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CHIRALITY IN α- AND β-ADRENOCEPTOR AGONISTS AND ANTAGONISTS

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CONTENTS

1. INTRODUCTION

Stereoselectivity for α- and β-adrenoceptor agonists and antagonists is a complex subject since the adrenergic neuroeffector junction, unlike other neuroeffector junctions, contains a vast array of stereoselective processes. A hypothetical adrenergic neuroeffector junction is presented in Fig. 1 depicting several of the many processes known to occur at this site, most of which show some degree of stereoselectivity.

Postjunctional α_1-, β_1- and β_2-adrenoceptors have been known for many years. Recently, postsynaptic α_2-adrenoceptors have also been identified.[1] These four adrenoceptor subtypes (process 1) possess their own particular stereochemical requirements for the natural neurotransmitter, *R*(−)-noradrenaline, as well as for exogenously administered drugs. The existence of a prejunctional α_2-adrenoceptor which regulates neurotransmitter release via a negative feedback system (process 2) is also known,[2] and this has become the target of recent stereochemical investigations.[3–8] Up to 70–90% of the natural neurotransmitter, *R*(−)-noradrenaline, liberated by adrenergic nerve terminals is removed from the synaptic cleft by an amine uptake pump ($uptake_1$), which has been proposed to be only weakly stereoselective (process 3). In addition, cytoplasmic *R*(−)-noradrenaline in the sympathetic nerve terminal is rapidly accumulated by adrenergic storage vesicles (process 4) which display a high degree of stereoselectivity. Enzymatic inactivation of noradrenaline by monoamine oxidase (MAO), located primarily in the cytoplasm of the sympathetic nerve terminal (process 5), and by catechol-O-methyltransferase (COMT), an extraneuronal enzyme (process 7), have been investigated for their stereochemical requirements, as has the extraneuronal uptake process itself ($uptake_2$; process 6). It is clear, therefore, that an understanding of the stereochemical requirements of adrenergic drugs must involve consideration of the configurational requirements of each of these varied processes for adrenergic drugs possessing one or more points of asymmetry. In addition, the conformational requirements for these different processes must also be addressed.

For directly-acting sympathomimetic amines which stimulate the α- and β-adrenoceptor subtypes directly, the stereochemical requirements at the level of the postjunctional and prejunctional adrenoceptors are most critical, although $uptake_1$, $uptake_2$, MAO and COMT may all affect drug activity by altering synaptic concentrations of the active species. For indirectly-acting sympathomimetic amines, which work through the liberation of endogenous stores of catecholamines in the adrenergic nerve terminal, stereoselective considerations of $uptake_1$ as well as uptake into adrenergic storage vesicles are most critical since these sites regulate the access of the indirectly-acting sympathomimetic amine to those compartments within the sympathetic nerve terminal from where they release the neurotransmitter.

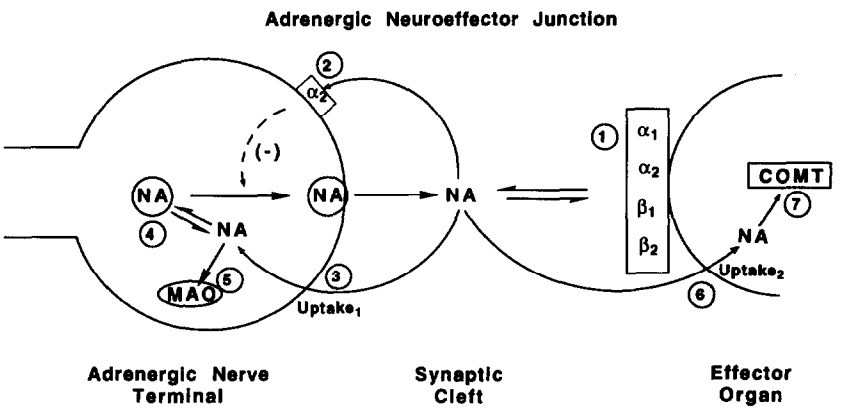


Fig. 1. Hypothetical adrenergic neuroeffector junction depicting several processes known to occur at this site. Many of these processes are stereoselective. NA refers to noradrenaline, MAO refers to monoamine oxidase and COMT refers to catechol-O-methyltransferase.

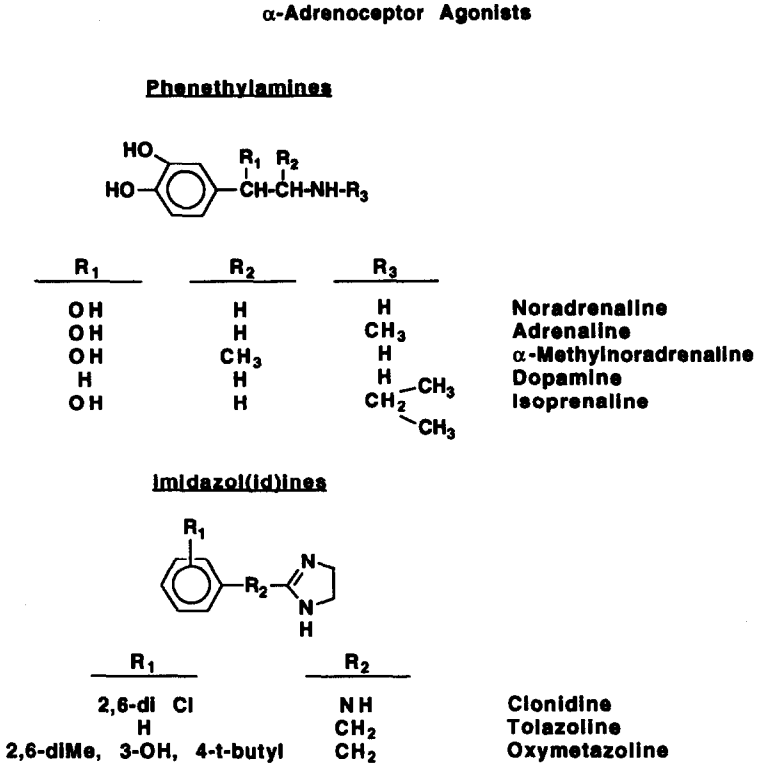


Fig. 2. Chemical structures for important members of the phenethylamine and imidazol(id)ine classes of α-adrenoceptor agonists.

2. STEREOCHEMICAL REQUIREMENTS FOR DIRECTLY-ACTING α- AND β-ADRENOCEPTOR AGONISTS

The vast majority of α- and β-adrenoceptor agonists fall into two distinct chemical categories, the phenethylamines and the imidazolines or the imidazolidines (Fig. 2). The phenethylamines include such compounds as the natural neurotransmitter, *R*(−)-noradrenaline, and the natural circulating hormone, *R*(−)-adrenaline, as well as the synthetic agonists, phenylephrine, methoxamine and isoprenaline. The imidazolines and the imidazolidines include such compounds as clonidine, naphazoline and oxymetazoline. As a general rule, the similarities between these two classes of α- and β-adrenoceptor agonists far exceed their differences. Nonetheless, the differences are significant when one considers the stereochemical requirements made by the α- and β-adrenoceptors for these two classes of agonists, and these differences contribute most to our understanding of how these drugs interact with the α- and β-adrenoceptor subtypes. In particular, the stereochemical demands made by the α- and β-adrenoceptors for the phenethylamines and imidazolines differ markedly, and these differences have led to the proposal that these two classes of drugs interact differently with the α- and β-adrenoceptors.

2.1. *Configurational requirements for directly-acting α- and β-adrenoceptor agonists of the phenethylamine class*

2.1.1. *Phenethylamines with asymmetry at the β-carbon atom (benzylic position).* The potency ratio between the (−)- and (+)-enantiomers of optically active drugs is referred to as the enantiomeric activity ratio, and this ratio is useful in characterizing and subclassifying α- and β-adrenoceptors. In general, β-hydroxy-substituted phenethylamines (i.e., hydroxyl substitution at the 1

position) have enantiomeric activity ratios at both α- and β-adrenoceptors in excess of 100-fold. In contrast, the enantiomeric activity ratios obtained for similarly substituted imidazolines are low, generally less than 10-fold. These differences indicate that α- and β-adrenoceptors are better able to discriminate between enantiomers of optically active phenethylamines than between enantiomers of optically active imidazolines, and that the stereochemical requirements of the α- and β-adrenoceptors are relatively less stringent for the imidazolines than for the phenethylamines.

2.1.1.1. *Easson–Stedman Hypothesis*

The most important theory governing the activity of phenethylamines possessing one point of asymmetry at the β-carbon atom (i.e., benzylic position) is the Easson–Stedman hypothesis[10–12] (for reviews see Refs 13–18). This hypothesis proposes that a three-point attachment is involved in the binding of a sympathomimetic amine possessing an asymmetric β-carbon atom to what was then simply called the adrenergic receptor (α- and β-adrenoceptor subtypes were not described until the classic study of Ahlquist[19]). For *R*(−)-adrenaline, these three functional groups were proposed to be (a) the protonated nitrogen atom common to all sympathomimetic amines, (b) the phenyl group whose binding to the receptor was proposed to be enhanced by *meta* and/or *para* phenolic hydroxyl substitution, and (c) the benzylic hydroxyl group attached to the β-carbon atom (Fig. 3). According to the Easson–Stedman hypothesis,[10] as modified by Blaschko[11] and Beckett,[12] these three functional groups are in a most favorable stereochemical configuration for interaction with the adrenoceptors for only the *R*(−)-enantiomer of adrenaline. As far as *S*(+)-adrenaline and its β-desoxy derivative, epinine, are concerned, the β-hydroxyl group is incorrectly oriented or absent, respectively, and therefore not available for interaction with the adrenoceptors. Thus, only a two point attachment is considered possible for *S*(+)-adrenaline and epinine. This presumably would account for the lower activities of the *S*(+)-enantiomer and corresponding desoxy derivative of adrenaline relative to the *R*(−)-enantiomer, and would also account for the fact that the *S*(+)-enantiomer and corresponding β-desoxy derivative are equal in potency to each other. Thus, the Easson–Stedman hypothesis predicts the following rank order of potency for optically active phenethylamines with asymmetry at the β-carbon atom: *R*(−)-enantiomer > *S*(+)-enantiomer = desoxy derivative.

The Easson–Stedman hypothesis is illustrated schematically in Fig. 3 for the enantiomers of noradrenaline and the corresponding desoxy derivative, dopamine. This hypothesis has been shown by Patil *et al.*[20,21] to apply to virtually all sympathomimetic amines of the phenethylamine class. However, the application of the Easson–Stedman hypothesis is only valid when indirect sympathomimetic activity (i.e., release of endogenous catecholamines), which is marked for the desoxy

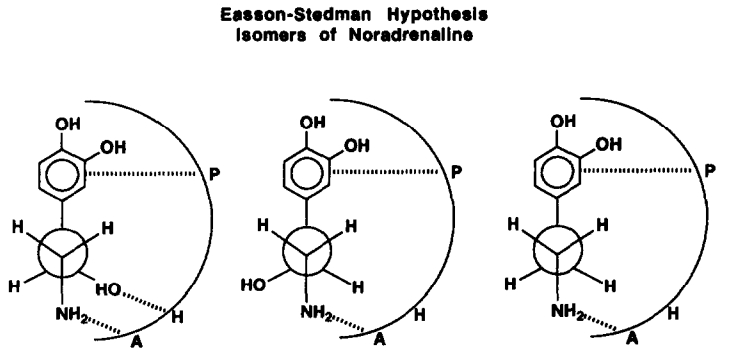


Fig. 3. Schematic representation of the Easson–Stedman hypothesis for interaction of the *R*(−)- and *S*(+)-enantiomers of noradrenaline, and the corresponding β-desoxy derivative, dopamine, with adrenoceptors. P, H, and A represent three hypothetical binding sites to which attach the phenyl, hydroxyl and amino functional groups of phenethylamines, respectively.

derivatives relative to the $S(+)$-enantiomers,[22,23] is eliminated such that only direct postjunctional receptor mediated effects are considered. Recent studies have shown that the Easson–Stedman hypothesis applies to all four of the known adrenoceptor subtypes (i.e., α_1, α_2, β_1, β_2),[5,13,14,20,21] and no exceptions to the Easson–Stedman hypothesis are known among the phenethylamine class.

The significance of the Easson–Stedman hypothesis in relation to the imidazolines is not as clear as for the phenethylamines. When imidazolines structurally related to tolazoline are hydroxy substituted at a position analogous to the β-carbon atom of the phenethylamines (i.e., benzylic position), activity at α_1- and α_2-adrenoceptors is either unchanged or markedly decreased.[24–28] According to the Easson–Stedman hypothesis, one would have anticipated that α-adrenoceptor activity would increase dramatically if the hydroxyl group, when oriented in the optimal *R* stereochemical configuration, was critical for the attachment of the agonist to the α-adrenoceptor subtypes. Furthermore, the difference in activity between the $R(-)$- and $S(+)$-enantiomers of imidazolines with asymmetry at the benzylic carbon atom, which is analogous to the β-carbon atom of the phenethylamines, is small or nonexistent,[18,25–28] in contrast to the much larger enantiomeric potency differences of two to three orders of magnitude observed for the phenethylamines.[14,21,29] These observations have prompted the suggestion that the α-adrenoceptor mediated effects of the imidazolines do not adhere to the Easson–Stedman hypothesis[24–28,30] in contrast to the phenethylamines which most definitely do.[14,21] In spite of the fact that the α-adrenoceptor mediated effects of the imidazolines do not conform to the Easson–Stedman hypothesis, recent findings indicate that the weak β-adrenoceptor mediated effects of optically active imidazolines do indeed adhere to the Easson–Stedman hypothesis.[24–26]

Based on these observations, it has been proposed that while the phenethylamines interact with the adrenoceptors by a three-point attachment,[10,12] the imidazolines appear to interact with α-adrenoceptors by only a two-point attachment.[18] The possibility must be considered that the phenethylamines and imidazolines interact differently with α-adrenoceptors,[25,28,31–34] and this has recently been confirmed at the molecular level in which the binding of phenethylamines to the active site of cloned α-adrenoceptors has been shown to differ from that of the imidazolines.

2.1.2. *Phenethylamines with asymmetry at the α-carbon atom.* The enantiomers of α-methyldopamine are commonly employed to study the stereochemical demands made by α- and β-adrenoceptors for phenethylamines with asymmetry at the α-carbon atom (i.e., 2-position; see Fig. 2). Patil and Jacobowitz[35] have shown that $2S(+)$-α-methyldopamine is more potent than the corresponding $2R(-)$-enantiomer at β_2-adrenoceptors. Likewise, the β_1-adrenoceptor mediated effects of α-methyldopamine are also confined primarily to the $2S(+)$-enantiomer.[36] However, in contrast to the high degree of enantioselectivity observed for the β_1- and β_2-adrenoceptor mediated effects of α-methyldopamine, it was observed that both enantiomers of α-methyldopamine are extremely weak agonists at α_1-adrenoceptors, with no difference between the enantiomers being detected.[35] In agreement with these findings are those of Ruffolo and Waddell[6] who found that the enantiomers of α-methyldopamine are equiactive at α_1-adrenoceptors in guinea pig aorta. However, in contrast to the equal potencies of the enantiomers of α-methyldopamine at α_1-adrenoceptors, a marked preference was shown by α_2-adrenoceptors in field-stimulated guinea pig ileum for the $2S(+)$-enantiomer over the $2R(-)$-enantiomer.[6]

As a result of the high degree of stereoselectivity shown by the α_2-adrenoceptor, but not by the α_1-adrenoceptor, for the enantiomers of α-methyldopamine, marked differences exist between the enantiomers of α-methyldopamine in their α_2/α_1-adrenoceptor selectivities. Thus, while $2R(-)$-α-methyldopamine showed only a two-fold preference for α_2-adrenoceptors over α_1-adrenoceptor, its enantiomer, $2S(+)$-methyldopamine, displayed a 23-fold preference for the α_2-adrenoceptor.[6]

According to the Easson–Stedman hypothesis, dopamine, which lacks the β-hydroxyl group, will attach to both the α_1- and α_2-adrenoceptors by only a two point attachment involving the catechol ring and aliphatic nitrogen atom.[14,20,21] Since the two enantiomers of α-methyldopamine are equipotent to dopamine at α_1-adrenoceptors,[6] it is logical to conclude that both enantiomers of

α-methyldopamine likewise bind to the α_1-adrenoceptor by only a two point attachment, and that the α_1-adrenoceptor does not interact significantly with the methyl substituent at the α-carbon atom when present in either the 2*R*(−)- or 2*S*(+)-configuration. Conversely, the 2*S*(+)-enantiomer of α-methyldopamine is significantly more potent at the α_2-adrenoceptor than either its 2*R*(−)-enantiomer or dopamine (desmethyl analog), with the two latter compounds being equal in activity to each other. These results indicate that the α_2-adrenoceptor, in marked contrast to the α_1-adrenoceptor, has the ability to interact with, or at least accommodate, the α-methyl group of α-methyldopamine when it is present and in the optimal 2*S* stereochemical configuration. Since dopamine would appear to bind to the α_2-adrenoceptor by only two points of attachment (i.e., Easson–Stedman hypothesis is also valid for α_2-adrenoceptors), it follows, therefore, that 2*R*(−)-α-methyldopamine likewise binds to the α_2-adrenoceptor by only two points since this enantiomer is equipotent with dopamine at the α_2-adrenoceptor.[6] However, the 2*S*(+)-enantiomer of α-methyldopamine, which is more potent than either the 2*R*(−)-enantiomer or dopamine, may bind to the α_2-adrenoceptor by a three-point mode of attachment involving the catechol ring, the protonated nitrogen atom and the α-methyl group.[6]

This hypothesis involving asymmetry at the α-carbon atom of phenethylamines is presented schematically in Fig. 4 for the enantiomers of α-methyldopamine as it would apply differentially to α_1- and α_2-adrenoceptors. The model calls for an additional recognition site existing only on the α_2-adrenoceptor which can interact with and/or accommodate, the α-methyl group of phenethylamines so substituted (i.e., α-methyldopamine, α-methylnoradrenaline, etc.) and when oriented in the

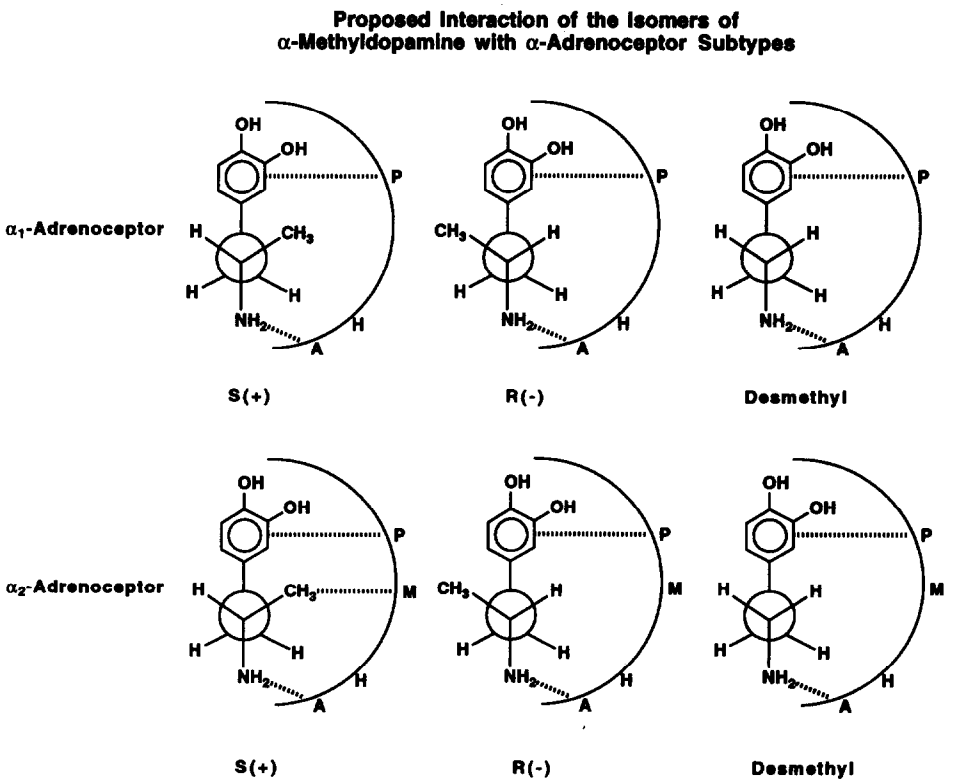


Fig. 4. Schematic representation of the possible interaction of α-methyl substituted phenethylamines with α_1- and α_2-adrenoceptors. The hypothetical binding sites, P, H, and A are the same as indicated in Fig. 3, in addition to which the site M is proposed to exist only on α_2-adrenoceptors to accommodate the α-methyl substituent.

2*S*(+)-configuration. 2*R*(−)-α-Methyldopamine, which also possesses the α-methyl group, but in the incorrect orientation, and the desmethyl derivative (i.e., dopamine) which does not possess the α-methyl group, are predicted to be less active than the 2*S*(+)-enantiomer at α_2-adrenoceptors (but not α_1-adrenoceptors) presumably because these two compounds may only bind to the α_2-adrenoceptor by only a two-point attachment as illustrated in Fig. 4.

2.1.3. *Phenethylamines with asymmetry at both the α- and β-carbon atoms.* The stereochemical requirements made by α- and β-adrenoceptors for phenethylamines with two asymmetric centers are more complicated than those with one asymmetric center since four diastereoisomers (i.e., two enantiomeric pairs) exist. The four possible enantiomers of α-methylnoradrenaline are shown in Fig. 5. The α- and β-adrenoceptors are strict in their configurational requirements for phenethylamine agonists with two chiral centers. Patil and Jacobowitz[35] have established that β_2-adrenoceptors are highly selective for only the 1*R*,2*S*(−)-*erythro*-enantiomer of α-methylnoradrenaline, with the remaining three enantiomers being inactive. Goldberg *et al.*,[7] employing radioligand binding techniques, have shown that the same stereochemical requirements apply to the β_1-adrenoceptor for the stereoisomers of α-methylnoradrenaline. Likewise, the α_1- and α_2-adrenoceptors are also highly selective for the 1*R*,2*S*(−)-*erythro*-enantiomer of α-methylnoradrenaline.[5,7,35]

Comparison of the stereochemical requirements of α_1- and α_2-adrenoceptors illustrate several important quantitative differences between these two α-adrenoceptor subtypes. The enantiomeric activity ratio of the enantiomers of noradrenaline is 107-fold for α_1-adrenoceptors and 479-fold for α_2-adrenoceptors, with the 1*R*(−)-enantiomer being most potent in both cases.[5] These findings suggest that quantitative differences exist in the configurational demands made by α_1- and α_2-adrenoceptors for phenethylamines possessing only one chiral center, with the stereochemical demands made by α_2-adrenoceptors being far more stringent than those made by α_1-adrenoceptors. The case for the enantiomers of α-methylnoradrenaline is even more illustrative of these differences. For the 1*R*,2*S*(−)-*erythro*- and 1*S*,2*R*(+)-*erythro*-enantiomers of α-methylnordrenaline, an enantiomeric activity ratio of 60-fold exists at α_1-adrenoceptors, whereas the enantiomeric activity ratio at α_2-adrenoceptors is 550-fold.[3] These observations support the notion that the stereochemical demands made by α_2-adrenoceptors are far more stringent than those made by α_1-adrenoceptors, especially for phenethylamines possessing two asymmetric centers.[5]

In the previous section it was argued that the α_2-adrenoceptor could selectively recognize and/or bind α-methyl substituents of phenethylamine agonists when oriented in the optimal 2*S*(+)-configuration (see Section 2.1.2.). Conversely, the α_1-adrenoceptor does not have this ability. This hypothesis may be extended to include the interactions of α-methylnoradrenaline (having two asymmetric centers) with α_1- and α_2-adrenoceptors, which would also account for the significant pharmacological differences that exist between α-methylnoradrenaline and noradrenaline. The most

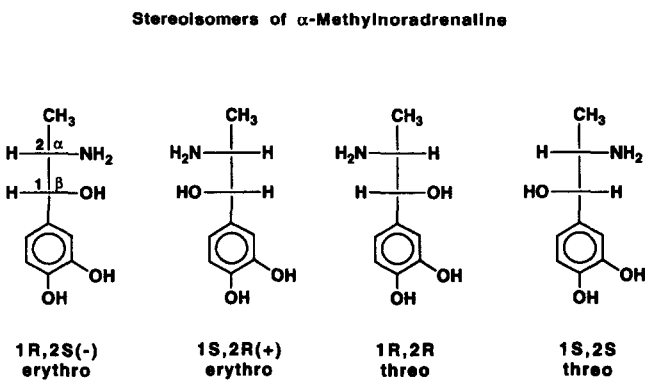


Fig. 5. The four diastereoisomers of α-methylnoradrenaline presented in the Fischer projection.

active enantiomers of α-methylnoradrenaline and noradrenaline at α_1- and α_2-adrenoceptors are those in which the β-hydroxyl group is in the *R* absolute configuration.[5,7,21] Previous investigations which have shown that the Easson–Stedman hypothesis is valid for both the α_1- and α_2-adrenoceptor subtypes[5] indicate that a three-point attachment is likely for 1*R*(−)-noradrenaline interacting with both α_1- and α_2-adrenoceptors. Since the α_1-adrenoceptor mediated effects of 1*R*(−)-noradrenaline are not increased, or may even be decreased,[5,18,21,37–39] by α-methyl substitution (Section 2.1.2.), it is concluded that the 1*R*,2*S*(−)-*erythro*-enantiomer of α-methylnoradrenaline also binds by a three-point attachment to α_1-adrenoceptors, consistent with the hypothesis presented above which suggests that the α_1-adrenoceptor lacks the ability to interact with the α-methyl substituent in any configuration. Conversely, addition of an α-methyl group to noradrenaline significantly enhances activity at α_2-adrenoceptors,[5–7,37] indicating that this receptor subtype can recognize the α-methyl substituent when oriented in the optimum *S* absolute configuration, as was observed for α-methyldopamine. Since it appears that the α_2-adrenoceptor can also recognize the catechol, β-hydroxyl and amino groups (i.e., Easson–Stedman hypothesis is valid for α_2-adrenoceptors[5]), it is proposed that the 1*R*,2*S*(−)-*erythro*-enantiomer of α-methylnoradrenaline binds to the α_2-adrenoceptor by a four-point mode of attachment involving the catechol, β-hydroxyl, amino and α-methyl groups, in contrast to the α_1-adrenoceptor where only three of these functional groups (i.e., catechol, β-hydroxyl and amino groups) are involved in the binding of this same compound. These proposed interactions of 1*R*(−)-noradrenaline and 1*R*,2*S*(−)-*erythro*-α-methylnoradrenaline with the α_1- and α_2-adrenoceptor subtypes are presented schematically in Fig. 6 to illustrate these significant differences. Note the proposed three-point interaction of both 1*R*(−)-noradrenaline and 1*R*,2*S*(−)-*erythro*-α-methylnoradrenaline with α_1-adrenoceptors. In contrast, at the α_2-adrenoceptors, 1*R*(−)-noradrenaline will still interact by only a three-point attachment, whereas 1*R*,2*S*(−)-*erythro*-α-methylnoradrenaline is proposed to interact with the α_2-adrenoceptor by a four point attachment which includes also the α-methyl substituent. This model is consistent with the observations that 1*R*(−)-noradrenaline is equipotent to 1*R*,2*S*(−)-*erythro*-α-methylnoradrenaline at α_1-adrenoceptors,[5,6] whereas 1*R*,2*S*(−)-*erythro*-α-methylnoradrenaline is significantly more potent than 1*R*(−)-noradrenaline at α_2-adrenoceptors.[5,37] A slightly modified hypothesis involving different conformations of noradrenaline and α-methylnoradrenaline has been offered by Triggle.[16]

2.1.4. *Asymmetry at the N-substituent of phenethylamines.* Simple phenethylamines, such as noradrenaline, adrenaline and α-methylnoradrenaline, have only two carbon atoms separating the aromatic ring and aliphatic nitrogen atom. For these simple phenethylamines, up to two points of asymmetry may exist; at the α- and/or β-carbon atoms. However, several phenethylamines with relatively large N-substituents have been synthesized, and many of these compounds possess an additional asymmetric center. Few such compounds have been resolved into their component enantiomers and evaluated at α- and β-adrenoceptors in order to assess the configurational requirements made by receptors for these different asymmetric centers on the N-substituent. Dobutamine, however, is one compound possessing an unusual point of asymmetry in which the individual enantiomers have been evaluated in detail with some striking results. Dobutamine (Fig. 7) is an inotropic agent capable of increasing myocardial contractility at doses that have little or no effect on heart rate[40–43] or blood pressure.[43,44] The point of asymmetry in dobutamine exists on the rather bulky N-substituent. While it is generally observed that the individual enantiomers of phenethylamines possess qualitatively similar pharmacological activities and differ mainly in potency,[14] dobutamine is unusual in that the individual enantiomers display marked qualitative differences in their overall pharmacological profiles as well as large differences in potency, all of which contribute to the efficacy of this agent clinically. *In vitro* studies of the individual enantiomers of dobutamine[45] indicate that the (−)-enantiomer is a potent α_1-adrenoceptor agonist and a weak β_1- and β_2-adrenoceptor agonist in contrast to the (+)-enantiomer which is a strong β_1- and β_2-adrenoceptor agonist and possesses no agonist activity at α-adrenoceptors. In fact, (+)-dobutamine has been found to be a weak α-adrenoceptor antagonist capable of blocking, in part, the potent α-adrenoceptor

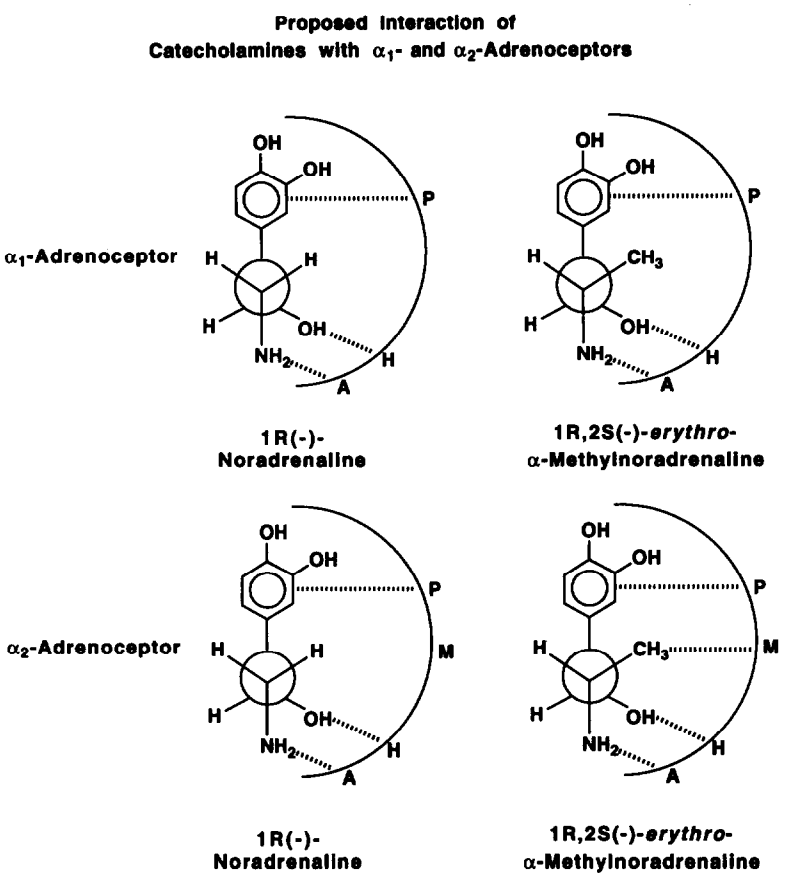
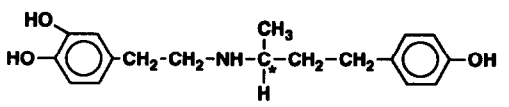


Fig. 6. Possible interactions of noradrenaline and α-methylnoradrenaline with α_1- and α_2-adrenoceptors. The proposed sites of interaction, P, H, M, and A, are the same as in Figs 3 and 4.

agonist effects of the (−)-enantiomer. These results have been confirmed *in vivo*.[47] Thus, the (−)-enantiomer of dobutamine is a relatively potent and selective α_1-adrenoceptor agonist, whereas the (+)-enantiomer of dobutamine is a strong β_1- and β_2-adrenoceptor agonist.

The qualitatively different effects mediated by the enantiomers of dobutamine at α- and β-adrenoceptors are presented in Table 1. Although the inotropic effect of dobutamine is generally attributed to selective stimulation of myocardial β_1-adrenoceptors by the (+)-enantiomer, recent studies have shown clearly that the α_1-adrenoceptor mediated effects of dobutamine, which exists in the (−)-enantiomer, also contribute, at least in part, to the inotropic selectivity of the compound as well as to its vascular effects. Several lines of evidence exist to support the hypothesis that both enantiomers of dobutamine in the racemic mixture used clinically contribute to the overall activity of the drug. Thus, recent studies show that myocardial α_1-adrenoceptors exist and mediate a positive



Dobutamine

Fig. 7. Chemical structure of the inotropic agent, dobutamine. The asterisk denotes the point of asymmetry.

Table 1. Possible Mechanism of Action of Dobutamine

Enantiomers	Pharmacologic Activity	Effect in Heart[a]		Vascular[b] Tone
		Force[c]	Rate[d]	
(+)-Dobutamine	β_1/β_2	+	+	-
(-)-Dobutamine	α_1	+	0	+
(±)-Dobutamine[e]	$\alpha_1/\beta_1/\beta_2$	++	+	0

a +, increase; -, decrease

b Vascular tone refers to vasodilation (-) or vasoconstriction (+). 0 refers to no net change in vascular tone.

c Force of myocardial contraction (contractility or inotropic activity).

d Rate of myocardial contraction

e (±)-refers to the racemic mixture of dobutamine used clinically.

inotropic response with little or no change in heart rate. These myocardial α_1-adrenoceptors are, indeed, activated by (−)-dobutamine when the racemic mixture is given *in vitro* and *in vivo*.[46a] Furthermore, in spite of the fact that the β-adrenoceptor mediated effects of dobutamine are generally believed to be responsible for the inotropic activity of the drug, it is the (−)-enantiomer of dobutamine that is most selective as an inotropic agent *in vitro* and *in vivo*,[46a] and the inotropic selectivity of dobutamine *vis-à-vis* heart rate is attenuated, in part, by α-adrenoceptor blockade, as is the ability of dobutamine to increase cardiac output, left ventricular dp/dt_{max}, and stroke volume.[46a] It is apparent, therefore, that both enantiomers of dobutamine contribute to the inotropic activity of the drug by stimulating different adrenoceptors in the heart, with the (−)-enantiomer activating primarily the α_1-adrenoceptor, whereas the (+)-enantiomer stimulates mainly the β_1-adrenoceptor. Since only the β_1-adrenoceptor contributes to increases in heart rate (*vis-à-vis* inotropic activity), the racemic mixture of dobutamine used clinically displays inotropic selectivity over heart rate (e.g., compare the effects on force and heart rate in Table 1). Likewise, the lack of effect of racemic dobutamine on blood pressure is also the result of the individual effects of the enantiomers used clinically. Thus, the α_1-adrenoceptor mediated vasoconstrictor effects produced by the (−)-enantiomer of dobutamine are exactly offset by the β_2-adrenoceptor mediated vasodilatory effects of the (+)-enantiomer, resulting in no net change in blood pressure (Table 1). As such, dobutamine represents a most unusual circumstance in which both enantiomers of this optically active phenethylamine possess qualitatively different pharmacological profiles, and both enantiomers of dobutamine contribute to the beneficial effects of the racemic mixture used clinically in human patients with congestive heart failure.[46a] Interestingly, animal studies show that neither enantiomer of dobutamine alone can mimic the effects produced by the racemic mixture used clinically.[46a]

2.2. *Configurational requirements of α- and β-adrenoceptors for agonists of the imidazol(id)ine class*

2.2.1. *Asymmetry at the benzylic carbon atom of imidazolines and imidazolidines.* Optically active centers in imidazoline agonists are rare. However, a few examples of optically active imidazolines are known and provide some insight into how this unique class of adrenoceptor agonists interacts

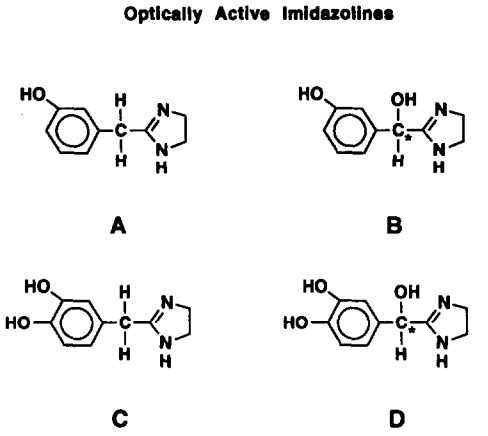


Fig. 8. Chemical structures of optically active imidazolines possessing benzylic hydroxyl groups, and their corresponding desoxy derivatives. The asterisk denotes the point of asymmetry.

with α- and β-adrenoceptors. As stated earlier, the Easson–Stedman hypothesis predicts the following rank order of potency for phenethylamines possessing an asymmetric, hydroxyl-substituted benzylic carbon atom: *R*(−)-enantiomer > *S*(+)-enantiomer = desoxy derivative. The Easson–Stedman hypothesis has been shown to be valid for all phenethylamines tested to date,[14] but does not apply to any of the imidazolines. The imidazolines in Fig. 8 possess asymmetric benzylic carbon atoms at positions analogous to the β-carbon of phenethylamines. Ruffolo *et al.*[24,25] have shown that hydroxyl substitution of compound A (desoxy) to yield compound B [(±)-racemate] results in a 4- to 10-fold decrease in activity as opposed to the two order of magnitude increase in activity predicted by the Easson–Stedman hypothesis. Furthermore, hydroxyl substitution of compound C (desoxy) to yield the *R*(−)- and *S*(+)-enantiomers of compound D have also been synthesized and studied in detail. In a variety of α_1-adrenoceptor test systems, the rank order of potency for these compounds was as follows: desoxy derivative ≥ *R*(−)-enantiomer > *S*(+)-enantiomer.[26,28,30,48,49] This order of potency is clearly different than that predicted by the Easson–Stedman hypothesis. Likewise, at α_2-adrenoceptors, the *R*(−)-enantiomer of compound D was found to be only 6-fold more potent than the *S*(+)-enantiomer; however, the corresponding desoxy derivative was found to be an extremely potent α_2-adrenoceptor agonist,[26] leading to a rank potency order of: desoxy derivative ≫ *R*(−)-enantiomer > *S*(+)-enantiomer, which also deviates from the Easson–Stedman hypothesis. Thus, it may be concluded that the Easson–Stedman hypothesis does not apply to either the α_1- or α_2-adrenoceptor mediated effects of imidazoline agonists in spite of the fact that this hypothesis does accurately predict the α-adrenoceptor mediated effects of the phenethylamines.

Interestingly, however, these optically active imidazolines possess weak β_1- and β_2-adrenoceptor agonist effects, which surprisingly do adhere to the Easson–Stedman hypothesis. Thus, at β_1- and β_2-adrenoceptors, both *in vitro* and *in vivo*, the *R*(−)-enantiomer of these optically active imidazolines is consistently more potent than either the *S*(+)-enantiomer or the corresponding desoxy derivative, with the two latter compounds being roughly equivalent in potency. This rank order of potency of *R*(−)-enantiomer > *S*(+)-enantiomer = desoxy derivative has now been shown to apply to several optically active imidazolines interacting with β_1- and β_2-adrenoceptors in spite of the fact that these compounds do not adhere to the Easson–Stedman hypothesis at α_1- or α_2-adrenoceptors. This interesting discrepancy in which optically active imidazolines adhere to the Easson–Stedman hypothesis at β-adrenoceptors, but not at α-adrenoceptors, highlights the marked qualitative differences that exist in the stereochemical demands made by α- and β-adrenoceptors for agonists of the imidazoline class (but not the phenethylamine class where qualitative differences do not exist between

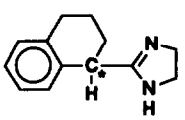
Optically Active Amidines

Fig. 9. Chemical structure of an optically active amidine possessing a benzylic hydroxyl group, and the corresponding desoxy derivative. The asterisk denotes the point of asymmetry.

the stereochemical demands made by the α- and β-adrenoceptor subtypes). Identical results have been obtained for optically active amidine derivatives (Fig. 9) which are structural analogs of the imidazolines. Thus, the imidazolines and amidines have the capacity to discriminate between α- and β-adrenoceptors with respect to the Easson–Stedman hypothesis, as opposed to the phenethylamines which adhere to the Easson–Stedman hypothesis at both α- and β-adrenoceptors.

One point of asymmetry also exists at the benzylic carbon atom of tetrahydrozoline (Fig. 10). It has been demonstrated *in vitro*[25,50] and *in vivo*[51] that the activity of tetrahydrozoline resides predominantly in the (−)-enantiomer. The enantiomeric activity ratio for the enantiomers of tetrahydrozoline is low, generally less than 10-fold,[25,50] characteristic of all optically active imidazolines studied to date. This contrasts with the phenethylamines which display enantiomeric activity ratios of 100- to 1000-fold when chirality exists at the benzylic carbon atom.

2.2.2. *Asymmetry at the imidazoline ring.* Optically active imidazolines with asymmetry existing at the 4 position of the imidazoline ring have also been synthesized and tested (Fig. 11). The two major substituents that have been placed at this position are methyl and benzyl groups. Both substitutions decrease intrinsic activity and thereby change potent agonists into antagonists.[52,53] Virtually no stereoselectivity exists between the enantiomers of either the methyl or benzyl substituted imidazolines.[50,52,53]



Tetrahydrozoline

Fig. 10. Chemical structure of tetrahydrozoline. The asterisk denotes the point of asymmetry.

Optically Active Naphazoline Derivatives

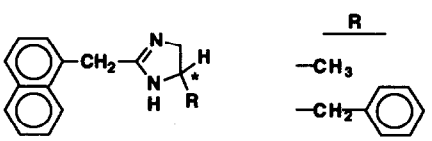


Fig. 11. Derivatives of naphazoline with points of asymmetry (asterisk) on the imidazoline ring.

2.3. *Comparison of the configurational requirements of α- and β-adrenoceptors for optically active phenethylamines and imidazolines*

Based on the previous discussion it is apparent that several important differences exist in the stereochemical requirements made by α- and β-adrenoceptors for optically active phenethylamines and imidazolines. Enantiomeric activity ratios for phenethylamines are, in general, quite large, whereas enantiomeric activity ratios for optically active imidazolines are quite small and often nonexistent. Thus, enantiomeric activity ratios for phenethylamines with one point of asymmetry at the β-carbon atom (benzylic position) are typically in excess of 100-fold, and enantiomers of phenethylamines with two points of asymmetry (i.e., as in α-methylnoradrenaline) may show enantiomeric activity ratios in excess of 500-fold. In contrast, optically active imidazolines rarely show enantiomeric activity ratios larger than 10-fold, and a complete lack of stereoselectivity is not uncommon among imidazolines.

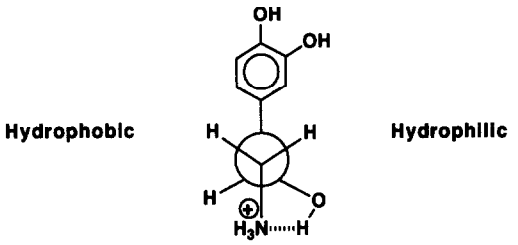
Furthermore, the Easson–Stedman hypothesis applies strictly to all phenethylamines that interact with α_1-, α_2-, β_1- and β_2-adrenoceptors. In contrast, no optically active imidazolines (or amidines) studied to date adhere to the Easson–Stedman hypothesis at α_1- or α_2-adrenoceptors, whereas the β_1- and β_2-adrenoceptor mediated effects of these compounds do apparently adhere to the Easson–Stedman hypothesis, pointing out previously unrecognized differences in the stereochemical demands made by α- and β-adrenoceptors.

In general, it appears that the steric demands made by α- and β-adrenoceptors for phenethylamines are significantly more stringent than those for the imidazolines. It is likewise apparent that the phenethylamines and the imidazolines differ markedly with respect to their chiral interactions with α- and β-adrenoceptors, and these differences have led to the proposal that the phenethylamines and imidazolines interact differently with α- and β-adrenoceptors.

2.4. *Conformational requirements for directly-acting α- and β-adrenoceptor agonists*

2.4.1. *Conformational requirements of phenethylamines.* The most active enantiomer of noradrenaline and other phenethylamines at α- and β-adrenoceptors is the *R*(−)-enantiomer.[5,13,14,18,21] When two asymmetric centers exist on a phenethylamine, as in α-methylnoradrenaline (Fig. 5) or ephedrine, the 1*R*,2*S*(−)-*erythro*-enantiomer is most active.[5,6,13,14,18,21,35,54,55] The relative positions in space of the three important functional groups (i.e., phenyl, β-hydroxyl and aliphatic nitrogen) of a phenethylamine when bound to α- and β-adrenoceptors, is obtained from an analysis of the conformational demands made by these receptors. The exact conformation of a phenethylamine required for interaction with the α- and β-adrenoceptors is not known with certainty. However, consideration of the physical properties of these agonists in the solid state and in solution has allowed the energetically preferred conformations to be established. Theoretical calculations[56–62] indicate that the preferred conformation of *R*(−)-noradrenaline in solution is the extended-*trans* conformation in which the amino and phenyl groups are at a dihedral angle of 180° (Fig. 12). This conformation represents an energy minimum, and hence greater stability and a greater probability of existence at any point in time. This conformation also appears to be stabilized by an intramolecular electrostatic or hydrogen bonding interaction between the amino and β-hydroxyl groups[61,62] (Fig. 12). The 1*R*,2*S*-α-methyl, β-hydroxyl disubstituted phenethylamines, such as 1*R*,2*S*(−)-*erythro*-ephedrine and 1*R*,2*S*(−)-*erythro*-α-methylnoradrenaline, also prefer this same extended-*trans* conformation.[17,56,61,62] X-Ray crystallographic studies of (−)-noradrenaline and (−)-ephedrine in the solid state[63,64] indicate that in this state, as in solution, the preferred conformation is the extended-*trans* form.

These studies regarding the preferred conformations of sympathomimetic amines in solution and in the solid state have led to speculation concerning which conformation of a phenethylamine is required for binding to, and activation of, the α- and β-adrenoceptors. However, the preferred conformation of an agonist in solution or in the solid state is not necessarily the same conformation required for interaction with the receptor.[14,65] Furthermore, the preferred conformation of a phen-



1R(-)-Noradrenaline

Fig. 12. Newman projection of the 1*R*(−)-enantiomer of noradrenaline showing both hydrophilic and hydrophobic sides of the molecule. Note the proposed intramolecular hydrogen bond that forms between the β-hydroxyl group and the amino group which has been suggested to stabilize the molecule in the *trans*-extended conformation.

ethylamine in the relatively lipoidal region of the receptor, commonly called the 'biophase',[66,67] or near the 'active' site of the receptor where the physical environment may not resemble an aqueous solution, may differ from that confirmation found to predominate in solution.[65,68]

The molecular conformation of various biogenic amines has been reviewed in detail by Carlstrom *et al.*[64] These authors propose, based on a review of a large volume of literature dealing with conformations of biogenic amines, that potent, directly-acting phenethylamines should have the five following characteristics: (a) a six membered aromatic ring system, (b) an extended ethylamine side chain oriented approximately perpendicular to the aromatic ring system, (c) a positively charged nitrogen atom (at physiological pH) on the ethylamine side chain, (d) a hydrophilic and hydrophobic side of the molecule resulting from the β-hydroxyl group being oriented on the same side of the molecule (*cis*) as the *meta* phenolic hydroxyl group of the aromatic ring, and (e) an *R* absolute configuration at the β-carbon atom to which is attached a hydroxyl group (Fig. 12). According to Carlstrom *et al.*,[64] the amino, phenyl and β-hydroxyl groups, which the Easson and Stedman hypothesis[10] suggest are necessary for interaction with α- and β-adrenoceptors, will be in the appropriate configuration to interact with the receptors only when these five requirements are met, as is the case for the levorotatory, *R*-enantiomers of the phenethylamines. For the dextrorotatory, *S*-enantiomers, where the β-hydroxyl group is on the opposite side from the *meta* phenolic hydroxyl group, or for the corresponding desoxy derivative in which the β-hydroxyl group is absent, weaker activity should result, as predicted by the Easson–Stedman hypothesis[10] (see Section 2.1.1.1.).

Several attempts have been made to establish which of the infinite number of possible conformations that phenethylamines may adopt is required for interaction with the α-adrenoceptors. However, relatively little has been done to establish the conformational requirements of the β-adrenoceptors. The use of conformationally rigid or restricted analogs of noradrenaline has been attempted by Smissman and Gastrock.[69] These investigators have synthesized a series of conformationally restrained noradrenaline analogs that are derivatives of *trans* decalin (Fig. 13) for the purpose of establishing the conformational requirements of α-adrenoceptors for phenethylamines. However, the pharmacological activity of these conformationally restrained noradrenaline analogs yielded little new information due to the extremely low agonist activity that resulted from the additional bulk used to restrict conformation. Hence, these efforts have not provided the important information originally hoped for.

However, Erhardt *et al.*,[70] using two conformationally restricted analogs of dopamine (desoxynoradrenaline) which possess relatively little unnecessary bulk, have attempted to establish the conformational demands made by α_1-adrenoceptors. These investigators evaluated the α_1-adrenoceptor mediated effects of the *trans*-extended and *cis*-folded enantiomers of 2-(3,4-dihydroxyphenyl)-

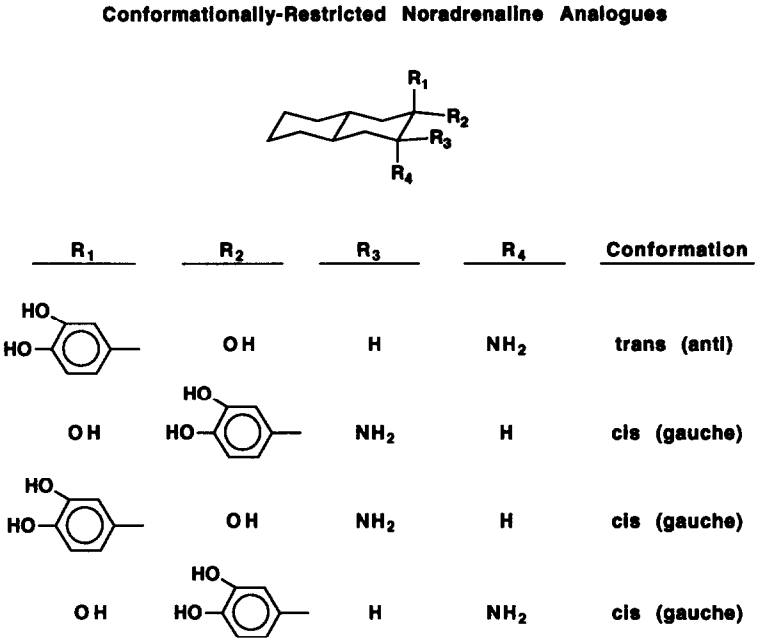


Fig. 13. Conformationally-restricted *trans*-decalin analogues of noradrenaline.

cyclopropylamine (Fig. 14) in rabbit aorta. The *trans*-extended form was found to be 5-fold more potent than the *cis*-folded analogue, strongly suggesting that the *trans*-extended conformation, which is the highly preferred conformation for phenethylamines in solution and in the solid state, is also that conformation preferred by the α_1-adrenoceptor.

In a similar manner, Ruffolo *et al.*[4] have investigated the conformational demands made by α_2-adrenoceptors by evaluating the *trans*-extended and *cis*-folded analogs of 2-(3,4-dihydroxyphenyl)-cyclobutylamine (Fig. 14) in field-stimulated guinea pig ileum. These results indicated that the pre-junctional α_2-adrenoceptor also preferred phenethylamines in the *trans*-extended conformation over the *cis*-folded form. It was concluded, therefore, that α_1- and α_2-adrenoceptors have similar conformational requirements for activation by agonists of the phenethylamine class.

A series of N-substituted *exo*- and *endo*-isomers of 2-amino-6,7-dihydroxybenzonorbornene

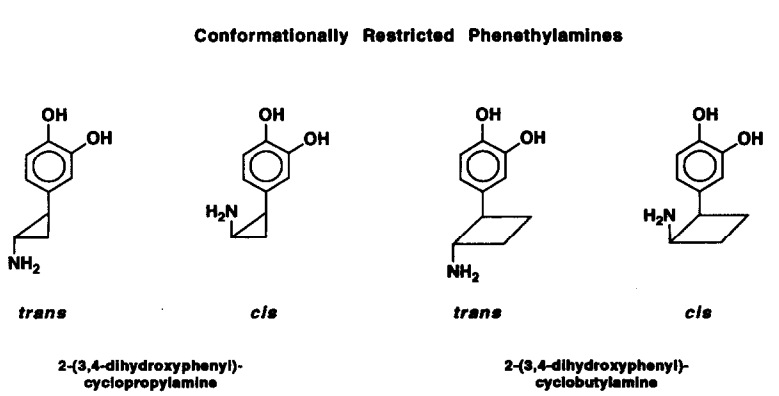


Fig. 14. Conformationally restricted cyclopropylamine and cyclobutylamine derivatives of dopamine used to establish the conformational requirements of α_1- and α_2-adrenoceptors, respectively.

2-Amino-6,7-dihydroxybenzonorbornene

exo **endo**

Fig. 15. Conformationally-restricted analogs of dopamine that were used to establish the conformational requirements of α_1- and α_2-adrenoceptors.

derivatives (Fig. 15) have been synthesized as rigid catecholamine analogs and have been investigated for activity at α_1- and α_2-adrenoceptors by Hicks *et al.*[70a] It was observed that the *endo*-isomer, which corresponded to the *cis*-folded conformation of a phenethylamine, was inactive at both α_1- and α_2-adrenoceptors, in contrast to the *exo*-isomer, which corresponded to the *trans*-extended conformation, which was extremely potent at both α_1- and α_2- adrenoceptors. These results are consistent with those described above which indicate that for activation of both α_1- and α_2-adrenoceptors, a fully extended-*trans* conformation of a phenethylamine is required.

2.4.2. *Conformational requirements of imidazolines and imidazolidines.* One of the first attempts to define the molecular conformation required for interaction of the imidazolines with α-adrenoceptors was made by Pullman *et al.*[59] In a quantum mechanical study of the conformational properties of naphazoline, these investigators concluded that the most stable conformation of naphazoline was one in which the naphthyl and imidazoline rings were mutually perpendicular with a dihedral angle of 90°. This conformation would place the aromatic ring and one of the imidazoline nitrogen atoms at a dihedral angle of approximately 180°, similar to what has been observed with the phenethylamines in the solid state and in solution. Consistent with this observation are reports that the free base of clonidine in solution prefers a conformation in which the phenyl and imidazoline rings assume a mutually perpendicular arrangement.[71–74] X-Ray crystallographic studies of clonidine hydrochloride in the solid phase also show a nearly perpendicular arrangement between the phenyl and imidazoline rings.[75] Although the perpendicular arrangement of the phenyl and imidazoline rings of clonidine has been attributed to steric forces around the relatively bulky *ortho* chlorine substituents,[76,77] it has recently been observed that even unsubstituted benzylimidazolines and phenyliminoimidazolines may also assume the same perpendicular arrangement of the phenyl and imidazoline rings in solution.[73,74]

Although the preferred conformation of the imidazolines in solution and in the solid state has been established and shown to resemble the *trans*-extended conformation of the phenethylamines, conformationally rigid imidazoline derivatives have not been studied in a highly quantitative manner in order to establish conclusively that this conformation is also the conformation preferred by the adrenoceptors for agonists and the imidazoline class.

3. STEREOCHEMICAL REQUIREMENTS FOR INDIRECTLY-ACTING SYMPATHOMIMETIC AMINES

3.1. *Indirectly-acting sympathomimetic amines*

It is now known that α- and β-adrenoceptor agonists may be divided into two classes based on their mechanisms of action. The first class, which has been discussed previously, is referred to as directly-acting sympathomimetic amines. These agonists interact directly with α- and β-adrenoceptors, and a study of the configurational and conformational requirements for these compounds provides insight into the stereochemical demands made by the pre- and postjunctional α- and β-adrenoceptors.

The second class of α- and β-adrenoceptor agonists is referred to as the indirectly-acting sympathomimetic amines, which include compounds such as tyramine, ephedrine and amphetamine. Agonists of this class do not interact directly with α- and β-adrenoceptors to a significant extent, but rather enter the adrenergic nerve terminal and release endogenous (largely 'cytoplasmic') stores of neurotransmitter from sympathetic nerve terminals.[78–80] Before endogenous stores of catecholamines (generally noradrenaline in postganglionic sympathetic nerve terminals) can be released, the indirectly-acting sympathomimetic amines must first be transported into the nerve terminal by the cocaine-sensitive amine uptake pump (uptake$_1$) located on the neuronal cell membrane[9] (Fig. 1). Thus, a configurational and conformational analysis of indirectly-acting sympathomimetic amines will provide valuable information concerning the stereochemical demands made by the uptake$_1$ pump. It should also be noted that some sympathomimetic amines act by both a direct and indirect mechanism,[21] and care must be taken in evaluating the stereochemical selectivities of these 'mixed' adrenoceptor agonists.

While most of the neurotransmitter released by indirectly-acting sympathomimetic amines is liberated from cytoplasmic pools, release of bound neurotransmitter from within adrenergic storage vesicles located in the sympathetic nerve terminal (Fig. 1) also occurs for some compounds.[80] Before vesicular release of neurotransmitter can occur, these indirectly-acting sympathomimetic amines must first be transported from the cytoplasm of the nerve terminal into the storage vesicle. Such compounds must, therefore, also be substrates for the vesicular transport system in addition to the neuronal uptake$_1$ process (Fig. 1). Both reserpine-sensitive and reserpine-resistant vesicular uptake processes have been identified, and it has been determined that certain indirectly-acting sympathomimetic amines release bound vesicular noradrenaline following transport into the storage vesicle via the reserpine-resistant system, while others use the reserpine-sensitive carrier.[80–83] Indirectly-acting sympathomimetic amines may therefore liberate noradrenaline from the cytoplasmic stores following uptake into the nerve terminal by the uptake$_1$ pump, or from vesicular stores following first accumulation by the uptake$_1$ pump and subsequent uptake into the adrenergic storage vesicles. As such, the configurational and conformational demands made by uptake$_1$ and by the vesicular uptake mechanism(s) must be considered.

3.2. *Configurational requirements for indirectly-acting phenethylamines*

3.2.1. *Phenethylamines with asymmetry at the β-carbon atom (benzylic position).* The ability of the neuronal uptake pump (uptake$_1$) to distinguish between enantiomers of optically active phenethylamines with an asymmetric β-carbon atom has been highly disputed. Early reports indicated that the neuronal uptake$_1$ pump, which transported indirectly-acting sympathomimetic amines into the sympathetic nerve terminal, showed some degree of stereoselectivity for *R*(−)-enantiomers of phenethylamines.[84–87] However, the differences observed between enantiomers were rather small (only 2- to 5-fold) and were not consistent from tissue-to-tissue and could not be observed in all tissues.[88,89] In fact, in certain tissues where neuronal uptake had previously been shown to be highly stereoselective for *R*(−)-enantiomers, this 'apparent' stereoselectivity was lost after inhibiting the vesicular uptake process which itself shows a high degree of stereoselectivity.[90–92] These results suggested that the stereoselectivity originally attributed to the neuronal uptake$_1$ pump may have in large part resulted from stereoselectivity occurring at the level of the adrenergic storage vesicle.

The question as to the absolute stereoselectivity of the neuronal membrane uptake$_1$ pump for phenethylamines possessing asymmetry at the β-carbon atom has never been completely resolved, but Iversen *et al.*[93] have argued convincingly that uptake$_1$ is in all probability stereoselective for *R*(−)-enantiomers of β-hydroxyl substituted phenethylamines. The enantiomeric activity differences displayed by the neuronal uptake$_1$ pump are at best only very small, and would seem to vary from tissue-to-tissue, with some tissues showing a complete absence of stereoselectivity. The relatively small differences in stereochemical preference shown by the neuronal uptake pump for optically active phenethylamines with chirality existing at the β-carbon atom, would seem to contrast with

the relatively large stereochemical differences of up to two to three orders of magnitude shown by the α- and β-adrenoceptors for the same compounds.

While the question of stereoselectivity at the neuronal uptake pump associated with the cell membrane remains somewhat of an enigma, there is little doubt that the transport system associated with the adrenergic storage vesicles for sympathomimetic amines with asymmetry at the β-carbon atom is a highly stereoselective process. von Euler and colleagues[94-97] have consistently demonstrated the existence of a stereochemical preference for *R*(−)-noradrenaline over the *S*(+)-enantiomer in storage vesicles isolated from bovine splenic nerves. Likewise, a similar stereochemical preference has been observed in isolated bovine chromaffin granules (i.e., adrenergic storage vesicles in adrenal chromaffin cells), or 'ghosts' prepared from them,[98,99] and in storage vesicles from rat heart.[100]

3.2.2. *Phenethylamines with asymmetry at the α-carbon atom.* Although the neuronal uptake$_1$ pump displays little or no stereoselectivity for optically active phenethylamines with the point of asymmetry existing at the β-carbon atom, such is not the case when asymmetry exists at the α-carbon atom where relatively large and reproducible differences in enantioselectivity have been observed for the uptake$_1$ transport system. Iversen[101] has reported a 20-fold difference in the abilities of 2*S*(+)- and 2*R*(−)-amphetamine to inhibit the neuronal uptake of noradrenaline, with the 2*S*(+)-enantiomer being the most potent. In addition, Marquardt *et al.*[102] investigated the ability of three phenethylamines with α-methyl substitutions to inhibit noradrenaline uptake into synaptosomes prepared from rat brain. For each of the three enantiomeric pairs, activity was approximately 3-fold greater for the 2*S*(+)-enantiomer compared to the corresponding 2*R*(−)-enantiomer.

Data are noticeably lacking concerning asymmetry at the α-carbon atom and stereoselectivity of phenethylamine uptake into adrenergic storage vesicles. However, inferences drawn from compounds with two asymmetric centers (see Section 3.2.3.) would tend to indicate that the vesicular uptake process also favors the 2*S*(+)-enantiomers over the 2*R*(−)-enantiomers for optically active phenethylamines with asymmetry at the α-carbon atom.

3.2.3. *Phenethylamines with asymmetry at both the α- and β-carbon atoms.* Patil and Jacobowitz[35] have determined by histochemical studies in iris from reserpine-pretreated rats that both the 1*R*,2*S*(−)-*erythro*- and 1*S*,2*R*(+)-*erythro*-enantiomers of α-methylnoradrenaline are substrates for neuronal uptake$_1$ with little or no stereochemical preference being observed for either enantiomer. While both *erythro*-enantiomers of α-methylnoradrenaline appear to be transported by uptake$_1$ to similar degrees, it was observed that neither of the *threo*-enantiomers of α-methylnoradrenaline (see Fig. 5) were potent substrates for uptake$_1$. Thus, while neuronal uptake$_1$ failed to distinguish between the 1*R*,2*S*(−)- or 1*S*,2*R*(+)-*erythro*-enantiomers of α-methylnoradrenaline, the membrane uptake pump could distinguish between the *erythro* and *threo* diastereoisomers. In spite of this apparent stereoselectivity in rat iris, similar rates of neuronal uptake between the *erythro* and *threo* diastereoisomers of α-methylnoradrenaline have been reported in mouse and rabbit hearts, although the *erythro* isomers were selectively retained, possibly indicating a high degree of stereoselectivity for the *erythro* isomers of α-methylnoradrenaline at the level of the vesicular uptake process.[103,104]

Several studies have shown that only the 1*R*,2*S*(−)-*erythro*-enantiomer of metaraminol can function as a false neurochemical transmitter.[105,106] For a compound to serve as a false neurotransmitter, it must first be accumulated by the neuronal uptake$_1$ mechanism and then subsequently transported from the cytoplasm into the adrenergic storage vesicle by the vesicular uptake pump.[107] Since all four optical isomers of metaraminol appear to be substrates for neuronal uptake, by inference it appears logical to conclude that only the 1*R*,2*S*(−)-*erythro*-enantiomer is subsequently accumulated and retained by the vesicular uptake pump of the adrenergic storage vesicles. It is concluded, therefore, that the adrenergic storage vesicles display a marked degree of stereoselectivity for only the 1*R*,2*S*(−)-*erythro*-isomer of phenethylamines with asymmetry at both the α- and β-carbon atoms.

A more direct assessment of vesicular uptake of the stereoisomers of metaraminol has been

made by Sugrue and Shore[83] who have demonstrated that only the 1*R*,2*S*(−)-*erythro*-enantiomer is a substrate for uptake into adrenergic storage vesicles. Consistent with this observation are the findings of Muscholl *et al.*[108] who demonstrated that while the initial rates of uptake of the optical isomers of α-methylnoradrenaline are the same, there is a selective retention of only the 1*R*,2*S*(−)-*erythro* form. The most likely interpretation of these results is that the initial accumulation of a sympathomimetic amine into the cytoplasm by neuronal uptake$_1$ is not a highly stereoselective process for these phenethylamines with two asymmetric centers (as was the case for phenethylamines with one point of asymmetry at the β-carbon atom; Section 3.2.1.), but that vesicular uptake and subsequent retention by the adrenergic storage vesicles are highly stereoselective processes. Results consistent with this interpretation have now been reported by others.[109,110]

The functional significance of these studies on the stereoselectivity of neuronal and vesicular uptake of phenethylamines with two points of asymmetry has been addressed by Patil *et al.*[20,21,54,55] who investigated the adrenoceptor-mediated effects of the four optical isomers of ephedrine in the rat vas deferens where the effects of the ephedrine isomers are predominantly indirect.[21] In this tissue, the indirect activity of ephedrine resides in only the 1*R*,2*S*(−)-*erythro*-isomer, with the three remaining isomers being only weakly active or completely inactive. It cannot be stated at the present time whether the stereoselectivity observed in these functional studies of the indirect effects of ephedrine result from stereoselectivity at uptake$_1$, stereoselectivity at the level of vesicular uptake, or both, or whether, in fact, the stereoselectivity occurs at some other process such as displacement of endogenous noradrenaline from one or more bound sites in the cytoplasm or adrenergic storage vesicle.

3.3. *Conformational requirements for neuronal uptake$_1$ and vesicular uptake of phenethylamines*

It has been proposed that the preferred conformation of noradrenaline for neuronal uptake$_1$ is the *trans*-extended conformation in which the phenyl ring and aliphatic nitrogen atom are at a dihedral angle of 180°.[111–114] As discussed previously (Section 2.4.1.), this is the same conformation of a phenethylamine that exists in solution and in the solid state. The use of conformationally restricted phenethylamine derivatives has largely confirmed that the *trans*-extended conformation is highly preferred by the neuronal uptake$_1$ pump. Horn and Snyder[115] and Tuomisto *et al.*[116] have shown that *trans*-2-phenylcyclopropylamine (Fig. 16) is 100- to 1000-fold more potent than the corresponding *cis*-folded form in inhibiting noradrenaline uptake$_1$ in the central nervous system. Miller *et al.*[117] have obtained similar results in peripheral tissues. Other studies using rigid or semirigid phenethylamine analogs also indicate a high degree of selectivity of the amine uptake pump for the *trans*-extended conformation of phenethylamines.[112,113,117–120] Some evidence exists, however, to indicate that gauche conformations may not be without activity.[14,121] The conformational demands made by the vesicular transport system for phenethylamines have not been

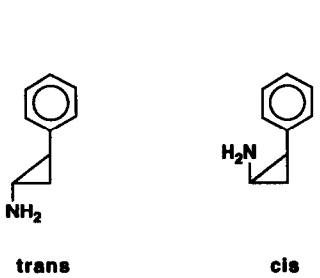


Fig. 16. Conformationally restricted cyclopropylamine derivatives used to establish the conformational requirements of neuronal uptake$_1$.

defined. It is interesting to note that although the neuronal uptake$_1$ process does not have stringent configurational requirements, especially for phenethylamines with asymmetry at the β-carbon atom, the uptake$_1$ pump is extraordinarily selective in its conformational requirements, with a clear preference for phenethylamines in the *trans*-extended conformation.

4. STEREOCHEMICAL REQUIREMENTS FOR α- AND β-ADRENOCEPTOR ANTAGONISTS

4.1. α-*Adrenoceptor antagonists*

Stereoselectivity in α-adrenoceptor antagonists has been a relatively disappointing area of research. One reason for this is the fact that the vast majority of α-adrenoceptor antagonists do not possess a chiral center. Furthermore, most α-adrenoceptor antagonists bear no resemblance to the agonists, and as such, it is difficult to draw parallels. Several optically active antagonists of the imidazoline class have been prepared, but differences in activity between enantiomers is either not commonly observed or is low.

4.1.1. *Benzodioxanes.* One particularly interesting class of α-adrenoceptor antagonists is the benzodioxanes which do possess one or more points of asymmetry. Several benzodioxanes have been synthesized and their absolute configurations have been determined by Nelson and co-workers.[122,123] Benzodioxanes, such as WB-4101, prosympal, and piperoxan (Fig. 17), possess one asymmetric center, and it has been determined that the *S* absolute configuration is always more potent than the *R* configuration.[50,122-124] Dibozane has two asymmetric centers (Fig. 17), and it has been determined that the *S,S*-enantiomer is more potent than the *R,R*-enantiomer.[50] The *meso* form (dibozane is a symmetrical molecule) is similar in potency to the *S,S*-enantiomer.[50] Nelson *et al.*[122] have argued convincingly that the conformational distribution of the aminoalkyl, oxygen and aromatic functional groups of the *S*-benzodioxanes is similar to that of *R*(−)-adrenaline, thus accounting for the α-adrenoceptor blocking activities and the observed stereoselectivities of this class of compounds.

4.1.2. *Yohimbine diastereoisomers.* Weitzell *et al.*[125] have reported marked and surprising differences in the prejunctional α_2- and postjunctional α_1-adrenoceptor blocking potencies of the yohimbine diastereoisomers (Fig. 18). Differences in potency as well as α-adrenoceptor subtype selectivity have been reported, and several of the yohimbine isomers are invaluable as pharmacological tools to subclassify α-adrenoceptors. McGrath[126] has indicated that the rank order of potency of

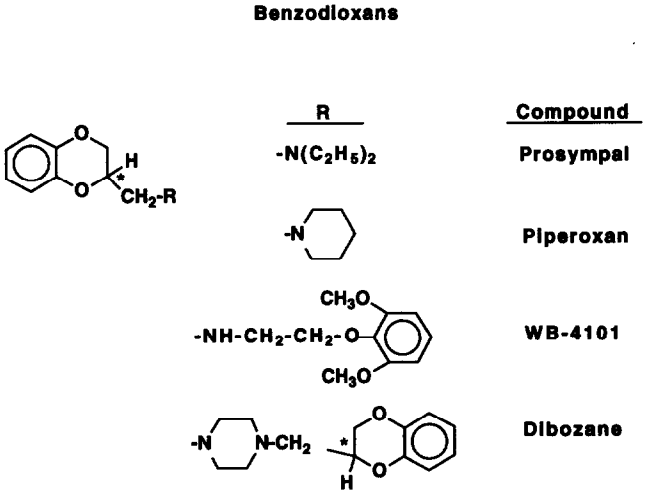


Fig. 17. Chemical structures of several optically active α-adrenoceptor blocking agents of the benzodioxan class. The asterisk denotes the point of asymmetry.

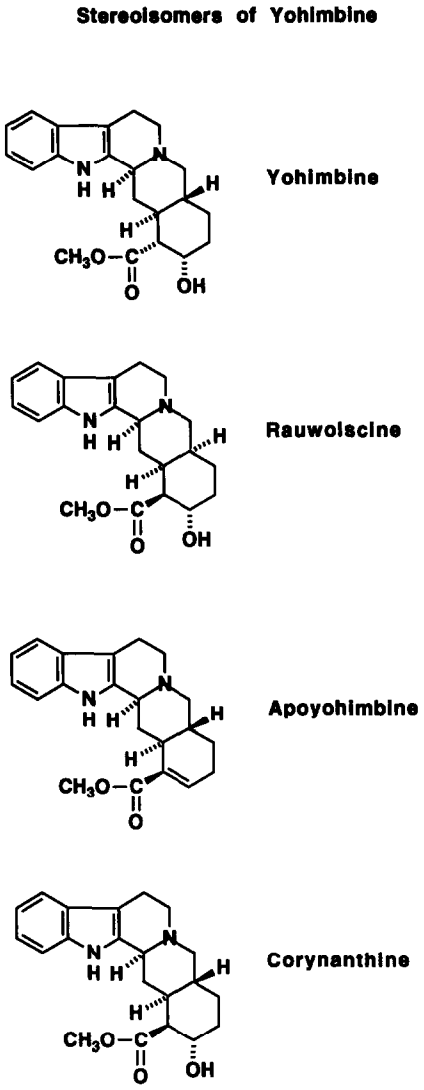


Fig. 18. Chemical structures of the stereoisomers of yohimbine that are used to characterize and subclassify α_1- and α_2-adrenoceptors.

the four isomers of yohimbine as antagonists of the α_1-adrenoceptors is; apoyohimbine > corynanthine > yohimbine > rauwolscine. Potencies at α_2-adrenoceptors are markedly different, with a rank order of: apoyohimbine > rauwolscine > yohimbine > corynanthine. As a result of these differences in the relative potencies at α_1- and α_2-adrenoceptors, the selectivities of these compounds for α_2- vs α_1-adrenoceptors is: rauwolscine > yohimbine > apoyohimbine > corynanthine. Thus, rauwolscine and yohimbine have become standard α_2-adrenoceptor antagonists, and corynanthine has become a prototype α_1-adrenoceptor antagonist.

4.1.3. *β-Haloalkylamines.* The enantiomers of the irreversible α-adrenoceptor antagonist, phenoxybenzamine (Fig. 19), have been prepared and evaluated by Portoghese *et al.*[127] A 15-fold difference was observed in the rate at which these enantiomers alkylate α-adrenoceptors, with the (+)-enantiomer having the faster rate. Portoghese *et al.*[127] have argued that the intrinsic alkylating

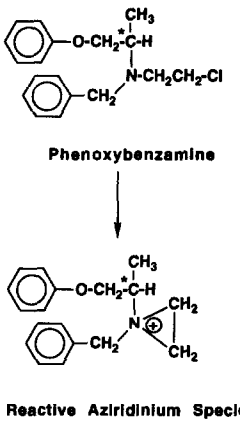


Fig. 19. Chemical structure of phenoxybenzamine and the reactive aziridium species that exists prior to covalent binding to α_1- and α_2-adrenoceptors. The asterisk denotes the point of asymmetry.

activities of the phenoxybenzamine enantiomers is the same, and that both enantiomeric forms interact with the α-adrenoceptor through the highly reactive chiral, aziridinium species which is formed from both enantiomers at the same rate (Fig. 19). It was proposed, therefore, that the observed enantiomeric activity difference resulted from differences in affinities of the asymmetric precursors for the α_1-adrenoceptor. In contrast, other irreversible α-adrenoceptor antagonists of the β-haloalkylamine class, such as N,N-dimethyl-β-chlorophenethylamine, have been shown to lack enantioselectivity,[128,129] since both enantiomers alkylate the α_1-adrenoceptor through one common, highly reactive, achiral intermediate in which asymmetry is also lost prior to alkylation of the receptor.

4.1.4. *Imidazolines.* Idazoxan (Fig. 20) is an imidazoline that has been identified as a highly potent and selective antagonist of peripheral and central α_2-adrenoceptors, with an α_2/α_1-adrenoceptor selectivity ratio of 100- to 1000-fold. In the rat vas deferens, (−)-idazoxan was found to be three times more potent than the (+)-enantiomer at antagonizing the prejunctional α_2-adrenoceptor mediated effects of clonidine. In contrast, (+)-idazoxan was 15 times more potent than (−)-idazoxan in blocking the postjunctional α_1-adrenoceptor mediated effects of phenylephrine, although both enantiomers are far less potent as α_1-adrenoceptor antagonists than α_2-adrenoceptor antagonists.[130] In the same study, (+)-idazoxan was found to be more potent than (−)-idazoxan in antagonizing the central α_2-adrenoceptor-mediated sedation produced by clonidine or azapexole. Since the physicochemical properties of (−)- and (+)-idazoxan are identical, and brain penetration of both compounds is therefore likely to be the same, these data suggest that differences may exist

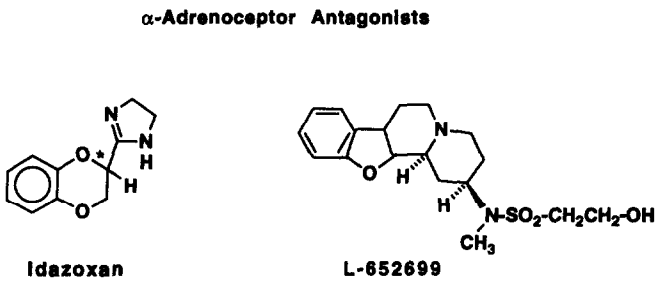


Fig. 20. Chemical structures of the α-adrenoceptor antagonists, idazoxan and L-652799. The asterisk denotes the point of asymmetry.

between central and peripheral α_2-adrenoceptor populations in their stereochemical requirements for optically active antagonists of the imidazoline class. As such, (−)-idazoxan appears to be the more potent α_2-adrenoceptor antagonist in the periphery, whereas (+)-idazoxan is the most potent enantiomer at blocking central α_2-adrenoceptors.

4.1.5. *Benzoquinolizines.* A series of hexahydrobenzofuroquinolizines have been synthesized and examined for relative affinities at α_1- and α_2-adrenoceptors.[131,132] Racemic N-(1,3,4,6,7,12*b*-hexahydro-2H-benzo[b]furo[2,3-*a*]quinolizin-2-yl)-N-methyl-2-hydroxy-ethane-sulfonamide (L-652699; Fig. 20) was identified as a selective α_2-adrenoceptor antagonist that exhibited a 150-fold preference for α_2- vs α_1-adrenoceptors. The (2*R*,12*bS*)-isomer of L-654284 proved to be twice as potent as the racemate, whereas the (2*R*,12*bR*)-enantiomer displayed little or no activity. The stereochemical configuration of the chiral centers of L-654284 are identical to the corresponding centers in yohimbine and rauwolscine (Fig. 18), to which L-654284 is structurally related.

4.2. *β-Adrenoceptor antagonists*

4.2.1. *Aryloxyethylamines.* Unlike α-adrenoceptor antagonists, β-adrenoceptor antagonists closely resemble the agonists in both chemical structure and stereochemical requirements. Thus, most β-adrenoceptor antagonists are ethylamines, or more appropriately, aryloxyethylamines, and possess a hydroxyl group in a position analogous to the β-hydroxyl group of the phenethylamines, such as isoprenaline. The most active enantiomers of β-adrenoceptor antagonists with asymmetry at this point have absolute stereochemical configurations identical to those of the active enantiomers of β-adrenoceptor agonists, such as 1*R*(−)-isoprenaline. Thus, the enantiomeric activity ratio for the optical isomers of propranolol (Fig. 21) is approximately 40-fold, with the (−)-enantiomer being most potent.[14] The similarity in stereochemical requirements for β-adrenoceptor agonists and antagonists suggests that β-adrenoceptor antagonists may be more specific in their attachment to β-adrenoceptors than α-adrenoceptor antagonists are in their attachment to α-adrenoceptors, where little similarity exists between agonists and antagonists.[14]

Methyl substitution of β-adrenoceptor antagonists at the α-carbon atom, as in butoxamine (Fig. 21), has been reported to decrease potency.[62] It would appear, however, that in these β-adrenoceptor antagonists where asymmetry exists at both the α- and β-carbon atoms, the most active enantiomer

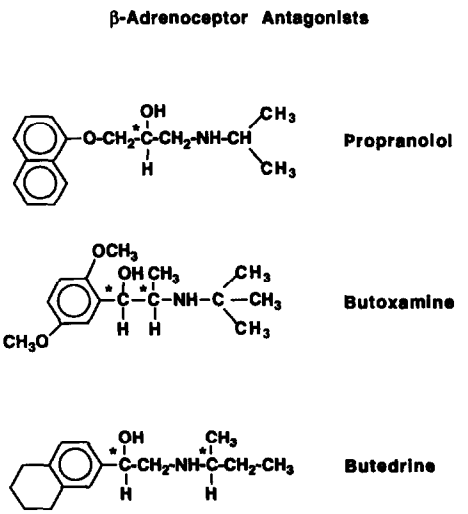


Fig. 21. Chemical structures of several β-adrenoceptor antagonists with one or two points of asymmetry. The asterisk notes the point of asymmetry.

will have the same absolute configuration as the structurally related agonists in the phenethylamine class, such as 1*R*,2*S*(−)-*erythro*-α-methylnoradrenaline. Butedrine (Fig. 21) is a β-adrenoceptor antagonist with two points of asymmetry that are remote from each other. At the 1 position is the hydroxyl group, and at the 4 position is the methyl substituent. All four optical isomers (i.e., two pairs of diastereoisomers) have been tested and both the 1*R*,4*R*- and 1*R*,4*S*-isomers are potent β-adrenoceptor antagonists, with the 1*R*,4*R*-enantiomer perhaps being slightly more potent.[14] The 1*S*,4*S*- and 1*S*,4*R*-isomers are relatively weak β-adrenoceptor antagonists presumably due to the incorrect stereochemistry at the 1 position.

4.3. *'Mixed' α- and β-adrenoceptor antagonists*

4.3.1. *Labetalol.* The most useful information concerning the stereoselectivity of α- and β-adrenoceptor antagonists comes from the relatively new class of antagonists that block both α- and β-adrenoceptors. Labetalol is the prototypic member of this class of 'mixed' antagonists and possesses two asymmetric centers (Fig. 22) giving rise to two pairs of diastereoisomers and four possible optical isomers. Recent studies with labetalol[133-136] have demonstrated that the α- and β-adrenoceptor blocking properties are not distributed uniformly among the individual stereoisomers. The four optical isomers of labetalol have now been resolved and their absolute configurations have been assigned. The α- and β-adrenoceptor blocking activities of the stereoisomers of labetalol have been evaluated in the dog by Brittain *et al.*,[133] and the relative potencies listed in Table 2 have been calculated from their results. It is clear that the α_1-adrenoceptor blocking activity of labetalol resides predominantly in the 1*S*,4*R*-isomer, whereas the β_1- and β_2-adrenoceptor blocking effects reside predominantly in the 1*R*,4*R*-isomer. That one of the four optical isomers of labetalol should be a selective α_1-adrenoceptor antagonist while another shows a distinct preference for β_1- and β_2-adrenoceptors is indeed remarkable. An interesting comparison may be made between labetalol and dobutamine (see Section 2.1.4. and Fig. 7). Both compounds are close structural analogs, but dobutamine has only one asymmetric center on the relatively bulky N-substituent, an asymmetric position also common to labetalol. In the case of dobutamine, it was observed that the (−)-enantiomer was predominantly an α_1-adrenoceptor agonist while the (+)-enantiomer was predominantly a β_1- and β_2-adrenoceptor agonist, which is similar to the situ-

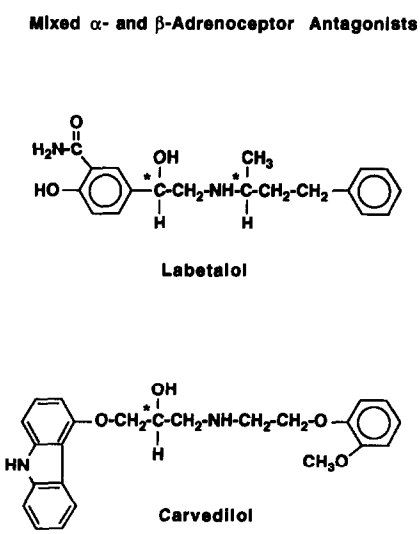


Fig. 22. Chemical structures of two 'mixed' α- and β-adrenoceptor antagonists, labetalol and carvedilol. The points of asymmetry are denoted by the asterisks.

Table 2. Relative α- and β-adrenoceptor blocking activities of the stereoisomers of labetalol in the dog

	Relative Potencies		
Absolute Configuration	α_1	β_1	β_2
Labetalol (1R,4R; 1S,4S; 1R,4S;1S,4R)	1	1	1
1R,4R-Labetalol	0.15	2.27	2.18
1S,4S-Labetalol	0.39	0.03	<0.02
1R,4S-Labetalol	0.23	0.15	0.09
1S,4R-Labetalol	1.74	0.04	0.02

ation with labetalol, except that the isomers of labetalol are antagonists. Thus, it may be that this relatively unusual position of asymmetry can impart differential α- and β-adrenoceptor selectivities upon the individual stereoisomers.

4.3.2. *Carvedilol.* Carvedilol (Fig. 22) is a multiple action drug now available for use clinically in the treatment of mild to moderate hypertension. The antihypertensive effects of carvedilol result primarily from α_1-, β_1- and β_2-adrenoceptor blockade, as well as from calcium channel blockade. Unlike labetalol, carvedilol has only one point of asymmetry, but as is the case with labetalol, the α- and β-adrenoceptor blocking effects of carvedilol reside in different enantiomers.[137] Thus, the potent β_1-adrenoceptor antagonist activity of carvedilol resides primarily in the $S(-)$-enantiomer, which is at least 100-fold more potent in this regard than the $R(+)$-enantiomer. Interestingly, the α_1-adrenoceptor blocking activity of carvedilol occurs in both enantiomers and, unlike the case for labetalol, is comparable in both enantiomers. Thus, $S(-)$-carvedilol is a potent and relatively selective β_1-adrenoceptor antagonist, whereas both enantiomers of carvedilol are equipotent α_1-adrenoceptor antagonists.[137] It is apparent, therefore, that the point of asymmetry in carvedilol is significantly more crucial with regard to the β-adrenoceptor blocking activity of the drug compared to the α-adrenoceptor antagonist activity of the compound. Furthermore, the point of asymmetry in carvedilol appears to occur at a position that is not recognized by the α_1-adrenoceptor. As is the case for labetalol, neither enantiomer of carvedilol alone can mimic the pharmacological profile of the racemic mixture used clinically.

5. SUMMARY AND CONCLUSIONS

The Easson–Stedman hypothesis [i.e., $R(-)$-enantiomer > $S(+)$-enantiomer = desoxy derivative] is the most generally applicable theory regarding the direct sympathomimetic activity of phenethylamines possessing one chiral center existing at the β-carbon atom (benzylic position). The theory proposes a three-point attachment to α- and β-adrenoceptors (via the nitrogen, β-hydroxyl and phenyl ring) for the $R(-)$-enantiomer of a phenethylamine, whereas the $S(+)$-enantiomer and corresponding desoxy derivative bind by only a two-point attachment (through the amino and phenyl groups), accounting for their lower activities. The Easson–Stedman hypothesis is valid for phenethylamines interacting with all adrenoceptor subtypes (i.e., α_1, α_2, β_1, β_2), but does not hold for the α-adrenoceptor mediated effects of the imidazolines. Phenethylamines with two asymmetric centers (e.g. α-methylnoradrenaline) have four stereoisomers, and activity resides primarily in the $1R,2S(-)$-*erythro*-isomer for all adrenoceptors. The α- and β-adrenoceptors would appear to prefer phenethylamines and imidazolines in the *trans*-extended conformation in which the aromatic ring and nitrogen atom are at a dihedral angle of 180°.

Indirectly-acting sympathomimetic amines act through liberation of endogenous stores of noradrenaline, and as such, a stereochemical analysis of these compounds provides information about the demands made by the neuronal uptake pump (uptake$_1$) and the vesicular uptake pump of the adrenergic storage vesicles. The neuronal uptake pump displays, at best, only a slight stereochemical preference for 1*R*(−)-enantiomers of β-hydroxyl substituted phenethylamines, while the adrenergic storage vesicles show a high degree of stereoselectivity in favour of the 1*R*(−)-enantiomer. Neuronal uptake$_1$ does show a stereochemical preference for the 2*S*(+)-enantiomers of α-methyl-substituted phenethylamines over the corresponding 2*R*(−)-enantiomers, and thus it follows that the major activity of indirectly-acting sympathomimetic amines with two points of asymmetry (e.g., ephedrine) resides predominantly in the 1*R*,2*S*(−)-*erythro*-isomer. The *trans*-extended conformation of a phenethylamine is highly preferred over the *cis*-folded conformation by the neuronal uptake$_1$ pump.

β-Adrenoceptor antagonists are structurally similar to the agonists, and similar stereochemical requirements are expected and, in fact, are observed. Competitive α-adrenoceptor antagonists of the benzodioxane, imidazoline, yohimbine and benzoquinolizine classes show some degree of stereoselectivity, but the degree of stereoselectivity is often low and not predictable. Most optically active irreversible α-adrenoceptor antagonists of the β-haloalkylamine class (with the exception of phenoxybenzamine) display no stereoselectivity, suggesting that both enantiomeric forms interact with α-adrenoceptors through one common, highly reactive asymmetrical intermediate. 'Mixed' α- and β-adrenoceptor antagonists, such as labetalol and carvedilol, possess one or two points of asymmetry. Differences in the relative α- and β-adrenoceptor blocking potencies exist among the individual stereoisomers of these 'mixed' antagonists. This relatively new class of antagonists with dual α- and β-blocking activities have proven to be useful in distinguishing between the stereochemical demands made by α- and β-adrenoceptors.

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