87 ± 13</td>
<td>75 ± 14**</td>
<td>−15</td>
<td>−27</td>
</tr>
<tr>
<td>p between treatments**</td>
<td>N.S.</td>
<td>N.S.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>7</td>
<td>67 ± 10</td>
<td>58 ± 8</td>
<td>54 ± 9</td>
<td>−13</td>
<td>−20</td>
</tr>
<tr>
<td>Oxybutynin (0.03)</td>
<td>7</td>
<td>89 ± 17</td>
<td>44 ± 10**</td>
<td>28 ± 6**</td>
<td>−51</td>
<td>−68</td>
</tr>
<tr>
<td>p between treatments</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N.S., not significant.

* p < 0.05 and ** p < 0.01 versus basal values (within groups).

**TABLE 4**

<table>
<thead>
<tr>
<th>Treatment (mg/kg/min)</th>
<th>No. of rats</th>
<th>−60 min (Basal I)</th>
<th>0 min (Basal II)</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
<th>Percent change vs. Basal II at 15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>7</td>
<td>0.67 ± 0.11</td>
<td>0.32 ± 0.05††</td>
<td>0.29 ± 0.04</td>
<td>0.21 ± 0.04**</td>
<td>0.22 ± 0.04**</td>
<td>0.18 ± 0.03**</td>
<td>−10</td>
<td>−32</td>
<td>−31</td>
<td>−42</td>
</tr>
<tr>
<td>Rec 15/3079 (0.3)</td>
<td>7</td>
<td>0.62 ± 0.05</td>
<td>0.24 ± 0.03††</td>
<td>0.30 ± 0.04</td>
<td>0.29 ± 0.03</td>
<td>0.24 ± 0.03</td>
<td>0.22 ± 0.03</td>
<td>22</td>
<td>19</td>
<td>−12</td>
<td></td>
</tr>
<tr>
<td>p within times</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>N.S.</td>
<td></td>
<td></td>
<td>22</td>
<td>19</td>
<td>−12</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>6</td>
<td>0.56 ± 0.07</td>
<td>0.30 ± 0.04†</td>
<td>0.24 ± 0.04</td>
<td>0.25 ± 0.04</td>
<td>0.24 ± 0.05</td>
<td>0.19 ± 0.04</td>
<td>−21</td>
<td>−15</td>
<td>−18</td>
<td>−33</td>
</tr>
<tr>
<td>WAY 100635 (0.3)</td>
<td>5</td>
<td>0.66 ± 0.18</td>
<td>0.29 ± 0.09†</td>
<td>0.30 ± 0.07</td>
<td>0.31 ± 0.06</td>
<td>0.23 ± 0.05</td>
<td>0.19 ± 0.05**</td>
<td>6</td>
<td>10</td>
<td>−18</td>
<td>−34</td>
</tr>
<tr>
<td>p within times</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>N.S.</td>
<td>N.S.</td>
<td></td>
<td></td>
<td>6</td>
<td>10</td>
<td>−18</td>
<td>−34</td>
</tr>
<tr>
<td>Vehicle</td>
<td>7</td>
<td>0.49 ± 0.04</td>
<td>0.27 ± 0.04†</td>
<td>0.24 ± 0.04</td>
<td>0.20 ± 0.04‡‡</td>
<td>0.21 ± 0.03</td>
<td>0.19 ± 0.04**</td>
<td>−12</td>
<td>−24</td>
<td>−21</td>
<td>−29</td>
</tr>
<tr>
<td>Indomethacin (1.0)</td>
<td>6</td>
<td>0.66 ± 0.13</td>
<td>0.28 ± 0.06†</td>
<td>0.24 ± 0.04</td>
<td>0.32 ± 0.05</td>
<td>0.35 ± 0.08*‡‡</td>
<td>0.36 ± 0.08*‡‡</td>
<td>−16</td>
<td>12</td>
<td>24</td>
<td>26</td>
</tr>
<tr>
<td>p within times</td>
<td>N.S.</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>N.S.</td>
<td></td>
<td></td>
<td>−16</td>
<td>12</td>
<td>24</td>
<td>26</td>
</tr>
</tbody>
</table>

N.S., not significant.

* p < 0.05 and † p < 0.01 vs. basal I values; † † p < 0.05 and † † † p < 0.01 vs. "basal II" values (within groups).

**p between treatments** represents, at each time, the significance of the difference between the trend (basal to time) observed in the saline and oxybutynin groups (analysis of variance of contrast variables).
but shifted the concentration-effect curve to 5-HT to the right, consistent with being a neutral competitive antagonist, like WAY 100635 (Newman-Tancredi et al., 1996; Testa et al., 1999). Accordingly, Rec 15/3079 antagonized 8-OH-DPAT-induced forepaw treading, a model considered representative of activity at postsynaptic 5-HT1A receptors (Fletcher et al., 1993) and antagonized 8-OH-DPAT-induced hypothermia in mice, a model dependent upon activation of somatodendritic 5-HT1A receptors (Fletcher et al., 1993).

In vivo studies show that Rec 15/3079 potently inhibits the frequency of the isovolumic bladder-voiding contractions in anesthetized rats. The rapid distension of the urinary bladder in urethane-anesthetized rats produces a series of rhythmic bladder-voiding contractions showing peak amplitude of 30 to 40 mm Hg and frequency of about 0.7 peaks/min (Guarneri et al., 1993). The frequency of these contractions is related to the sensory afferent arm of reflex micturition and to the integrity of the micturition center, whereas their amplitude is a property of the efferent arm of the reflex (Maggi et al., 1984, 1986). In this model system, compounds that act mainly on the central nervous system cause a block in voiding contraction, whereas drugs that act at the level of the detrusor muscle decrease the amplitude of the bladder contractions (Guarneri et al., 1993). After i.v. injection of morphine, the rhythmic bladder-voiding contractions rapidly disappear for a period of time depending on the injected dose (Dray and Metsch, 1984; Hisamitsu and deGroat, 1984). When these contractions restart, they exhibit the same amplitude and frequency as observed in the predrug (basal) period. On the contrary, oxybutynin injection induces a slight increase in the frequency of the contractions and a marked reduction of their amplitude. However, high doses of oxybutynin (>1000 µg/kg) abolish bladder contractions due to the saturation of bladder muscarinic receptors or to its calcium antagonist activity. When contractions restart, they show high frequency (higher than that observed in the basal period) and a marked reduction in their amplitude. This behavior is shared by several other antimuscarinic drugs such as propantheline and emepronium bromide (Guarneri et al., 1993). The effects of Rec 15/3079 in this experimental model were qualitatively and quantitatively similar to those of morphine (block of the contractions and no effects on their amplitude) and WAY 100635 (Testa et al., 1999). However, receptor binding studies and the lack of an inhibitory effect of naloxone (tested at a dose completely blocking the effect of morphine) in this model seem to exclude a modulation or an

![Fig. 6. Effect of Rec 15/3079 in different animal models of anxiety. Open bars, controls; dashed bars, Rec 15/3079 at 1, 3, and 10 mg/kg i.v., respectively; crossed bars, reference compounds (from top to bottom: clobazam, 16 mg/kg i.v.; clobazam, 8 mg/kg i.v.; and diazepam, 2 mg/kg i.v.). Numbers on bars represent the percent change versus controls. *p < 0.05; **p < 0.01; ***p < 0.001 versus controls.

![Fig. 7. Effect of Rec 15/3079 in different animal models of depression. Open bars, controls; dashed bars, Rec 15/3079 at 1, 3, and 10 mg/kg i.v., respectively; crossed bars, reference compound (imipramine, 8 mg/kg i.v.). Numbers on bars represent the percent change versus controls. *p < 0.05; **p < 0.01; ***p < 0.001 versus controls.
interaction of Rec 15/3079 with opiate receptors. Citalopram potentiated the effect of Rec 15/3079 in the voiding contraction model suggesting that 5-HT release could be involved in the mechanism of action of Rec 15/3079. Citalopram in fact increases the extracellular concentration of 5-HT, thereby activating inhibitory somatodendritic 5-HT1A receptors and consequently reducing the firing activity of 5-HT neurons and the release of extracellular serotonin. The combined treatment of citalopram and the 5-HT1A antagonist overrides the negative feedback increasing the extracellular concentration of 5-HT more than the former drug alone.

In conscious animals, micturition occurs in response to distension of urinary bladder wall. Once threshold tension is achieved, activation of afferents, conveyed by the pelvic nerve, triggers the relay center in the dorsolateral tegmentum of the pons, which has been referred as the pontine micturition center. Descending input from the pontine micturition center activates preganglionic neurons in the L6 and S1 spinal cord, which send axons in the pelvic nerve that convey excitatory input to the bladder. At least two types of afferent neurons innervate the urinary bladder. One type is mechanosensitive, with myelinated axons, and is activated by both low (non-nociceptive) and high (nociceptive) intraves-

ical pressure. The second type of afferents does not respond to bladder distension, possesses unmyelinated axons, and is activated by cold or chemical irritation of the bladder mucosa. These latter afferents are believed to have primarily nociceptive functions (Birder and deGroat, 1992).

In conscious rats with saline filled bladders (where mechanosensitive afferents are mainly involved), Rec 15/3079 increased bladder capacity without affecting bladder contractility. On the contrary, the antimuscarnic oxybutynin clearly decreased MP and had no effect on bladder capacity. In our experiments using diluted acetic acid as irritant, Rec 15/3079 showed a protective effect after i.v. administration of 0.3 mg/kg (i.e., the decrease of BVC induced by acetic acid was reversed). This effect was greater than that exerted by the same dose of indomethacin, a well-known antiinflammatory drug, and was similar to that of WAY 100635. Cystometry studies were also performed in conscious guinea pigs, as this animal model is particularly suitable for the evaluation of drugs on bladder contractility (Noronha-Blob et al., 1989). In this model, Rec 15/3079 was again active at increasing BVC with no marked effect upon MP whereas oxybutynin exhibited a potent depressant action on bladder contractility.

The in vivo effects of Rec 15/3079 are unlikely to involve actions at the level of the urinary bladder because only high concentrations of the drug influenced carbachol and noradrenaline-induced contraction of lower urinary tract tissues in vitro. Such concentrations of Rec 15/3079 are not achieved in vivo with the doses used in this study.

A number of therapeutic targets at the level of central nervous system have been proposed for 5-HT1A receptor antagonists based upon the activity of 5-HT1A ligands in preclinical models, developed neurochemical hypotheses, and the localization of the receptor in different brain regions (Schechter and Kelly, 1997). Beyond the positive effects on the lower urinary tract function observed (Testa et al., 1999; present study), therefore, there could be other potential central activity for 5-HT1A receptor antagonists.

Rec 15/3079, in doses at least 10-fold higher than those active on the bladder, does not exhibit significant anxiolytic effect. Similarly, only large doses of WAY 100635 (2.5–5 mg/kg) had a mild anxiolytic activity on the elevated plus maze in mice (Mendoza et al., 1999). On the other hand, WAY 100635 was not active in the conflict model, which has been very predictive of anxiolytic activity of novel compounds (Schechter and Kelly, 1997).

Rec 15/3079 had no effect in the hot plate test but increased the tail-flick latency in all doses tested. In this regard, it has been reported (Alhaider and Wilcox, 1993) that intrathecal administration of 5-HT1A agonists (e.g., 8-OH-DPAT, busipirone, etc.) significantly shortened the tail-flick reflex. This effect was reversed by the 5-HT1A antagonist pindolol (Corradetti et al., 1998), suggesting that 5-HT1A antagonists could inhibit the facilitatory role of spinal 5-HT1A receptors on nociceptive transmission. The effects of Rec 15/3079 on spinal analgesia suggest that part of the activity of this compound may be exerted at the spinal level, as previously reported for WAY 100635 (Kakizaki et al., 1998).

Summarizing, Rec 15/3079 can be considered a centrally acting compound affecting the central transmission of the voiding impulse without decreasing bladder contractility. Thus, Rec 15/3079 is in sharp contrast to oxybutynin and
other anticholinergic drugs, which act mainly by blocking bladder contractility. Rec 15/3079 does not exhibit relevant effects at central nervous system until one uses doses at least 10-fold higher than those active on the bladder.

A common cause of incontinence (about 33%) is impaired bladder contractile function along with detrusor hyperactivity (Resnick and Yalla, 1987). Depression of bladder contractility increases residual volume, an unwanted side effect of drugs with anticholinergic profile like oxybutynin (Cardozo et al., 1987; Andersson, 1988), and therefore should be avoided in patients with impaired detrusor function. Rec 15/3079, because of its lack of effects on micturition pressure, could represent an advantage over the existing therapies.

Acknowledgments

We thank Dr. D. E. Clarke for his important contribution to the discussion of the paper. The technical assistance of C. Destefani, S. Schiavi, I. Simonazzi, and R. Cova is acknowledged gratefully.

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