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Search for selective antagonists at α_1-adrenoreceptors: neutral or negative antagonism? [1]

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Abstract

In this article the use of competitive antagonists as tools in receptor characterization and classification is discussed. It is pointed out that caution is required in receptor characterization because negative antagonism (inverse agonism) rather than neutral antagonism could play a relevant role. This implies that antagonists should be evaluated not only with regard to their affinity, but also with regard to their ability to affect the equilibrium between the two receptor states, namely active and inactive states. Since affinity and efficacy of a negative antagonist are system dependent the use of negative antagonists as competitive antagonists in receptor characterization may give rise to false differences in receptor subtypes. Finally, this article summarizes recent developments in the design of new α_1-adrenoreceptor antagonists which are structurally related to prazosin or WB 4101.

Keywords: α_1-Adrenoreceptors; Receptor classification; Inverse agonism; Neutral antagonism

1. α_1-Adrenoreceptor classification

α_1-Adrenoreceptors are members of a family which includes two additional classes, β- and α_2-adrenoreceptors, and is activated by the neurotransmitters adrenaline and noradrenaline. Noradrenaline is released from neurons throughout the central nervous system (CNS) and periphery, while both adrenaline and noradrenaline are released from the adrenal medulla in response to stress. Noradrenaline and adrenaline participate in a variety of physiological functions which are mediated by adrenoreceptor subtypes and second messenger systems.

Adrenoreceptors belong to the superfamily of G-protein-coupled receptors [1]. β- and α_2-adrenoreceptors are positively and negatively coupled to adenylyl cyclase through G_s and G_i, respectively, whereas α_1-adrenoreceptors trigger an increase in intracellular Ca^{2+} concentration by coupling to the G_q class of G proteins to activate phospholipase C (PLC) (generating IP_3 and causing a release of intracellular Ca^{2+} stores) or by coupling to voltage-dependent Ca^{2+} channels in the plasma membrane (allowing influx of extracellular Ca^{2+}).

Pharmacological and binding studies have shown that α_1-adrenoreceptors can be classified into at least three subtypes, namely α_{1A}, α_{1B} and α_{1D} [2]. The α_{1A} subtype has high affinity for antagonists such as WB 4101, 5-methylurapidil and (+)-niguldipine and is insensitive to inactivation by chloroethylclonidine (CEC). The α_{1B} subtype displays lower affinity for the above antagonists, but is preferentially inactivated by the alkylating agent CEC and shows high affinity for (+)-cyclazosin, whereas the α_{1D} subtype has high affinity for the antagonist BMY 7378. Current evidence indicates that rat submaxillary gland, human liver and various tissues such as prostatic rat vas deferens, rabbit prostate and prostatic urethra contain predominantly the α_{1A}-adrenoreceptor, whereas rat liver and spleen are considered α_{1B}-adrenoreceptor preparations and the α_{1D}-adrenoreceptor mediates the contraction in rat aorta. Cloning studies have confirmed the existence of three distinct α_1-adrenoreceptors, which are now designated as α_{1a}, α_{1b} and α_{1d} subtypes. The recombinant α_{1a}-adrenoreceptor (formerly designated as α_{1c}), corresponds to the native α_{1A}-adrenoreceptor, the recombinant α_{1b} to the native α_{1B} and the α_{1d} (formerly designated as $\alpha_{1a/d}$ in some publications) to the native α_{1D}-adrenoreceptor recently

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characterized in rat aorta. Thus, α_1-adrenoreceptors are now classified as α_{1A} (α_{1a}), α_{1B} (α_{1b}) and α_{1D} (α_{1d}), with upper and lower case subscripts being used to designate native or recombinant receptor, respectively [2]. In addition to α_{1A}-, α_{1B}- and α_{1D}-adrenoreceptor subtypes, which share a high affinity for prazosin, the existence of additional α_1-adrenoreceptors has been proposed. These are called α_{1L}-adrenoreceptors and are characterized by a low functional affinity for prazosin. However, these receptors have not been cloned yet and their characterization is still difficult [3].

2. α_1-Adrenoreceptor antagonists

α_1-Adrenoreceptor antagonists can be divided into two broad categories according to their mechanism of action: (a) those that bind reversibly and thus prevent access of agonists to the receptor binding site (competitive or reversible antagonism), and (b) those that inhibit by forming a covalent bond with some component of the receptor (irreversible antagonism).

A vast array of structurally unrelated compounds interacts with α_1-adrenoreceptor subtypes which makes it inherently difficult to determine the structural requirements leading to receptor subtype selectivity [3–5]. β-Haloalkylamines and tetraamine disulfides, the prototype of which are phenoxybenzamine and benextramine, respectively, are the most investigated classes of α_1-adrenoreceptor irreversible antagonists [6,7]. However, the majority of α_1-adrenoreceptor antagonists display a competitive mechanism of action and belong to a variety of different structural classes such as yohimbanes, ergot alkaloids, quinazolines, *N*-arylpiperazines, imidazolines, phenylalkylamines, benzodioxanes, indoles, 1,4-dihydropyridines, hetero-fused 3-benzazepines, and dibenzoquinolizines. The structure of representative examples from these different classes of α_1-adrenoreceptor antagonists is shown in Figs. 1 and 2. α_1-Adrenoreceptor antagonists have been the subject of several reviews [3–7]. The aim of this short review is to update the knowledge on α_1-adrenoreceptor antagonists bearing a quinazoline or a benzodioxane moiety.

2.1. Prazosin-related antagonists

Prazosin, the prototype of quinazoline-bearing compounds, is a selective α_1-adrenoreceptor antagonist widely used not only as a pharmacological tool for α-adrenoreceptor subtype characterization but also as an effective agent in the management of hypertension [4,5]. Its antihypertensive activity depends on peripheral vasodilatation mediated by a post-junctional α_1-adrenoreceptor blockade. Moreover, given its high α_1-selectivity, prazosin lacks side effects such as tachycardia and hyperreninemia, which are connected with a presynaptic α_2-adrenolytic action. In addition, in contrast to certain β-adrenoreceptor antagonists, prazosin improves the plasma lipid profile. For these reasons, prazosin represents

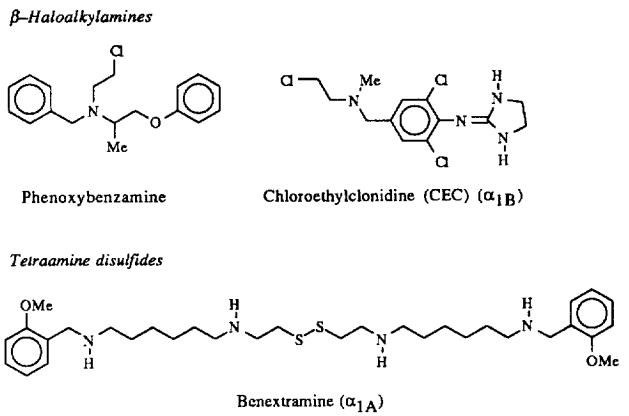


Fig. 1. Representative examples of α_1-adrenoreceptor irreversible antagonists. The subtype selectivity, if any, is indicated in brackets.

a valid tool to explore α_1-adrenoreceptor binding site topography and a lead compound in developing new therapeutically useful agents.

Our research group has long been involved in designing new α_1-adrenoreceptor antagonists structurally related to prazosin and in studying structure–affinity and structure–selectivity relationships with the goal of developing high-affinity, site-selective ligands for subtypes of the α_1-adrenoreceptor. A series of prazosin-related compounds has been investigated following the design strategy shown schematically in Fig. 3 [8–12].

The role of the piperazine ring of prazosin was investigated through its replacement by an α,ω-alkanediamine chain [8]. It turned out that the piperazine ring may not be essential for activity at α_1-adrenoreceptors and that activity and selectivity depend on the length of the alkane chain and *N*-methylation of both the amide and the 2-amino functions (Fig. 4).

The compound bearing a *N*,*N*′-dimethyl-1,6-hexanediamine moiety (**1**) was the most active of the series, being more potent than prazosin [8]. The chain length effect on potency allowed us to postulate that the rat vas deferens α_1-adrenoreceptor incorporates a lipophilic area, located between the binding sites for the quinazoline and the furan rings of prazosin, which is able to accommodate a hexane spacer optimally. To achieve information about the size and possible stereochemical requirements of this lipophilic area, we designed a series of compounds in which the very flexible polymethylene chain of **1** is incorporated partially or totally into a constrained structure [9]. The objective of this structural modification was to afford compounds in which the alkane moiety is forced to assume a definite arrangement while keeping the quinazoline and furan rings in a position likely to be similar to that of prazosin (Fig. 3).

Several prazosin-related compounds have been investigated for their blocking activity toward α-adrenoreceptors. The structural modification performed on the prazosin structure included the replacement of the piperazine ring with 2,3-dialkylpiperazine, 1,2-cyclohexanediamine or decahydroquinoxaline moieties [9]. It turned out that antagonist activity within *cis/trans* stereoisomeric compounds not only

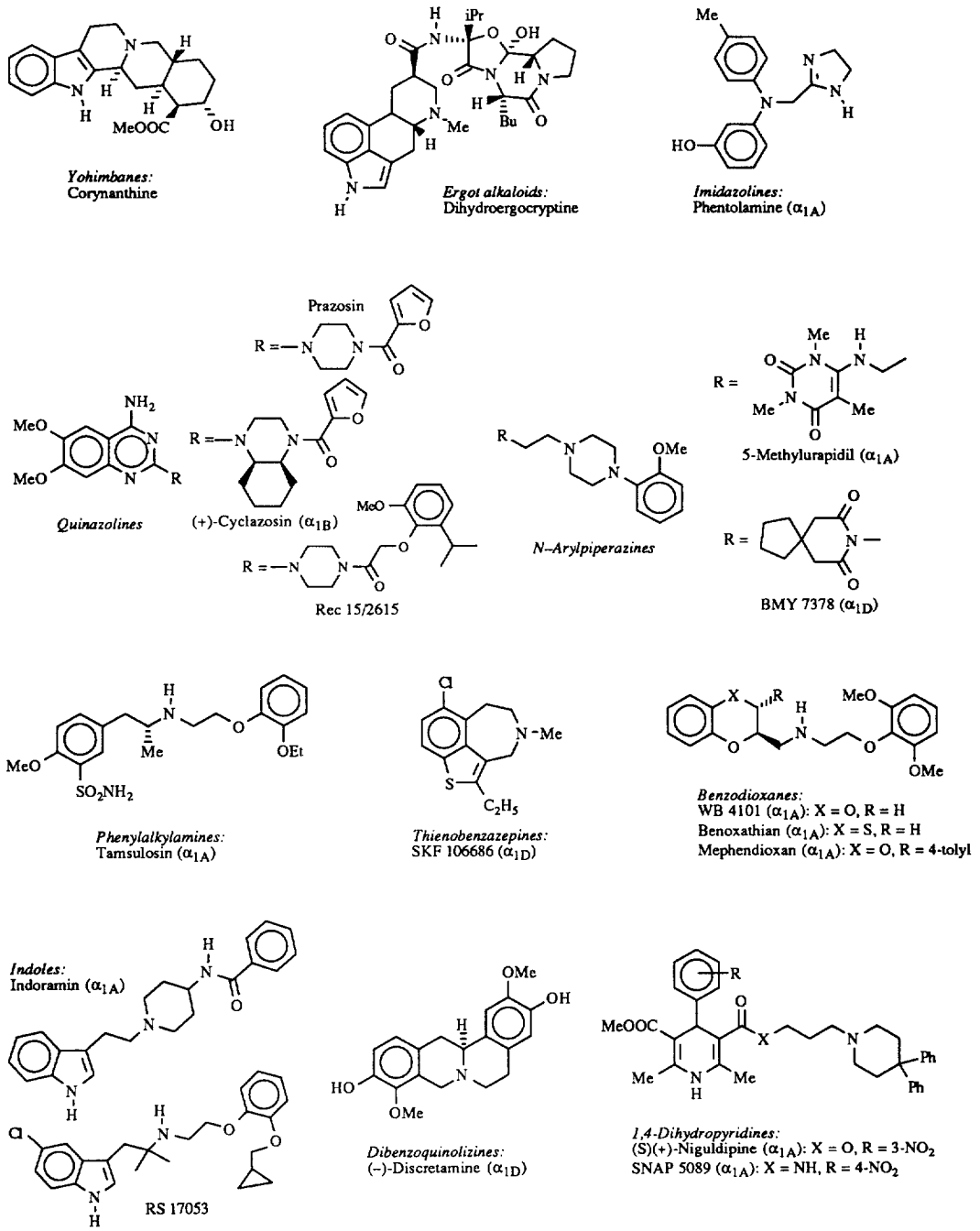


Fig. 2. Representative examples from different classes of α_1-adrenoreceptor competitive antagonists. The subtype selectivity, if any, is indicated in brackets.

supported the presence of a lipophilic binding area on the α_1-adrenoreceptor surface, but also suggested that the lipophilic pocket is endowed with a well-defined size and spatial orientation. Cyclazosin was the most potent and selective of the series with an α_1/α_2-selectivity ratio value of 7800. Furthermore, it showed a significant selectivity for α_{1B} (α_{1b})-adrenoreceptors with respect to the α_{1A} (α_{1a}) and α_{1d} subtypes as well as an interesting long-lasting hypotensive effect, very similar to that of doxazosin [9,10].

Since cyclazosin incorporates a decahydroquinoxaline nucleus in a *cis* relationship, which is responsible for the high affinity for α_1-adrenoreceptors we have synthesized its enantiomers to investigate whether the stereochemistry might increase the selectivity for α_1-adrenoreceptor subtypes [11]. The affinity profile displayed by the two enantiomers of cyclazosin at native α_{1A}- and α_{1B}- as well as at cloned α_{1a}-, α_{1b}-, and α_{1d}-adrenoreceptor subtypes was rather interesting and is reported in Table 1 and shown graphically in Fig. 5. (−)-Cyclazosin, although more potent than (+)-cyclazosin at all subtypes, was nearly devoid, like prazosin, of subtype selectivity, with the exception of a 12-fold higher affinity at native α_{1B}- relative to α_{1A}-adrenoreceptors. On

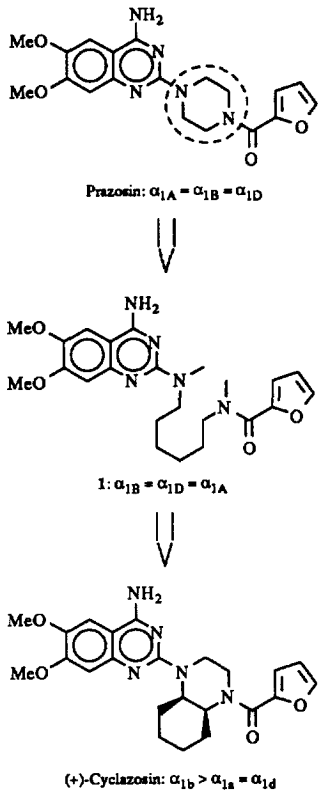


Fig. 3. Design strategy for the synthesis of prazosin-related compounds by replacing the piperazine ring of prazosin with an α,ω-alkanediamine chain or a decahydroquinoxaline moiety.

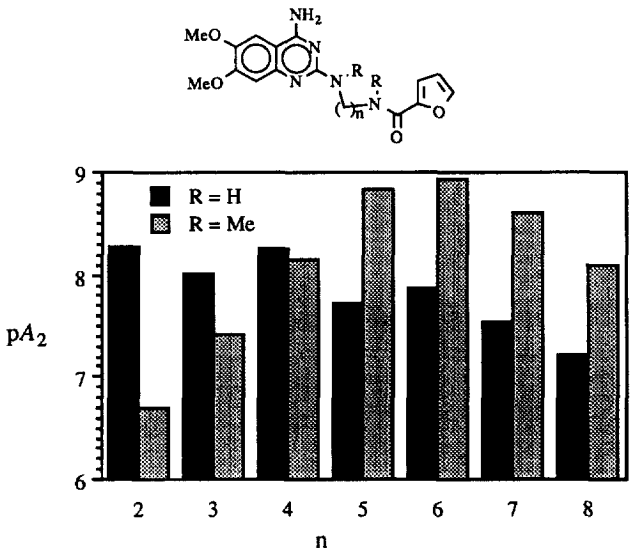


Fig. 4. Effect of chain length and *N*-methylation of prazosin-related compounds on the affinity for α_1-adrenoreceptors of isolated rat vas deferens.

the contrary, (+)-cyclazosin displayed high affinity ($pK_i = 9.16$) at cloned α_{1b}-adrenoreceptors and a significantly lower potency at both α_{1a} and α_{1d} subtypes ($pK_i = 7.48$ and 7.57, respectively). Furthermore, (+)-cyclazosin displayed selectivities of 1100-, 19 000-, and 12 000-fold in binding to α_{1b}-adrenoreceptors relative to α_2-adrenoreceptors and 5-HT_{1A} and D_2 receptors. Spiperone, which is considered a selective α_{1B}-adrenoreceptor antagonist, showed high affinity for other receptors as well, namely 5-HT_{1A} and D_2 receptors (Table 1).

An analysis of the affinity profile of the two enantiomers of cyclazosin reveals that stereochemistry plays a significant role at the three α_1-adrenoreceptor subtypes. Clearly, α_{1A} (α_{1a}) and α_{1d} subtypes, but not the α_{1B} (α_{1b})-adrenoreceptor, display a significant enantioselectivity for the two enantiomers. It appears that the stereochemical requirements for the α_{1B} (α_{1b}) subtype are satisfied by both enantiomers, whereas the α_{1A} (α_{1a}) and particularly the α_{1d} subtypes are markedly sensitive to the configuration of the *cis*-decahydroquinoxaline nucleus. Thus, (+)-cyclazosin emerges as the most interesting ligand of prazosin-related antagonists as it displayed high affinity, in the nanomolar range, like prazosin, and an unprecedented selectivity for α_{1B} (α_{1b})-adrenoreceptors, which is lacking in the antagonists presently available.

2.2. *WB 4101-related antagonists*

A benzodioxan nucleus bearing an appropriate substituent at position 2 can discriminate markedly among α-adrenoreceptor subtypes. In fact, WB 4101 and idazoxan (RX 781094), both carrying a 1,4-benzodioxan-2-yl moiety as a basic feature but having a different 2-substituent, are highly selective for α_1- and α_2-adrenoreceptors, respectively. However, WB 4101, although being highly potent toward α_1-

Table 1
Affinity estimates, expressed as pK_i, of the enantiomers of cyclazosin for native and cloned α_1-adrenoreceptor subtypes, native α_2-adrenoreceptors, and 5-HT_{1A} and D_2 receptors in comparison with prazosin and reference compound spiperone [a]

Compound	pK_i, native receptors (rat) [b]					pK_i, cloned receptors [c]		
	α_{1A}	α_{1B}	α_2	5-HT_{1A}	D_2	α_{1a}	α_{1b}	α_{1d}
(+)-Cyclazosin	7.73	9.68	6.13	4.89	5.08	7.48	9.16	7.57
(−)-Cyclazosin	8.77	9.85	5.86	5.21	<5	8.62	9.51	9.24
(±)-Cyclazosin	8.41	9.57	6.17	5.16	<5	8.18	9.23	9.28
Prazosin	9.03	9.44	6.83	5.63	<5	9.14	9.34	8.96
Spiperone	7.42	8.81	6.86	7.60	9.24	7.87	8.15	7.66

[a] Data from Ref. [11].
[b] Membranes were from hippocampus + 10 μM CEC (α_{1A}), liver (α_{1B}), cerebral cortex (α_2), hippocampus (5-HT_{1A}), and striatum (D_2).
[c] Membranes were from bovine brain (α_{1a}), hamster smooth muscle (α_{1b}), and rat brain (α_{1d}).

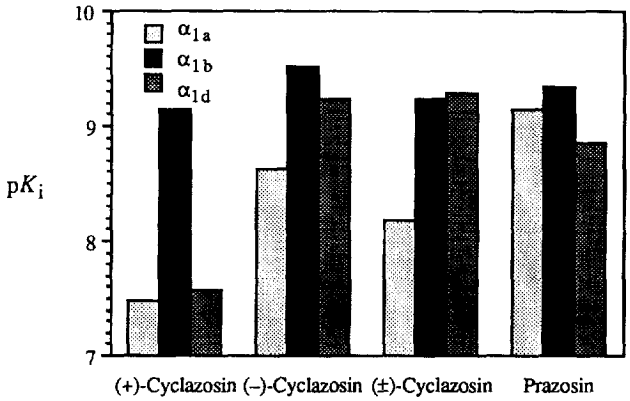


Fig. 5. Affinity estimates (pK_i) of racemic cyclazosin and its enantiomers for cloned α_1-adrenoreceptor subtypes (α_{1a}: bovine brain; α_{1b}: hamster smooth muscle; α_{1d}: rat brain) in comparison with prazosin.

adrenoreceptors, retains significant affinity for other receptor systems such as α_2-adrenoreceptors and 5-HT_{1A} receptors. A variety of WB 4101-related compounds have been studied, involving modifications of the benzodioxane ring, the amine function, or the (2,6-dimethoxyphenoxy)ethyl moiety. Although giving useful information on the structural requirements for an optimal interaction with α_1-adrenoreceptors, none of these manipulations performed on the structure of WB 4101 has led to a significant improvement of affinity or selectivity for α_1-adrenoreceptors.

The observation that replacement of a hydrogen at position 2 or 3 of idazoxan with a substituent such as a methyl can dramatically alter the potency at α_2-adrenoreceptors prompted us to introduce other substituents at position 3 of the benzodioxane ring of WB 4101 to verify whether this structural modification might decrease the affinity for α_2-adrenoreceptors while hopefully leaving unaffected that for α_1-adrenoreceptors (Fig. 6). Hence, a series of 3-substituted WB 4101 analogues has been investigated and, as expected, it turned out that the insertion of a phenyl ring at the 3-position of WB 4101 markedly affects the affinity for α_2-adrenoreceptors, whereas that for α_1-adrenoreceptors is only slightly decreased [13,14]. The overall result of this structural modification leading to phendioxan (Fig. 6) was a significant improvement in selectivity toward α_1-adrenoreceptors compared to the prototype WB 4101. It is evident that a 3-substituent (*trans*) may have a crucial role in the modulation of selectivity for α_1-adrenoreceptors [13].

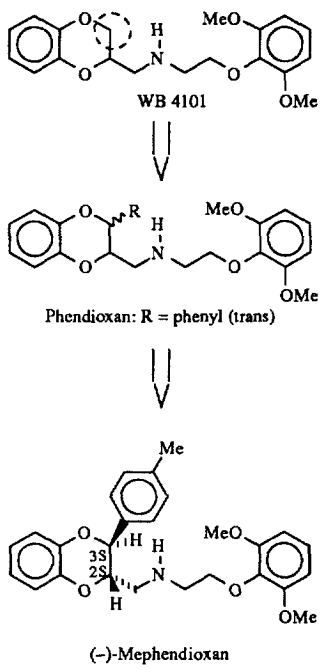


Fig. 6. Design strategy for the synthesis of WB 4101-related compounds by replacing a hydrogen atom at position 3 of WB 4101 with an aryl substituent.

The presence of a phenyl ring in phendioxan allowed us to examine the effect of selected aromatic substituents influencing different physicochemical parameters on both affinity and selectivity for α-adrenoreceptor subtypes. Among the analogues of WB 4101 bearing a 3-substituent the *p*-tolyl derivative mephendioxan (Fig. 6) resulted in the most potent and selective antagonist for the rat vas deferens α_1-adrenoreceptor subtype [14]. Since the enantiomers of WB 4101 have different affinities for α_1-adrenoreceptors, we investigated whether the enantiomers of mephendioxan, which have an additional chiral centre, might be able to discriminate among α_1-adrenoreceptor subtypes [15].

The affinity profile of the enantiomers of mephendioxan at cloned α_1-adrenoreceptor subtypes is reported in Table 2 and graphically in Fig. 7 in comparison with WB 4101.

Table 2
Affinity estimates, expressed as pK_i, of the enantiomers of mephendioxan for native and cloned α_1-adrenoreceptor subtypes, native α_2-adrenoreceptors, and 5-HT_{1A} and D_2 receptors in comparison with WB 4101 and reference compound 5-methylurapidil [a]

Compound	pK_i, native receptors (rat) [b]					pK_i, cloned receptors [c]		
	α_{1A}	α_{1B}	α_2	5-HT_{1A}	D_2	α_{1a}	α_{1b}	α_{1d}
WB 4101	8.89	7.59	7.83	8.14	6.91	9.21	7.24	8.20
(±)-Mephendioxan	8.27	6.99	5.45	6.22	7.08	9.04	7.29	7.93
(+)-Mephendioxan	7.39	6.11	5.67	5.90	6.08	8.08	6.71	6.72
(–)-Mephendioxan	8.76	7.20	5.37	6.05	7.06	9.46	7.68	8.18
5-Methylurapidil	8.33	6.66	6.36	8.92	6.03	8.69	5.98	6.76

[a] Data from Ref. [15].
[b] Membranes were from hippocampus + 10 μM CEC (α_{1A}), liver (α_{1B}), cerebral cortex (α_2), hippocampus (5-HT_{1A}), and striatum (D_2).
[c] Membranes were from bovine brain (α_{1a}), hamster smooth muscle (α_{1b}), and rat brain (α_{1d}).

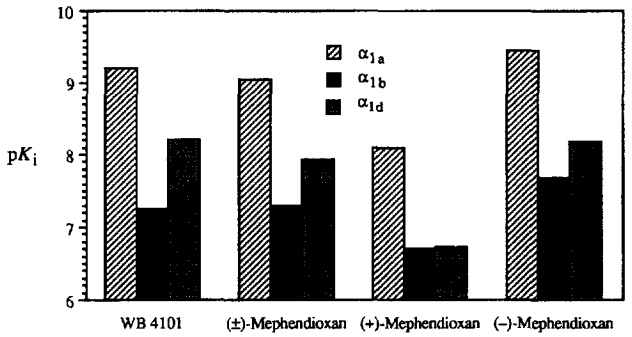


Fig. 7. Affinity estimates (pK_i) of racemic mephendioxan and its enantiomers for cloned α_1-adrenoreceptor subtypes (α_{1a}: bovine brain; α_{1b}: hamster smooth muscle; α_{1d}: rat brain) in comparison with WB 4101.

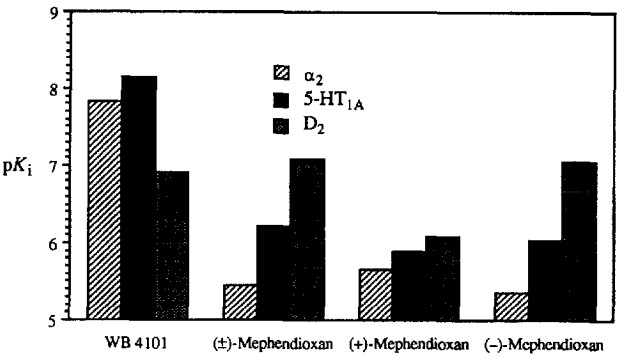


Fig. 8. Affinity estimates (pK_i) of racemic mephendioxan and its enantiomers for native receptors (α_2: rat cerebral cortex; 5-HT_{1A}: rat hippocampus; D_2: rat striatum) in comparison with WB 4101.

Clearly, (−)-mephendioxan was significantly more potent than the other enantiomer toward α_1-adrenoreceptor subtypes. The observed stereoselectivity of the enantiomers of mephendioxan is similar to that reported for the enantiomers of WB 4101. Furthermore, the 2*S*,3*S* configuration of (−)-mephendioxan is consistent with a 2*S* configuration for the most active enantiomer of WB 4101, as one would expect if related compounds act on the same receptor site.

Interestingly, (−)-mephendioxan was also 12 000-, 2500-, and 250-fold selective in binding to α_{1a}-adrenoreceptors relative to α_2-adrenoreceptors and 5-HT_{1A} and D_2 receptors, respectively. On this basis, it can be concluded that the insertion of a *trans-p*-tolyl substituent at position 3 of WB 4101 affording mephendioxan increases affinity and selectivity for α_{1A}-adrenoreceptors while significantly decreasing the affinity for α_2-adrenoreceptors and 5-HT_{1A} and D_2 receptors in comparison with the prototype WB 4101, as shown in Fig. 8.

2.3. α_{1A}- and α_{1L}-adrenoreceptor subtypes

As mentioned in Section 1, a prazosin low-affinity subtype of the α_1-adrenoreceptor has been reported by different authors, and is currently defined as α_{1L} [16–18]. Recently, experimental evidence has been given that several α_1-antagonists, in addition to prazosin, are endowed with high affinity for the α_{1a} (and α_{1A}) adrenoreceptor in radioreceptor binding and functional tests and exhibit much lower affinity as antagonists in functional assays in different tissues, formerly classified as α_{1A} [18,19]. These antagonists come from structurally different series (Fig. 2) and their relevant pharmacological data are listed in Table 3 where tamsulosin is also listed as an example of an unchanged profile. It should be emphasized that the number of these discriminating compounds is limited number with regard to all the compounds utilized for correlation between the different considered assays [19], but nevertheless their contribution to reclassification of the investigated tissues is fundamental.

Despite the evidence from functional pharmacological tests for the existence of the α_{1L}-adrenoreceptor subtype, all the attempts to sequence this receptor by molecular biology tools have so far failed, raising strong doubts as to the nature of this site as a distinct receptor. Based on the study of binding and functional responses of intact Chinese hamster ovary

Table 3
Binding and functional data for α_1-antagonists showing a clear separation in potency for the α_{1a}- and α_{1L}-adrenoreceptor subtypes

Compound	pK_i, radioreceptor binding			pA_2 (or pK_b*), functional assays		
	Human α_{1a}	Bovine α_{1a}	Dog prostate [b]	Rat perfused kidney (α_{1A}) [a]	Man lower urinary tract [a]	Rabbit urethra [b]
Prazosin	9.9 [a]	9.9 [a]	8.5	9.5	8.7	8.1*
	9.2 [b]	9.1 [b]				
5-Methylurapidil	9.2 [a]	9.4 [a]	–	9.2	8.2	8.0* [c]
WB 4101	9.8 [a]	10.0 [a]	–	10.3	8.9	–
SNAP 5089	–	8.7 [a]	7.7	–	<6.5	5.5*
	9.4 [b]	9.3 [b]				
RS 17053	9.2 [a]	9.5 [a]	7.4	9.8	7.3	5.6*
	9.2 [b]	8.8 [b]				
Rec 15/2615	8.7 [b]	8.1 [b]	7.1	–	–	6.5*
(*S*)-Niguldipine	9.7 [a]	9.8 [a]	–	10.5	7.3	–
Tamsulosin	10.4 [a]	10.4 [a]	9.8	10.2	10.4	9.3*
	10.3 [b]	9.8 [b]				

[a] Data from Ref. [18].
[b] Data from Ref. [19].
[c] Data from Ref. [28].

(CHO) cells transfected with the human α_{1a} clone, in contrast to what happens with the membranes obtained from the same cellular line, Ford et al. [20,21] observed that the intact cells showed an α_{1L} subtype behaviour whereas their membranes conserved an α_{1a} binding response. Also in this study, the compounds deviating from an α_{1a} profile were the same as those reported in Table 3 (prazosin, RS 17053, WB 4101, 5-methylurapidil and *S*-niguldipine) and the conclusion given by these authors was that the α_{1a} and α_{1L} subtypes represent two different 'pharmacological states' of the same receptor protein encoded by the gene of the α_{1a}-adrenoreceptor subtype.

More recently, Walden et al. [22] found that despite the high levels of mRNA for the α_{1a}-adrenoreceptor in the bladder urothelium from monkey, no receptor protein was found in this tissue, presumably due to translational repression. An alternative explanation could be that the α_{1a} mRNA was subjected to RNA editing, originating a different adrenoreceptor protein. The proposal that RNA editing may be a new mechanism for modulating the different cellular functions that are mediated by members of the G-protein-coupled receptor superfamily was made by Burns et al. [23], who studied the regulation of the receptor 5-HT_{2C}.

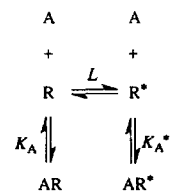
In addition to the two above hypotheses, a third possibility to explain the α_{1a}–α_{1L} issue may be found in the two-state receptor model, as follows.

2.4. Neutral and negative antagonism versus receptor classification

Competitive antagonists are a powerful and reliable tool for receptor characterization and classification. The pharmacological consequence of antagonist–receptor complex formation is the inhibition of the interaction of agonists with their sites, thus preventing receptor activation. The only relevant parameter which accounts for the antagonist capability to recognize a receptor and form a complex with it is the affinity which is an intrinsic characteristic of that antagonist. According to the theory, the affinity of an antagonist does not depend on the tissue and type of assay used for its determination; in other words, the affinity of antagonists is agonist and system independent. Thus, an affinity value assessed in functional assays should not differ from that determined in binding experiments using both native and recombinant receptors. This peculiar characteristic makes antagonists better tools in receptor classification than agonists for which, in addition to affinity, other pharmacological parameters (e.g. intrinsic activity, efficacy, receptor reserve, etc.) must be considered.

However, recent advances indicate that the classical concept of antagonism may require some redefinition. Currently, there is evidence that some receptor systems, namely those that express relatively high receptor levels with associated higher effector activity, can be activated even in the absence of an agonist [24–26]. This has been explained by admitting that receptors spontaneously interconvert between an active (R*) and an inactive (R) state: the higher the number of R*, the greater the spontaneous activity of a receptor. In this context, agonists and antagonists are defined by how much, and in which direction, they influence the equilibrium between R and R*. Consequently, antagonists may behave in two different ways: (a) those that can block agonist-independent responses by binding preferentially to R, thus shifting the equilibrium in favour of the inactive state, and (b) those that only block agonist-dependent responses because they do not distinguish between R and R* and do not affect the equilibrium between the two states. According to these action mechanisms, they can be defined as negative or neutral antagonists, respectively, and the ability of decreasing agonist-independent responses has been termed negative antagonism or inverse agonism. This implies that antagonists do not simply block the action of an agonist but can also possess an entire spectrum of efficacies, ranging from negative antagonism to neutral antagonism.

The situation described above has been discussed in detail by Leff [24] and can be represented schematically as follows:



where R and R* represent the inactive and active state, respectively, and the equilibrium between the two states is controlled by the equilibrium constant L in the absence of ligand. The interaction of an agonist (A) with the receptor alters the equilibrium between the two states according to its dissociation equilibrium constants at the two receptor states, namely K_A and K_A*. When A has higher affinity for R* it is an agonist, whereas when A has higher affinity for R it is an inverse agonist (negative antagonist). Consequently, affinity and efficacy are determined by the constants K_A and K_A*. However, these parameters are system-dependent quantities because they are the result of the basal R:R* ratio which, in turn, depends on the constant L. Since a variation of L is possible among tissues, it arises that an agonist may have different affinity as well as efficacy and potency according to the system, such as to indicate false differences in receptor types. Similarly, Leff [24] pointed out that the use of negative antagonists (inverse agonists) as neutral antagonists may, like agonists, give rise to problems since their estimated affinities are system dependent. Thus, for negative antagonists the affinity values estimated in functional assays may not necessarily be comparable with those obtained in binding experiments in which affinity is system independent. It is evident that the use of a negative antagonist in receptor classification may not be a reliable tool.

According to the two-state model, neutral antagonists are reliable tools in receptor classification since they are supposed to interact with agonist's and inverse agonist's binding sites

with the same affinity. It arises that the affinity of competitive antagonists is agonist and system independent. However, this crucial assumption deserves comment. Intuitively, R and R* states must have binding sites with different structural requirements to recognize agonists or inverse agonists selectively. Notwithstanding the diversity of the two binding sites, however, competitive antagonists are able to interact with either site with the same affinity. To account for this peculiar behaviour it becomes necessary to admit that for competitive antagonists the two receptor states retain a similar, if not identical, binding site which does not suffer any modification in the transition from one state to another. Although this possibility may not be excluded, we are intuitively inclined to favour the hypothesis that competitive antagonists recognize R and R* binding sites with an affinity which is hardly the same for the two states. If this reasoning were true, it becomes apparent that competitive antagonism may represent only a part of the scenario, if not an exception.

A survey of literature has revealed that some of the so-called competitive antagonists behave as negative antagonists when tested in the appropriate model. In the field of α_1-adrenoreceptor antagonists, prazosin, WB 4101 and benoxathian were shown to be negative antagonists in a vascular model [27]. Thus, negative antagonism rather than neutral antagonism could also be operating for the other antagonists discriminating α_{1a} and α_{1L} subtypes. The difference which is often observed for functional and binding affinities of antagonists might be explained by the fact that these compounds are negative antagonists and hence their affinity is system dependent.

In conclusion, much care is needed not only with agonists but also with antagonists in receptor subtype characterization, and analysis of their behaviour in the two-state receptor model could be a further issue to consider.

3. Conclusions

Despite the impressive results obtained by molecular biology studies, the availability of selective ligands, able to recognize only one among the α_1-adrenoreceptor subtypes, is limited, in particular for α_{1B} and α_{1D} subtypes, owing to a high percentage of amino acids which are identical in the active binding pocket of the different α_1-adrenoreceptor subtypes. Achievement of subtype selectivity is inherently more difficult for the agonists than for the antagonists, probably because the agonists are relatively small molecules, mostly interacting in the same way and with the same conserved amino acids in the receptor subtype regions that are presumed to bind agonists. The antagonists have a larger and in particular a longer structure than agonists, which may result in an increased number of specific contacts with receptor regions unique to one α_1-adrenoreceptor subtype, rather than to another, thus leading to selectivity. Although several so-called selective α_1-adrenoreceptor antagonists are available for the α_{1A} subtype, it should be emphasized, however, that the ideal selective ligands for the other subtypes are not yet available and remain a formidable challenge to medicinal chemists. The picture is further complicated by the fact that it is still unknown whether most of the so-called competitive antagonists are actually neutral or negative antagonists. Thus, pharmacotherapy and practical medicine are still waiting for the possible advantages arising from the identification of the three presently known α_1-adrenoreceptor subtypes.

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