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Pharmacological profile of enantiomerically pure chiral muscarinic agonists Desoxymuscarines

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Abstract

The enantiomers desoxymuscarine 6 were tested *in vitro* on guinea pig tissues, and their muscarinic potency was evaluated at M_2 (heart force and rate) and M_3 (ileum and bladder) receptor subtypes together with the enantiomers of the parent compound muscarine 1. The eutomers (+)-1 and (+)-6 and distomers (−)-1 and (−)-6 were also assayed *in vivo* on pithed rat. Affinity, relative efficacy and enantioselectivity were also determined for the compounds under study at M_2 (heart force and rate) and M_3 (ileum and bladder), in order to investigate muscarinic receptor heterogeneity. The results of this study have been discussed in comparison with the data previously reported for the structurally related fluoromuscarine (+)-4 and difluoromuscarines (+)-5 and (−)-5.

Keywords: Muscarinic receptor subtypes; Muscarine enantiomers; Desoxymuscarine enatinomers

Introduction

Molecular handedness is a crucial structural feature of biologically active compounds, since opposite configurations at pharmacophoric groups are frequently influencing the biological response, mainly in terms of affinity, toxicity and receptor subtype selectivity (1).

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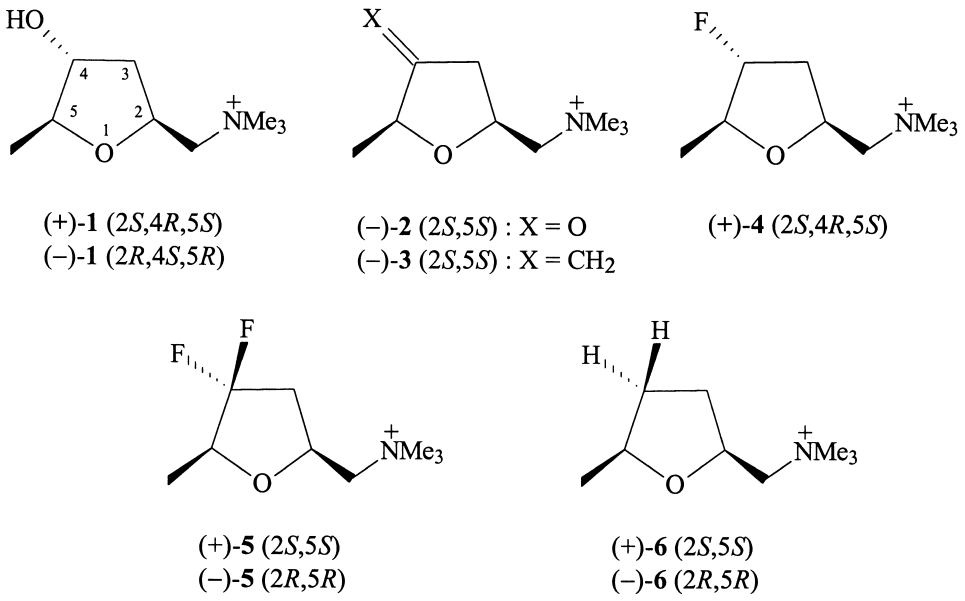
Therefore, the stereoisomeric composition of drugs is currently receiving considerable attention owing to its pharmacological as well as industrial and regulatory implications (2,3).

The muscarinic receptor population represents a family of cholinergic receptors abundant in the parasympathetic and in central nervous systems, where they mediate both excitatory and inhibitory effects (4). At present, the pharmacological classification of muscarinic receptors distinguishes among M_1, M_2, M_3 and M_4 subtypes. A fifth subtype, termed M_5, has been predicted by molecular cloning studies, even though its physiological role is yet unknown (5,6). The characterization of muscarinic receptor subtypes is based essentially on the discovery of selective antagonists (7–11). However, the development of new highly potent and subtype-selective muscarinic receptor agonists could provide novel therapeutic agents useful, for example, in the treatment of pain and Alzheimer's disease (12,13).

Well defined stereochemical requirements in the molecular structure of muscarinic ligands characterized by the presence of a tetrahydrofuran ring, i.e. muscarine and muscarone (14–16), are essentials for their interaction with the complementary receptor subsites. All the major chiral muscarinic agonists show in fact a high value of the eudismic ratio (ER) and a spatial arrangement around the chiral centers matching those of natural muscarine (+)-1 (17) (Fig. 1). These outcomes emerged also from our studies on the eight chiral muscarine stereoisomers (18), as well as the four chiral isomers of muscarone (2) and methylenemuscarone (3). The absolute configuration of the most potent stereoisomer of muscarone (19) and methylenemuscarone (20) is depicted in Fig.1. With the aim of studying further the relationship between the muscarinic activity/selectivity and the different substitution patterns at position 4 ("muscarinic subsite"), we synthesized and tested (2*S*,4*R*,5*S*)-4-deoxy-4-fluoromuscarine (+)-4 (fluoromuscarine, Fig. 1) and the two enantiomers of 4-deoxy-4,4-difluoromuscarine (+)-5 and (−)-5 (difluoromuscarine, Fig. 1).



(+)-**1** (2*S*,4*R*,5*S*)
(−)-**1** (2*R*,4*S*,5*R*)

(−)-**2** (2*S*,5*S*) : X = O
(−)-**3** (2*S*,5*S*) : X = CH_2

(+)-**4** (2*S*,4*R*,5*S*)

(+)-**5** (2*S*,5*S*)
(−)-**5** (2*R*,5*R*)

(+)-**6** (2*S*,5*S*)
(−)-**6** (2*R*,5*R*)

Fig. 1.

The pharmacological profile of these new chiral muscarinic agonists was investigted by means of *in vivo* and *in vitro* assays, and the results compared to those of racemic muscarine (±)-1 (21,22). The replacement of the hydroxy group of muscarine with a fluorine atom, i.e. (+)-4, caused a significant change only in the receptor-ligand interaction at the cardiac M_2 muscarinic receptors controlling rate (21), whereas the introduction of two fluorine atoms at position 4, i.e. (+)- and (−)-5, produced significant differences in the affinity and relative efficacy at M_2 (heart rate) and M_3 (ileum) receptor subtypes, with a parallel drop of the enantioselectivity (22).

In order to deepen the investigation of the stereoelectronic requirements at the "muscarinic subsite", recently we prepared the chiral forms of desoxymuscarine 6 (Fig.1), whose racemate is a moderately potent muscarinic agonist (23). Both enantiomers of 6 were obtained with enantiomeric excess higher than 98% through a synthetic sequence based on a chemoenzymatic approach (24). In this paper we report the results of the pharmacological characterization of (+)-and (−)-6 at M_2 (heart) and M_3 (ileum and bladder) muscarinic receptor subtypes.

Results and discussion

Muscarine and desoxymuscarine enantiomers (+)-1, (−)-1, (+)-6 and (−)-6, prepared according to published procedures (18,24), were tested *in vitro* on guinea pig tissues, and their muscarinic potency (pD_2) was evaluated at M_2 (heart force and rate) and M_3 (ileum and bladder) receptor subtypes. All the compounds behaved as full agonists ($\alpha = 1$, Table 1). In order to evaluate their muscarinic activity (ED_{50}) at ganglionic M_1 and cardiac (heart rate) M_2 receptors, the derivatives under study were also tested *in vivo* on pithed rat (Table 2). Since the only comparison of agonist potencies on different tissues is not sufficient to speculate on the heterogeneity of muscarinic receptors, affinity and relative efficacy were calculated (Table 3). Finally, the enantioselectivity, which is a parameter of utmost importance in the characteriza-

Table 1
Potencies, intrinsic activities and eudismic ratios of muscarines (+)-1/(−)-1 and desoxymuscarines (+)-6/(−)-6, at M_2 and M_3 muscarinic receptors

	Tissue							
	Guinea pig heart (M_2)				Guinea pig ileum (M_3)		Guinea pig bladder (M_3)	
	force		rate					
	pD_2[a]	α[b]	pD_2[a]	α[b]	pD_2[a]	α[b]	pD_2[a]	α[b]
(+)-1	7.68 ± 0.10	1.0	7.07 ± 0.14	1.0	7.05 ± 0.10	1.0	5.58 ± 0.05	1.0
ER[c]	302		214		63		4.3	
(−)-1	5.20 ± 0.22	1.0	4.74 ± 0.24	1.0	5.25 ± 0.12	1.0	4.95 ± 0.29	1.0
(+)-6	6.44 ± 0.06	1.0	6.59 ± 0.14	1.0	6.87 ± 0.15	1.0	5.22 ± 0.09	1.0
ER[c]	1.2		1.1		18		58	
(−)-6	6.36 ± 0.06	1.0	6.54 ± 0.18	1.0	5.62 ± 0.09	1.0	3.46 ± 0.11	1.0

[a] $-\log ED_{50}$. The results are the mean ± SEM, and the number of observations varies between 6 and 10.

[b] Intrinsic activity, measured by the ratio between the maximum response of the compound and the maximum response of (±)-muscarine.

[c] Eudismic ratio: the ratio between the potency of the more potent and the less potent enantiomer.

Table 2
Potencies of (+)-1, (−)-1, (+)-6 and (−)-6 at ganglionic M_1 receptors mediating tachycardia and at cardiac M_2 receptors mediating bradycardia in the pithed rat

	ED_{50} (μg/kg),pithed rat[a]	
	Increase in heart rate (M_1)	Decrease in heart rate (M_2)
(+)-1	3.0 ± 0.50	4.5 ± 0.75
ER[b]	179	14
(−)-1	536 ± 61	65.0 ± 7.2
(+)-6	13.9 ± 1.9	23.8 ± 1.8
ER[b]	27	10
(−)-6	377.8 ± 35.4	238.0 ± 93

[a] The results are the mean ± SEM, and the number of observations varies between 5 and 9.
[b] Eudismic ratio: the ratio between the potency of the more potent and the less potent enantiomer.

tion of receptor subgroups, was evaluated as the eudismic ratio (ER) both in *in vitro* and *in vivo* assays.

Inspection of the data gathered in Table 1 reveals that natural muscarine (+)-1 proved to be the most potent agonist at all the studied tissues. Furthermore, as expected, (+)-6, which shares with (+)-1 the absolute configuration at the two common stereogenic centers, emerged as the eutomer of desoxymuscarine.

Interestingly, muscarine and desoxymuscarine did not display the same potency trend among the tested receptor subtypes, and their ER values were accordingly affected. Indeed, the couple (+)-1/(−)-1 showed high and comparable ER values in the two M_2 assays, i.e. guinea pig heart force (ER=302) and rate (ER=214), whereas the values were lower and quite different in the two typical M_3 tests, i.e. guinea pig ileum (ER=63) and urinary bladder (ER=4.3). Conversely, the couple (+)-6/(−)-6 displayed the highest ER values at M_3 receptors, i.e. guinea pig urinary bladder (ER=58), and no enantioselectivity at cardiac M_2 subtypes (ER=1.2 and 1.1).

As expected, the *in vivo* data, obtained according to the method reported by Angeli et al. (22,25), showed that natural muscarine (+)-1 is more potent than (+)-6, the eutomer of desoxymuscarine, at ganglionic M_1 and cardiac M_2 receptors subtypes (Table 2). It is worth pointing out that the pronounced enantioselectivity observed for muscarine in the in vitro M_2 assays (ER=214) is drastically reduced in the in vivo test (ER=14). The opposite trend holds true for desoxymuscarine, which gained a moderate enantioselectivity in the in vivo assay (ER=10). As already postulated (21,22), the different results between the *in vitro* and *in vivo* assays could be attributed to differences in metabolism and pharmacokinetics among the agonists in the two preparations.

In order to evaluate a possible heterogeneity within the M_2 (heart force and rate) and M_3 (ileum and bladder) muscarinic receptor populations (21), further pharmacological parameters such as affinity (K_D) and relative efficacy (e_r) have been determined, as shown in Table 3. In the same table the data previously reported for fluoromuscarine (+)-4 and difluoromuscarines (+)-5 and (−)-5 have been included.

The comparison of the responses of the structurally related ligands gathered in Table 3 further supports previous studies on the nature of the interaction involving the binding of the hy-

Table 3
Pharmacological parameters of (+)-1, (−)-1, (+)-4, (+)-5, (−)-5, and (−)-6 in the guinea pig heart force and rate (M_2) and ileum and bladder (M_3)

	Tissue											
	Heart				Heart				Heart			
	Force pD_2[a]	Rate pD_2[a]	Ileum pD_2[a]	Bladder pD_2[a]	Force pK_D[a]	Rate pK_D[a]	Ileum pK_D[a]	Bladder pK_D[a]	Force e_r[b]	Rate e_r[b]	Ileum e_r[b]	Bladder e_r[b]
(+)-1	7.68 ± 0.10	7.07 ± 0.14	7.05 ± 0.10	5.58 ± 0.05	5.30 ± 0.12	3.67 ± 0.32	5.74 ± 0.23	4.81 ± 0.09	1.0	1.0	1.0	1.0
ER[c]	302	214	63	4.3	93	6	372	23	3	33	0.2	0.2
(−)-1	5.20 ± 0.22	4.74 ± 0.24	5.25 ± 0.12	4.95 ± 0.29	3.33 ± 0.19	2.89 ± 0.30	3.17 ± 0.19	3.45 ± 0.30	0.3	0.03	6.0	4.8
(+)-4[d]	6.73 ± 0.08	6.87 ± 0.04	7.36 ± 0.03	5.66 ± 0.03	4.67 ± 0.12	5.77 ± 0.17	5.72 ± 0.11	4.39 ± 0.09	0.5	0.005	2.0	2.8
(+)-5[e]	6.56 ± 0.14	6.58 ± 0.13	5.80 ± 0.07	5.23 ± 0.17	4.37 ± 0.21	3.39 ± 0.15	4.70 ± 0.08	4.40 ± 0.16	0.65	0.6	0.6	1.1
ER[c,e]	6.3	1.9	1.9	26	5.4	0.8	1.7	4.9	1.2	2.0	1.0	3.7
(−)-5[e]	5.76 ± 0.13	6.31 ± 0.15	5.53 ± 0.04	3.82 ± 0.06	3.64 ± 0.14	3.48 ± 0.16	4.47 ± 0.14	3.71 ± 0.06	0.55	0.3	0.6	0.3
(+)-6	6.44 ± 0.06	6.59 ± 0.14	6.87 ± 0.15	5.22 ± 0.09	4.40 ± 0.25	4.08 ± 0.09	4.35 ± 0.06	4.30 ± 0.17	0.5	0.1	15.5	1.4
ER[c]	1.2	1.1	18	58	29	13	9	7	0.05	0.07	2.0	4.7
(−)-6	6.36 ± 0.06	6.54 ± 0.18	5.62 ± 0.09	3.46 ± 0.11	2.94 ± 0.37	2.96 ± 0.32	3.39 ± 0.22	3.46 ± 0.31	11.0	1.5	8.0	0.3

[a] The results are the mean ± SEM, and the number of observations varies between 6 and 10.
[b] Relative efficacy [(+)-1 = 1].
[c] Eudismic ratio: the ratio between the potency , the affinity and the relative efficacy of eutomer and distomer.
[d] Reference 21: α values ranging from 0.88 to 0.98.
[e] Reference 22: α values ranging from 0.83 to 1.0.

droxy group of muscarine to the complementary receptor subsite (18,21,22,26–28). The stereogenic center with the (*R*) configuration at position 4, i.e. (+)-1 and (+)-4, is the structural requirement which gives the highest contribution to the potency of the ligands. Interestingly, at the M_2 receptors regulating heart rate, the replacement of the hydroxy group with a fluorine atom, while leaving unaffected the potency of (+)-1 and (+)-4, causes a 126-fold increase of the affinity together with a 200-fold reduction of the efficacy. Moreover, the suppression of the chiral center at the "muscarinic subsite", i.e. (+)-5 and (+)-6, induces a decrease of the values of all the pharmacological parameters, when compared to those determined for natural muscarine. In particular, the introduction of two geminal fluorine atoms at position 4, that is (+)-5, causes an overall lowering of the hydrogen bonding and of the dipole-dipole interaction between the fluorine atom of (+)-4 and the complementary receptor subsite (22). The effect is particularly evident at the M_2 subtype regulating heart rate, where (+)-4 displays a 240-fold higher affinity and a 120-fold lower efficacy than difluoromuscarine (+)-5. An opposite trend is detected, with the exception of the results at bladder, when the data on distomers (−)-1, (−)-5 and (−)-6 are taken into account. Hence, the overall enantioselectivity of muscarines 1, and presumably of fluoromuscarines 4, is significantly higher than that of difluoromuscarines 5 and desoxymuscarines 6. Therefore, the role of the absolute configuration at C-2 and C-5 is enhanced by the presence of a suitably oriented polar group (or atom) at C-4.

As far as tissue selectivity is concerned, based on the Furchgott's assumption that differences in pK_D of at least 0.5 may be indicative of receptor heterogeneity (29), we can deduce that (+)-1 is able to discriminate within M_2 and M_3 receptor subtypes. On the contrary, the affinity data of (+)-6 suggest that desoxymuscarine is not able to discriminate the same receptor populations. Moreover, the affinity values of distomers (−)-1 and (−)-6 are almost superimposable and characterized by a lack of any subtype selectivity. As a consequence, muscarines maintained a relevant enantioselectivity at heart force (ER=93) and reached a high value of eudismic ratio (ER=372) at ileum. The different ER values of (+)-1 and (−)-1 within both the M_2 and M_3 populations (93 versus 6 and 372 versus 23, respectively) further put in evidence the distinct structural requirements of the muscarinic receptor subtypes mediating, on one side, heart force and rate, and, on the other one, those involved in the ileum and bladder.

By taking into account the results obtained with fluoromuscarine (+)-4, the replacement of the hydroxy group of natural muscarine with a fluorine atom brings about significant changes at heart force potency as well as at heart rate affinity and efficacy. As a matter of fact, an inversion of the affinity profile at the M_2 receptor subtypes is observed on passing from (+)-1 to (+)-4 and, in addition, (+)-4 is 100 times more efficacious at receptors mediating heart force than at those mediating heart rate. However, differences in relative efficacy cannot be taken as a proof of receptor heterogeneity, since they may reflect different levels of receptor reserve. Unfortunately, the enantioselectivity parameters are not available for fluoromuscarine, since (−)-4 has not been prepared yet.

On the whole, the estimation of potency as well as affinity and relative efficacy of muscarine, difluoromuscarine and desoxymuscarine enantiomeric pairs further stresses the difficulty of interpreting the variations of these parameters as a function of molecular structure (30). However, the structure-activity relationships of this set of chiral muscarinic ligands, confirm that the M_2 and M_3 receptor subtypes are endowed with distinct structural requirements, that

is the muscarinic populations within the cardiac M_2 (heart force and rate) and M_3 (ileum and bladder) subtypes are characterized by a certain degree of heterogeneity. Among the compounds investigated in this study, natural muscarine (+)-1 and fluoromuscarine (+)-4 represent the pharmacological tools which better put in evidence such a heterogeneity. Since the "muscarinic subsite" is able to host a number of substituents of different size (28), whose influence on the pharmacological profile is not easily predicted, we are now elaborating the functional data of new chiral analogues of muscarine with lipophilic and steric hindered groups at this position.

Experimental section

All animal testing was carried out according to the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Pharmacology

In vitro tests

General considerations. Male guinea pigs (200–300 g) were killed by cervical dislocation, and the organs required were set up rapidly under 1 g of tension in 20-mL organ baths containing physiological salt solution (PSS) kept at an appropriate temperature (see below) and aerated with 5% CO_2-95% O_2. Two dose-response curves were constructed by cumulative addition of the reference agonist [(±)-muscarine]. The concentration of agonist in the organ bath was increased approximately 3-fold at each step, with each addition being made only after the response to the previous addition had attained a maximal level and remained steady. Following 30 min of washing, a new dose-response curve to the agonist under study was obtained. Responses were expressed as a percentage of the maximal response obtained in the control curve. The results are expressed in terms of pD_2, which is the $-\log ED_{50}$, the concentration of agonist required to produce 50% of the maximum contraction. Contractions were recorded by means of a force transducer connected to a two-channel Gemini polygraph (U. Basile). In all cases, parallel experiments in which tissues received only the reference agonist were run in order to check any variation in sensitivity.

Determination of dissociation constants. Dissociation constants (K_D) and relative efficacies (e_r) for compounds (+)-6 and (−)-6 were determined as previously described (29,22) according to the method of Furchgott and Bursztyn (31) for full agonists ($\alpha=1$).

Guinea pig ileum. Two-centimeter-long portions of terminal ileum were taken at about 5 cm from the ileum-cecum junction and mounted in PSS at 37 °C. The composition of PSS was the following (mM): NaCl (118), $NaHCO_3$ (23.8), KCl (4.7), $MgSO_4.7H_2O$ (1.18), KH_2PO_4 (1.18), $CaCl_2$ (2.52), glucose (11.7). Tension changes were recorded isotonically. Tissues were equilibrated for 30 min, and dose-response curves to (±)-muscarine were obtained at 30 min intervals, the first one being discarded and the second one being taken as the control.

Guinea pig bladder. A 2 mm wide longitudinal strip of bladder from urethra to the apex of the bladder was cut, excluding the portion under the urethra orefice, and mounted in PSS (the same used for ileum) at 37 °C. Contractions were recorded isometrically. Tissues were equilibrated for 30 min (see protocol for ileum).

Guinea pig stimulated left atria. The heart was rapidly removed and the right and left atria were separately excised. Left atria were mounted in PSS (the same used for ileum) at 30°C and stimulated through platinum electrodes by square-wave pulses (1 ms, 1 Hz, 5–10 V) (Tetra Stimulus, N. Zagnoni). Inotropic activity was recorded isometrically. Tissues were equilibrated for 2 hours and a cumulative dose-response curve to (±)-muscarine was constructed.

Right atria. Right atria were equilibrated for 1h at the above conditions (see guinea pig stimulated left atria for PSS and temperature). Contractions were recorded isometrically.

In vivo tests

Pithed rat. Male normotensive rats (270–330 g) were housed five per cage and maintained on a 12 h light/dark cycle. Food and water were available ad libitum. The animals were anaesthetized with equithesin (9.6 g nembutal sodium, 42.6 g chloral hydrate, 21.2 g $MgSO_4$, 400 mL propylene glycol, 50 mL ethyl alcohol and water to 1000 mL) 3 mL/kg of body weight ip. The right jugular vein was cannulated (PE 10 polyethylene tubing) for drug administration. Blood pressure was measured from the left common carotid artery through a PE 50 catheter connected to a pressure transducer (P23 ID, Statham, Hato Rey, Puerto Rico). The heart rate was measured continuously by means of a rate meter (Basile) which was triggered by the blood pressure pulse in the carotid artery. After catheterization of the trachea, heparin (150 IU/kg) was given iv to prevent blood coagulation. Temperature was maintained at approximately 37°C throughout the experiment by means of an overhead heating lamp. The rats were then pithed by insertion of a steel rod (1.5 mm in diameter) through the skull and foramen magnum down into the spinal canal (32). The animals were respired artificially by means of a Harvard Apparatus Model 681 rodent respirator at a frequency of 60 cycles/min with a volume of 1 mL/100 g. The preparation was allowed to equilibrate for at least 30 min before drug administration, until mean heart rate had stabilized. The basal heart rate amounted to 300 ± 8 beats/min (n = 50). Changes in heart rate were measured for individual doses of the agonist given iv (0.1 mL/100 g). Full recovery from the pressor and cardiac effects with return to preinjection values was allowed between successive doses. After drug injection, the venous cannula was flushed with 50 μL of isotonic saline solution.

Experimental protocol

All drugs were dissolved in saline (0.9% w/v) and injected iv in a volume of 0.1 mL/100 g. Because of desensitization phenomena, when compounds (+)-1, (−)-1, (+)-6 and (−)-6 were employed, only one single dose-response curve was assessed in each preparation. ED_{50} values were determined graphically from the resultant dose-response curves and represent the dose causing 50% of the maximum response of the compound under study. Pretreatment, iv, with antagonists was carried out 20 min before the administration of agonist. This interval was selected because preliminary experiments showed that after this time the antagonistic effects of pirenzepine (50 μg/kg iv) and tripitramine (30 μg/kg iv), respectively, were constant during the whole experiment.

Statistical analysis

Data are presented as means ± SEM of *n* experiments. Student's t test was used to assess the statistical significance of the difference between two means.

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References

1. R. CROSSLEY, Tetrahedron **48** 8155–8178 (1992).
2. *Drug Stereochemistry*, I.W. Wainer (Ed), Marcel Dekker Inc., New York (1993).
3. *Chirality in Industry II*, A.N. Collins, G.N. Sheldrake and J. Crosby (Eds), John Wiley & Sons, New York (1997).
4. R.M. EGLEN and N. WATSON, Pharmacol. Toxicol. **78** 59–68 (1996).
5. M.P. CAULFIELD, Pharmacol. Therap. **58** 319–379 (1993).
6. M.P. CAULFIELD, N.J.M. BIRDSALL, International Union of Pharmacology. XVII, Pharmacol. Rev. **50** 279–290 (1998).
7. C. SCHUDT, R. BOER, M. ELTZE, R. RIEDEL, G. GRUNDLER and N.J.M. BIRDSALL, Eur. J. Pharmacol. **165** 87–96 (1989).
8. B. SCHULTE, C. VOLZ-ZANG, E. MUTSCHLER, C. HORNE, D. PALM, A. WELLSTEIN and H.F. PITSCHNER, Clin. Pharmacol. Therap. **50** 372–378 (1991).
9. H.N. DOODS, R. QUIRION, G. MIHM, W. ENGEL, K. RUDOLF, M. ENTZEROF, G.B. SCHIAVI, H. LADINSKY, W.D. BECHTEL, H.A. ENSINGER, K.D. MENDLA and W. EBERLEIN, Life Sci. **52** 497–503 (1993).
10. C. MELCHIORRE, M.L. BOLOGNESI, A. CHIARINI, A. MINARINI and S. SPAMPINATO, J. Med. Chem. **36** 3734–3737 (1993).
11. R.M. WALLIS, Life Sci. **56** 861–868 (1995).
12. H.E. SHANNON, D.E. WOMER, F.P. BYMASTER, D.O.CALLIGARO, N.C. DELAP, C.H.MITCH, J.S.WARD, C.A.WHITESITT, M.D.B.SWEDBERG, M.J.SHEARDOWN, A.FINK-JENSEN, P.H.OLESEN, K.RIMVAL and P.SAUERBERG, Life Sci. **60** 969–976 (1997).
13. A. FISHER, E. HELDMAN, D. GURWITZ, R. HARING, Y. KARTON, H. MESCHULAM, Z. PITTEL, D. MARCIANO, R. BRANDEIS, E. SADOT, Y. BARG, R.PINCAS-KRAMARSKI, Z. VOGEL, I. GINZBURG, T.A. TREVES, R. VERCHOVSKY, S. KLIMOWSKY and A.D. KORCZYN, Ann. N.Y. Acad. Sci. **777** 189–196 (1996).
14. P.G. WASER, Pharmacol.Rev. **13** 465 (1961).
15. D.J. TRIGGLE and C.R. TRIGGLE, *Chemical Pharmacology of the Synapse*, 291–335, Academic Press, New York (1976).
16. R. DAHLBOM, *Stereochemistry and Biological Activity of Drugs*, E.J. Ariens (Ed), 127–142, Blackwell Scientific, Oxford (1983).
17. P. ANGELI, Il Farmaco **9** 565–577 (1995), and references cited therein.
18. M. DE AMICI, C. DALLANOCE, C. DE MICHELI, E. GRANA, G. DONDI, H. LADINSKY, G.B. SCHIAVI and F. ZONTA, Chirality **4** 230–239 (1992).
19. M. DE AMICI, C. DALLANOCE, C. DE MICHELI, E. GRANA, A. BARBIERI, H. LADINSKY, G.B. SCHIAVI and F. ZONTA, J.Med.Chem. **35** 1915–1920 (1992).
20. M. DE AMICI, C. DE MICHELI, T. GIANFERRARA, E. GRANA, F. ZONTA, H. LADINSKY and G.B. SCHIAVI, Il Farmaco **48** 1349–1357 (1993).
21. P. BRAVO, G. RESNATI, P. ANGELI, M. FRIGERIO, F. VIANI, A. ARNONE, G. MARUCCI and F. CANTALAMESSA, J.Med.Chem. **35** 3102–3110 (1992).
22. P. ANGELI, F. CANTALAMESSA, R. CAVAGNA, P. CONTI, M. DE AMICI, C. DE MICHELI, A. GAMBA and G. MARUCCI, J.Med.Chem. **40** 1099–1103 (1997).
23. A.H. BECKETT, N.J. HARPER and J.W. CLITHEROW, J. Pharm. Pharmacol. **15** 362–371 (1963).
24. P. CONTI, C. DALLANOCE, M. DE AMICI, C. DE MICHELI, G. CARREA and F. ZAMBIANCHI, Tetrahedron: Asymmetry **9** 657–665 (1998).

25. P. ANGELI, L. BRASILI, F. CANTALAMESSA, G. MARUCCI and J. WESS, Naunyn-Schmiedeberg's Arch. Pharmacol. **342** 625–629 (1990).
26. P. ANGELI, L. BRASILI, M. GIANNELLA, F. GUALTIERI, M.T. PICCHIO and E. TEODORI, Naunyn-Schmiedeberg's Arch.Pharmacol. **337** 1099–1103 (1988).
27. M. DE AMICI, C. DE MICHELI, E. GRANA, R. RODI, F. ZONTA and M.G. SANTAGOSTINO BARBONE, Eur.J.Med.Chem. **24** 171–177 (1989).
28. A. PIERGENTILI, M. PIGINI, W. QUAGLIA, S.K. TAYEBATI, F. AMENTA, M. SABBATINI and M. GIANNELLA, Bioorg. Med.Chem. **4** 2193–2199 (1996), and references cited therein.
29. R.F. FURCHGOTT, *Handbook of Experimental Pharmacology*, H. Blaschko, E. Muscholl (Eds), 283–335, Springer, Berlin, Heidelberg, New York (1972).
30. P. ANGELI, L. BRASILI, M. GIANNELLA, F. GUALTIERI and M. PIGINI, Br.J.Pharm. **85** 783–786 (1985).
31. R.F. FURCHGOTT and P. BURSZTYN, Ann. N. Y. Acad. Sci. **144** 882–899 (1967).
32. R.E. SHIPLEY and J.H. TILDEN, Proc Soc. Exp. Biol.Med. **64** 453–455 (1947).