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Report

Receptor Binding Studies of the Flavone, REC 15/2053, and Other Bladder Spasmolytics

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The new flavone derivative REC 15/2053, a compound with spasmolytic activity on the lower urinary tract, was examined for its *in vitro* interaction with alpha- and beta-noradrenergic receptors, dopaminergic, muscarinic, serotoninergic, and opiate receptors, and calcium-channel binding sites labeled with 1,4-dihydropyridines from normal rat brain. All the investigated receptors are directly or indirectly involved in the nervous control of the lower urinary tract functions. The activity of REC 15/2053 on these receptors was studied in comparison to the most common drugs used in the management of urinary bladder disorders such as flavoxate, emepronium bromide, oxybutynin, terodiline, and imipramine. REC 15/2053 showed only weak binding to [^{3}H]nitrendipine sites (IC_{50} = 14 μM) and muscarinic receptors (IC_{50} = 18 μM), whereas flavoxate was slightly active only at muscarinic receptors (IC_{50} = 12.2 μM). Emepronium bromide, oxybutynin, and terodiline were active only at muscarinic receptors, with IC_{50} values of 236, 5.4, and 588 nM, respectively. Oxybutynin showed a weak affinity to [^{3}H]nitrendipine binding sites (IC_{50} = 44.4 μM). Imipramine was active at alpha 1-adrenergic and muscarinic receptors (IC_{50} = 248 and 653 nM, respectively). The activity of REC 15/2053 at muscarinic receptors and 1,4-dihydropyridine binding sites seems too low to account for its mechanism of action.

KEY WORDS: Rec 15/2053; receptor profile; micturitional disorders; bladder; spasmolytics.

INTRODUCTION

In the past few years, disturbances of the lower urinary tract functions, such as incontinence, have attracted increasing interest (1,2). Many drugs have been used for the management of these disturbances (1,3,4), but their efficacy is limited and adverse reactions are common (3).

REC 15/2053 (5), a new flavone derivative chemically related to flavoxate (see Scheme I), has been reported to display an antispasmodic activity at the bladder level, since it antagonized $BaCl_2$-induced rat bladder contractions after i.v. administration in a noncompetitive way, with a pD'_2 = 4.73 (flavoxate, 4.51) (6). In anesthetized rats the compound, administered orally, inhibited saline-induced bladder voiding contractions, removing the micturition stimuli (6).

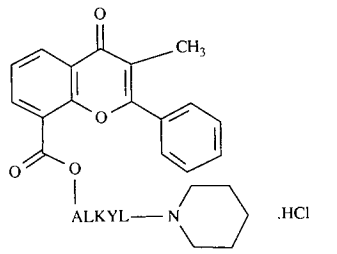
In this test REC 15/2053 and oxybutynin displayed almost equal activity, with ED_{30} values of 31.5 and 27.4 mg/kg, respectively. A positive performance on urodynamic parameters, such as the bladder volume capacity and micturition pressure in conscious rat cystometry, was also reported (6).

Recently many papers have been published describing the detailed anatomy and innervation of bladder and urethra (7–9). Alpha- and beta-adrenergic receptors, as well as dopaminergic, serotoninergic, muscarinic, and opiate receptors together with voltage-sensitive calcium channels, have been shown to be directly or indirectly involved in the nervous control of lower urinary tract functions (10–23 and references therein).

We therefore examined the effects of REC 15/2053 on the above-reported neurotransmitter receptors and binding sites. For comparison, we have extended this study to the most commonly utilized drugs in the management of urinary bladder disorders, such as flavoxate, emepronium bromide, oxybutynin, terodiline, and imipramine (1,3).

METHODS (See Table I for Details and References)

Male Sprague Dawley rats weighing 180–230 g (Charles River, Italia) were decapitated and the brains were removed



	FLAVOXATE	Rec 15 / 2053
ALKYL	-CH_2 CH_2-	CH_3 | -CCH_2- | CH_3

Scheme I

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Table I. Binding Assays: Summary of Methods[a]

Receptor	Alpha 1	Alpha 2	Beta 1 + 2	DA2	5-HT2	Muscarinic M1 + M2	Opiates	Ca^{2+} channels
Tissue	Brain – cerebellum	Brain – cerebellum	Cortex	Striatum	Cortex	Brain – cerebellum	Cortex	Brain – cerebellum
mg tissue/ml	10	10	10	5	10	0.5	7	5
Buffer (0.05 *M*)	Tris/HCl (a)	Tris/HCl (a)	Tris/HCl (b)	Tris/HCl (c)	Tris/HCl (e)	Na/K phosphate	Tris/HCl (d)	Tris/HCl
pH (25°C)	7.4	7.4	8.0	7.4	7.7	7.4	7.4	7.7
^{3}H-Ligand	Prazosin	Clonidine	DHA	Spiperone	Spiperone	QNB	Naloxone	Nitrendipine
Conc. (n*M*)	0.3	1	1	0.3	1	0.06	1	0.4
Definition of specific binding	Prazosin	1-NE	Pro-pranolol	(+)-Buta-clamol	Methy-sergide	Atropine	Naloxone	Nifedipine
Conc. (nM)	1,000	100,000	2,000	1,000	5,000	5,000	36,000	1,000
Inc. time (min.)	30	30	10	15	15	60	30	60
Incubation temp. (°C)	25	25	37	37	37	25	25	25
K_d of ligand (n*M*)	0.12	1.6	2.5	0.6	0.4	0.1	1.8	0.43
Reference(s)	28	29	30, 31	32	33	34	35	36, 37

[a] (a) buffer + 10 μ*M* pargyline + 0.1% ascorbic acid; (b) buffer + 3 m*M* $MgCl_2$; (c) buffer + 120 m*M* NaCl + 5 m*M* KCl + 2 m*M* $CaCl_2$ + 1 m*M* $MgCl_2$ + 10 μ*M* pargyline + 0.1% ascorbic acid; (d) buffer + 50 m*M* NaCl; (e) buffer + 1 m*M* $MgCl_2$. DHA, dihydroalprenolol; QNB, quinuclinidylbenzilate; 1-NE, 1-norepinephrine.

rapidly. Dissected cortical tissue (beta, serotonin-2, and opiate receptors), striatal tissue (dopamine-2 receptors), or the whole brain minus the cerebellum (alpha-adrenergic and muscarinic receptors as well as voltage-sensitive calcium channels) was homogenized on ice in buffer using a Polytron homogenizer. The homogenates were centrifuged (48,000*g*) and resuspended three times in fresh buffer to wash out endogenous ligands. In the nitrendipine binding assay, a 1000*g* centrifugation was run previously to precipitate nuclei and gross cell debris, which contribute nonspecifically to the binding.

The final pellet was resuspended in an appropriate volume of buffer to obtain the expected protein concentration in the incubation mixture [evaluated by the method of Bradford (24)].

Incubation was generally done in 10-ml polyethylene tubes. Tritiated ligand (20 μl) and the compound under evaluation (20 μl) were added before the aliquot (1–2 ml) of tissue suspension. All the drugs were initially tested at a 1 μ*M* concentration. In the presence of significant displacing activity, a complete competition curve was performed. Following incubation, cold buffer was added to each sample, and the contents were rapidly vacuum-filtered through Whatman GF/B glass-fiber filters. The filters were then rapidly washed three times with 5 ml of buffer, placed in scintillation vials, and shaken with 10 ml of Filtercount scintillation mixture.

Specific binding was defined as the total binding minus the binding in the presence of the displacing agent. Binding in the presence of various concentrations of test compound

Table II. Effects of REC 15/2053 and Reference Drugs on Rat Brain Receptors (Data Represent the IC_{50} (n*M*)[a]

Receptor	Alpha 1	Alpha 2	Beta	DA2	5-HT2	Ach. (muscarinic)	Opiates	Ca^{2+} channels
^{3}H-Ligand	Prazosin	Clonidine	DHA	Spiperone	Spiperone	QNB	Naloxone	Nitrendipine
REC 15/2053	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
Flavoxate	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
Emepronium bromide	>1000	>1000	>1000	>1000	>1000	236	>1000	>1000
Oxybutynin	>1000	>1000	>1000	>1000	>1000	5.4	>1000	>1000
Terodiline	>1000	>1000	>1000	>1000	>1000	588	>1000	>1000
Imipramine	248	>1000	>1000	>1000	>1000	653	>1000	>1000
Phenoxybenzamine	3.5	263	>1000	880	53	>1000	>1000	>1000
Yohimbine	>1000	7.3	>1000	>1000	>1000	>1000	>1000	>1000
Propranolol	>1000	>1000	7.3	>1000	>1000	>1000	>1000	>1000
Haloperidol	43	>1000	>1000	5.5	>1000	>1000	>1000	>1000
Methysergide	>1000	>1000	>1000	597	5.3	>1000	>1000	>1000
Atropine	>1000	>1000	>1000	>1000	>1000	58	>1000	>1000
Morphine	>1000	>1000	>1000	>1000	>1000	>1000	428	>1000
Nifedipine	>1000	>1000	>1000	>1000	>1000	>1000	>1000	2.4

[a] DHA, dihydroalprenolol; QNB, quinuclinidylbenzilate.

was expressed as a percentage of the specific binding with no drug present. The IC_{50} values and comparison among different displacing curves were evaluated by simultaneous nonlinear curve fitting according to the logistic equation as reported by De Lean *et al.* (25).

All radioactive chemicals were purchased from New England Nuclear. The other compounds and reagents were purchased from standard commercial sources.

RESULTS AND DISCUSSION

The effects of the different drugs considered in this study on adrenergic, muscarinic, dopaminergic, and serotoninergic receptors as well as on voltage-sensitive calcium channel and opiate receptors are summarized in Table II. Data on the activity of the most commonly used displacers of the receptors studied are also included in Table II.

REC 15/2053 was devoid of activity against alpha- and beta-adrenergic receptors.

None of the compounds commonly utilized in the management of urinary bladder dysfunctions (namely, flavoxate, emepronium bromide, oxybutynin, terodiline, and imipramine) inhibited the binding of [^{3}H]DHA to beta-adrenergic receptors or that of [^{3}H]clonidine to alpha 2-adrenergic receptors. The only compound inhibiting the binding of [^{3}H]prazosin to alpha 1-adrenergic receptors was imipramine, and its IC_{50} (248 n*M*) is in agreement with results reported previously (26,27).

All the drugs tested showed no activity or a displacing activity of less than 50% against DA2, 5-HT2, or opiate binding sites, indicating very little interaction with these receptors.

A number of compounds displaced [^{3}H]QNB from the muscarinic receptors. Almost all the reference drugs were active in the nanomolar range: oxybutynin (IC_{50} = 5.4 n*M*) > emepronium bromide (IC_{50} = 236 n*M*) > terodiline (IC_{50} = 588 n*M*) = imipramine (IC_{50} = 653 n*M*). Since a major part of bladder contractions seems to be mediated via muscarinic cholinoceptors, REC 15/2053 and flavoxate were tested at concentrations higher than 1 μ*M*. Both compounds were active at the micromolar level, with IC_{50} values of 18 and 12.2 μ*M*, respectively. Displacement of tritiated QNB by different concentrations of all the therapeutically relevant compounds studied is depicted in Fig. 1.

It is well known that part of the bladder contraction elicited by electrical stimulation of the pelvic nerves or by transmural electrical stimulation is resistant to atropine. The atropine-resistant part of the contraction can be blocked by the addition of a calcium entry blocker such as nifedipine (2). Owing to this fact, all the reference drugs as well as REC 15/2053 were tested at concentrations higher than 1 μ*M* on the Ca^{2+} channels binding sites. The displacement curves obtained by unconstrained nonlinear fitting of all the drugs tested on [^{3}H]nitrendipine binding, according to the logistic equation (25), are shown in Fig. 2.

REC 15/2053 and oxybutynin showed IC_{50} values higher than 10 μ*M* (14 and 44.4 μ*M*, respectively), indicating that very little interaction with this binding site should be expected at therapeutic doses. Flavoxate and terodiline displaced [^{3}H]nitrendipine at meaningless concentrations higher than 100 μ*M* (IC_{50} = 139 and 245 μ*M*, respectively). Imipramine and emepronium bromide were inactive up to 100 μ*M*.

In conclusion, on the basis of these results, REC 15/2053 displayed no relevant affinity for the receptors studied, with a weak displacing activity on the 1,4-dihydropyridine binding site that is too low to justify entirely its pharmacological activity. The low affinity for muscarinic receptors, in contrast to the reference drugs, may be indicative of the absence of the typical anticholinergic side effects in incontinence therapy, such as dryness of the mouth, accommodation disturbances, and tachicardia.

Finally, if the results obtained on the receptors consid-

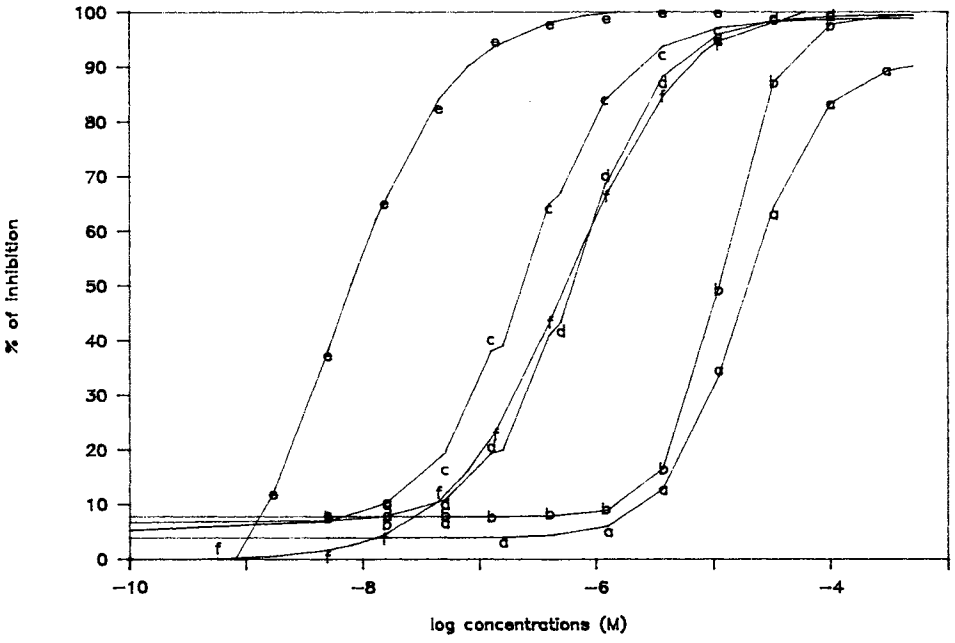

Fig. 1. Displacement of [^{3}H]QNB by different concentrations of REC 15/2053 (a), flavoxate (b), emepronium bromide (c), imipramine (d), oxybitynin (e), and terodiline (f).

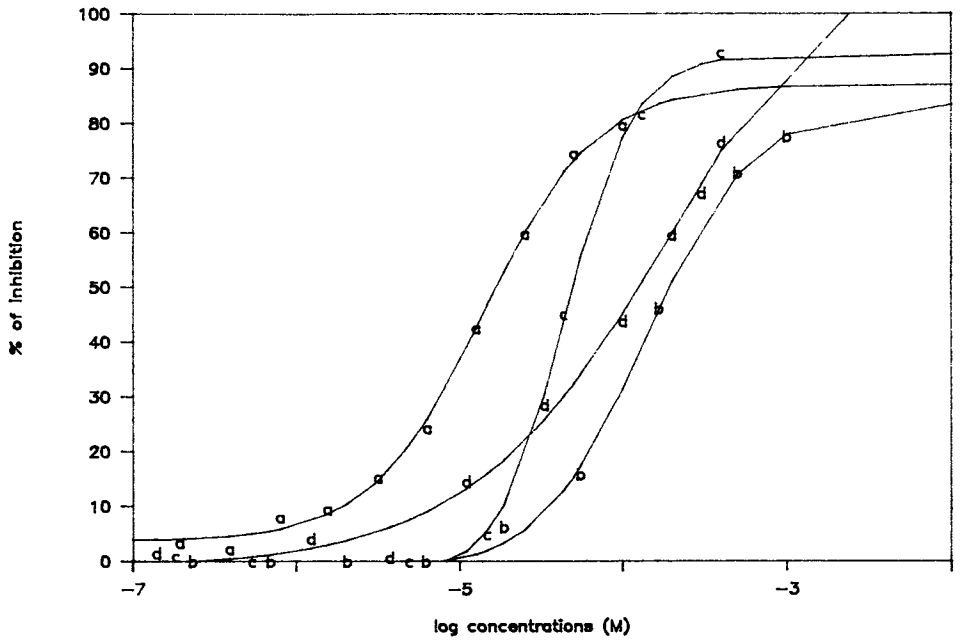

Fig. 2. Displacement of [^{3}H]nitrendipine by different concentrations of REC 15/2053 (a), flavoxate (b), oxybutynin (c), and terodiline (d).

ered herein cannot explain the mechanism of action of REC 15/2053, they can exclude undesirable side effects due to an interaction with the receptors studied.

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REFERENCES

1. N. M. Resnick and S. V. Yalla. *N. Engl. J. Med.* 313:800–805 (1985).
2. K. E. Andersson. *T.I.P.S.* 5:521–523 (1984).
3. K. E. Andersson and C. Sjogren. *Prog. Neurobiol.* 19:71–89 (1982).
4. K. E. Andersson and U. Ulmsten. *Acta Pharmacol. Toxicol.* 46:7–11 (1980).
5. D. Nardi, A. Tajana, R. Pennini, P. Cazzulani, G. Graziani, and S. Casadio. British Patent No. 2104507 (1981).
6. C. Pietra, P. Cazzulani, and R. Ceserani. *Neurol. Urodyn.* 6:247–248 (1987).
7. E. E. Daniel, W. Cowan, and V. P. Daniel. *Can J. Physiol. Pharmacol.* 61:1247–1273 (1983).
8. A. Elbadawi. *Neurol. Urodyn.* 1:3–50 (1982).
9. J. A. Gosling, J. S. Dixon, and J. R. Humpherson. *Functional Anatomy of the Urinary Tract,* University Park Press, London and New York, 1982.
10. G. Tsujimoto, P. F. Timmins, and B. B. Hoffman. *J. Pharmacol Exp. Ther.* 236:384–389 (1986).
11. S. A. Awad, A. W. Bruce, G. Carro-Ciampi, J. W. Downie, and M. Lin. *Br. J. Pharmacol.* 50:525–529 (1974).
12. R. M. Levin, F. S. Shofer, and A. J. Wein. *J. Pharmacol. Exp. Ther.* 212:536–540 (1980).
13. S. Vaidyanathan, M. S. Rao, B. C. Bapna, P. L. Sharma, K. S. N. Chary, and R. P. Swamy. *Ann. Clin. Res.* 12:49–51 (1980).
14. H. A. Bom, P. R. Saxena, and J. D. Bieseebek. *Br. J. Pharmacol.* 89:547P (1986).
15. P. Klarskov and J. H. Petersen. *Proc. 15th I.C.S.* 3(6):154 (1985).
16. L. Nilvebrant. *Acta Pharmacol. Toxicol.* 59:Suppl. 1 (1986).
17. G. F. Anderson and B. H. Marks. *J Pharmacol. Exp. Ther.* 221:598–603 (1982).
18. R. Rubinstein, I. Nissenkorn, and S. Cohen. *Eur. J. Pharmacol.* 100:21–27 (1984).
19. A. Dray, L. Nunan, and W. Wire. *Br. J. Pharmacol.* 85:717–726 (1985).
20. T. Hisamitsu and W. C. deGroat. *Brain Res.* 298:51–65 (1984).
21. A. Dray and R. Metsch. *J. Pharmacol. Exp. Ther.* 231:254–260 (1984).
22. F. B. Yousif, G. T. Bolger, A. Ruzycky, and D. J. Triggle. *Can. J. Physiol. Pharmacol.* 63:453–462 (1985).
23. A. Forman, K. E. Andersson, L. Henriksson, T. Rud, and U. Ulmsten. *Acta Pharmacol. Toxicol.* 43:111–118 (1978).
24. M. M. Bradford. *Anal. Biochem.* 72:248–254 (1976).
25. A. De Lean, P. J. Munsen, and D. Rodbard. *Am. J. Physiol.* 235:E97–E102 (1978).
26. E. Richelson and A. Nelson. *J. Pharmacol. Exp. Ther.* 230:94–102 (1984).
27. H. Hall and S. O. Ogren. *Eur. J. Pharmacol.* 70:393–407 (1981).
28. P. Greengrass and R. Bremner. *Eur. J. Pharmacol.* 55:323–326 (1979).
29. D. C. U'Prichard, D. A. Greenberg, and S. H. Snyder. *Mol. Pharmacol.* 13:454–473 (1977).
30. D. B. Bylund and S. H. Snyder. *Mol. Pharmacol.* 12:568–580 (1976).
31. L. H. Greenberg and B. Weiss. *J. Pharmacol. Exp. Ther.* 211:309–316 (1979).
32. M. P. Matres, M. Bandry, and J. L. Schwartz. *Life Sci.* 23:1781–1784 (1978).
33. I. Creese and S. H. Snyder. *Eur. J. Pharmacol.* 49:201–202 (1978).
34. H. Y. Yamamura and S. H. Snyder. *Proc. Natl. Acad. Sci.* 71:1725–1729 (1974).
35. K. Stengaard-Pedersen and L. I. Larsson. *Acta Pharmacol. Toxicol.* 48:39–46 (1981).
36. G. T. Bolger, P. Gengo, R. Klockowski, E. Luchowshi, H. Siegel, R. A. Janis, A. M. Triggle, and T. J. Triggle. *J. Pharmacol. Exp. Ther.* 225:291–310 (1983).
37. K. M. M. Murphy and S. H. Snyder. *Eur. J. Pharmacol.* 77:201–202 (1982).