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Review Article

Analysis of Efficacy of Chiral Adrenergic Agonists

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007.

ABSTRACT The origin of terms, affinity, intrinsic activity (or efficacy) and spare receptors has been reviewed. The Easson–Stedman theory (1933) in relation to the activation of adrenoceptors by agonists proved to be useful in the analysis of affinity and efficacy. Eudismic ratios of agonists provided critical information about the receptor-mediated activation. The evidence from circular dichroism spectroscopy with a fluorescent-tagged adrenoceptor agonist indicates a stereoselective interaction with the receptor. Thus, the simplest definition of efficacy may include the rate of change of the specific conformation of the receptor by the agonist, leading to the organized response. The functional groups of the potent enantiomer are postulated to interact in a "preferred" sequence with the receptor. The 7TM GPCR protein crystal structure of bovine rhodopsin was used as a model to construct the agonist interacting amino acid residues for α_{1A}- and β_1-adrenoceptors. It was observed that both $-^{+}NH_3$ group and chiral —OH group of (−)-epinephrine interact with Asp^{106} TM III of α_{1A}-adrenoceptor. Similar interactions were observed for (+)-epinephrine but critical differences were observed. Enantiomers of epinephrine and oxymetazoline were also docked in the position at β_1-adrenoceptor to elucidate the conformational changes. Some unique information has emerged about the activation of adrenoceptors by agonists. The differences in the pharmacological efficacy of the enantiomers compare favorably with the dynamics of conformational changes by the agonist at α_{1A}- and β_1 adrenoceptors. *Chirality 20:529–543, 2008.*

KEY WORDS: adrenergic agonist; ionization; efficacy; functions; enantiomers of catecholamines; imidazolines; binding sites; 7TM α-β adrenoceptors; initial events; activation sites; molecular modeling; conformations

HISTORICAL PERSPECTIVES ON EFFICACY

Nearly 100 yr ago, the sympathomimetic hormone from the adrenal gland was isolated and characterized as adrenaline or epinephrine.[1] Pure enantiomers were resolved.[2] Naturally occurring (−)-epinephrine, then called suprarenin, was a more potent pressor substance than the (+)-form.[3] Around the same time, the concept of drug–receptors was introduced.[4] The interest in the new synthetic structure–activity studies emerged. Structural molecular basis for differences in the pharmacological activity between enantiomers of epinephrine was simply explained by comparing the pressor activity of (+)-epinephrine with epinine. The latter symmetrical molecule without the benzylic hydroxyl group is pharmacologically equiactive to (+)-epinephrine. These facts led Easson and Stedman to postulate the importance of functional groups around an asymmetric center for the higher biologic activity of the (−)-epinephrine over the (+)-form.[5] The validity of the steric structure–activity relationship in the Easson–Stedman hypothesis for the sympathomimetic class of substances has been investigated.[6] On both α- or β-adrenoceptor-containing preparations, the order of potency in terms of EC_{50} of these catecholamines were (−)-enantiomer > (+)-enantiomer = desoxy catecholamine (Fig. 1). In organs with spare receptors the maximum effects at their highest concentrations of these agonists appeared equal. Particularly, in the vascular system after reduction of the spare α-adrenoceptors, the order of the maximum effect, an expression of efficacy was (−)-enantiomer > (+)-enantiomer = desoxy analogue. In other words, the enantiomers not only differed in affinity but also in intrinsic activity.

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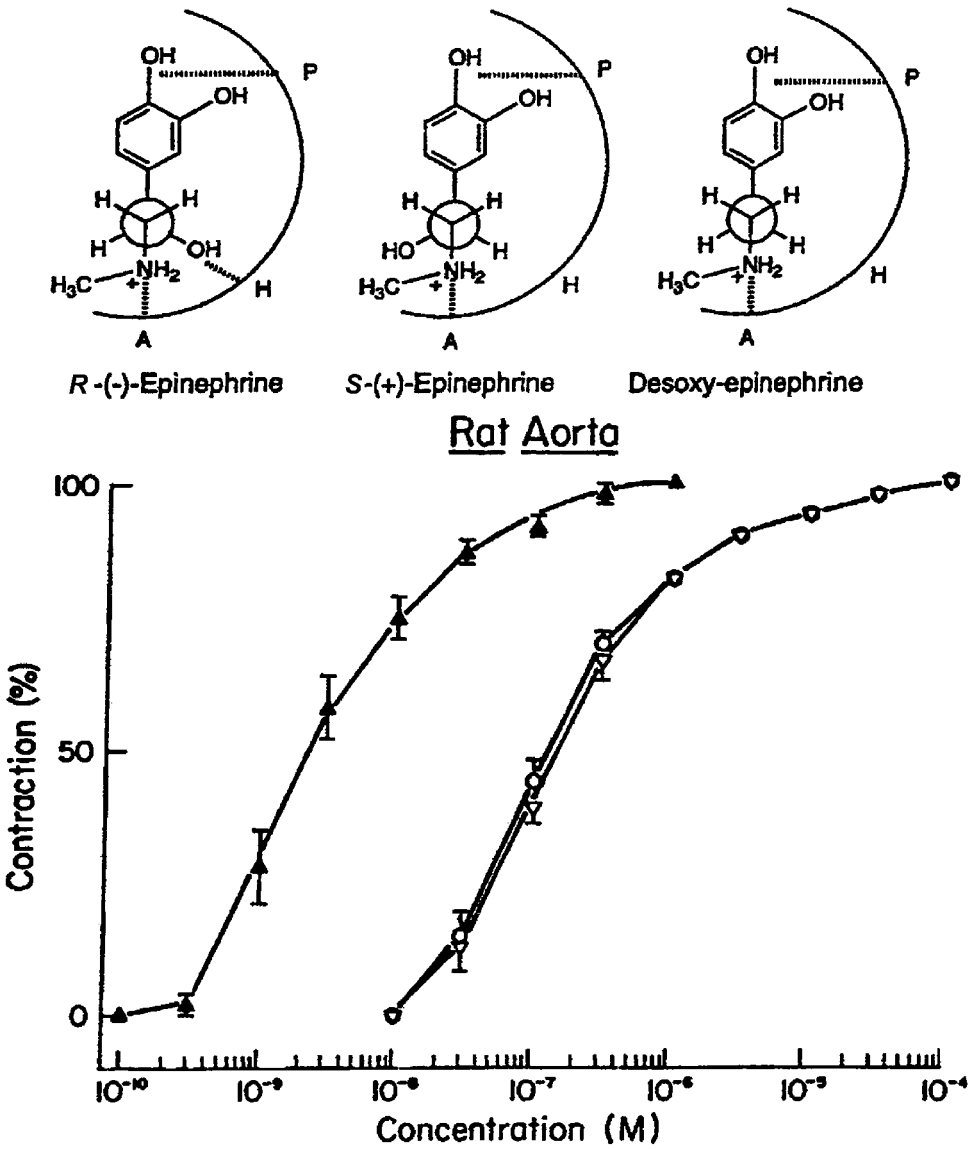


Fig. 1. Neuman projection of agonists. The Easson–Stedman hypothesis indicated that the high pharmacological activity of (−)-epinephrine was due to the sterically correct orientation of the benzylic OH with the receptor.[5] In (+)- form or in desoxy epinephrine (Epinine) such an interaction might be missing, resulting in low activity. The concentration response curve for rat aorta (α-adrenoceptor mediated response) is indicative of the greater higher activity of (−)-epinephrine (▲) and the low but equal potency of (+)-epinephrine (▽) and epinine (○).[7,8]

Ariëns[9] investigated the concentration–response relationship of a series of agonists on isolated organs. The terms affinity and intrinsic activity were used to explain drug action at the receptors. The maximum response at the highest concentration was referred to as the intrinsic activity. In an important publication, Stephenson recognized the fact that only a small number of total receptors in the organ were required by a potent drug to achieve maximum response.[10] The terms efficacy, spare receptors, and partial agonists were introduced. Intrinsic activity and efficacy are considered similar in their meaning. A molecule with low efficacy could interact with all receptors in the organ to produce a maximum response much below that produced by a highly potent agonist with low receptor occupancy. Such a drug is referred as a partial agonist. Thus, the EC_{50} of the partial agonist may represent the affinity parameter because the drug–receptor saturation kinetics can occur. But the EC_{50} of the highly potent molecule in the organ with spare receptors does not represent the affinity parameter because saturation of receptors at the maximum response does not occur.[11] Furchgott[12] inactivated a certain population of the receptors in an organ with various concentrations of the irreversible blocker dibenamine and observed a rectangular hyperbola of the agonist concentration–response curves. Subsequently, a unique method to calculate affinity and relative efficacies of agonists in organs with spare receptors was introduced.[13] The organ is treated with an irreversible blocker in such a way that the "fraction" of available receptors can be activated to produce a reduced concentration response

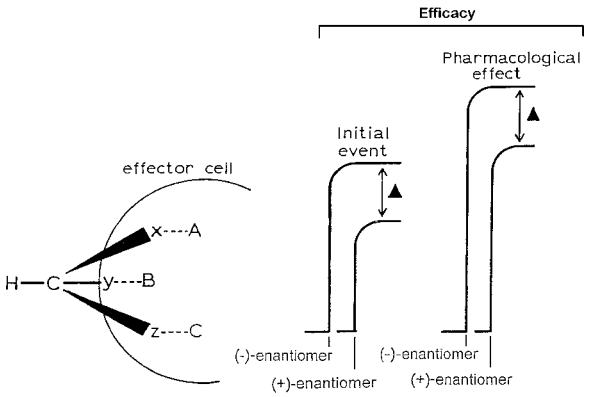


Fig. 2. Differential initial events and the subsequent mechanical pharmacologic effects produced by interaction of enantiomeric agonist with the hypothetical sites of the receptor. If both enantiomers activate two of the three interactive sites, then initial event or efficacy as ▲ = ▲-pharmacological effect can by postulated. On the isolated organs the efficacy is measured as the functional pharmacologic effect.[16,17]

for the so-called potent agonist. The saturation of the limited capacity of free receptors is possible and EC_{50} under these conditions represents the affinity of the drug for the receptor. The relative intrinsic efficacy can be calculated. The absolute intrinsic efficacy of a single agonist cannot be expressed in thermodynamic units. No such method is available so far. Differences in relative maximum effects of (−) and (+)-enantiomers observed in the concentration response curves generally represent differences in efficacy at the receptors.

Colquhoun[14] has carefully analyzed the problem of "affinity" and "efficacy" of agonists for the ion channel receptor. Accordingly the affinity constants determined in ligand binding experiments do not measure the receptor-related conformational changes that are intrinsically linked to the efficacy. He defines efficacy as simply the set of all the other microscopic equilibrium (or rate) constants, which describe all the transduction events that follow the initial binding reactions of receptor activation by the agonist. Such a definition encompasses all aspects of the transduction process. But, the molecular evolution of receptors indicates that the initial conformational change of the receptor by the agonist appears to be the most crucial one.

Thus, efficacy can be significantly related to the rate of change of specific conformation of the receptor and coupled cellular processes by the agonist in the physiologic milieu. A detailed understanding of the molecular events, as well as quantitative changes of receptor conformation by the agonist, is yet to be accomplished. Efficacy differences between enantiomers of epinephrine in all probability originate from nonlinear global conformational changes at the receptor. The signal is then coupled for the enlarged mechanical response via several second messengers collectively referred to as the transducer.[15] Interdependence of second messengers is spatially and kinetically complex. A simplified concept for the drug–receptor-related efficacy of enantiomers is illustrated in Figure 2. It is assumed that both enantiomers interact with the similar sites of the receptor. The enantiomeric activity ratios (or Eudismic ratios) provide an important parameter to discriminate adrenoceptor subtypes in various organs.[18–20] Snail neurons were more sensitive to dopamine, than either enantiomers of norepinephrine.[21] Dopamine-sensitive adenylate cyclase in mammalian neurons provided evidence for the dopamine receptor in substantia nigra.[22] (−)-Methoxamine is a potent α-adrenoceptor activator but blocks the β-adrenoceptor. Dobutamine enantiomers provide unusual pattern in the activation of the adrenoceptor.[23] Thus, in many cases, prediction of affinity and efficacy of catecholamine derivatives has been a difficult task. Therefore, steric structure–activity studies in the search of potent agonists remained empirical.

Stereoselective changes in the circular dichroism spectra of rat lung containing β-adrenoceptor protein provoked by enantiomers of epinephrine has been observed. Pharmacologically active (−)-epinephrine produced specific changes in the 205–220-nm region of the spectra indicating that the receptor helices may be perturbed.[16] The fluorescent tagged β-adrenoceptor also exhibits stereoselective changes with the enantiomers of isoproterenol.[24] The receptor-preferred conformation of the agonist, as well as resting conformation of the membrane receptor, are vital for the optimum efficacy. Over-expression of β_1-adrenoceptors in the heart of mouse by genetic engineering produces higher resting heart rate. It was concluded that the receptor spontaneously isomerized to the activated state without the agonist.[25,26] Such an abnormal base line cannot be used to compare the optimum efficacies of two agonists. Certainly a pathological condition is expressed.

MOLECULAR SPECIES IN ACTIVATION OF THE RECEPTOR

In addition to chirality, the initial events in drug–receptor interaction are governed by many factors. Ganellin[27] provided the most important insight of catecholamines, which can exist in four ionic species. Epinephrine has two p*K*a values, one for the catechol (8.39) and the other for the amino group (9.85). At pH 7.4 the majority (≥95.1%) of the amine exists as charged amino cation which may be responsible for the activation of the α-adrenoceptor.[28] The concentration of the zwitterionic species is about 3%. Is the latter species responsible for the interaction with the β-adrenoceptor? (see Fig. 3). It is important to note that the amino group of sympathomimetic drugs is not absolutely essential for the activation of the β-adrenoceptor. Kaumann et al.[29] observed the activation of the β-adrenoceptor in heart cells by 3′-4′dihydroxy-2-methylpropiophenone, an agonist without nitrogen. The efficacy was about half that of the potent agonist (−)-isoproterenol. Earlier, Larsen suggested a formation of the quinone methide species of catecholamines for the activation of α and β-adrenoceptors.[30] The interesting speculations are difficult to confirm.

Electron withdrawing fluorine (F) substitution at the 2 and 6 positions of the phenyl ring of norepinephrine significantly altered the receptor activating properties of the molecule. 6-F-(±)norephinephrine maintained high po-

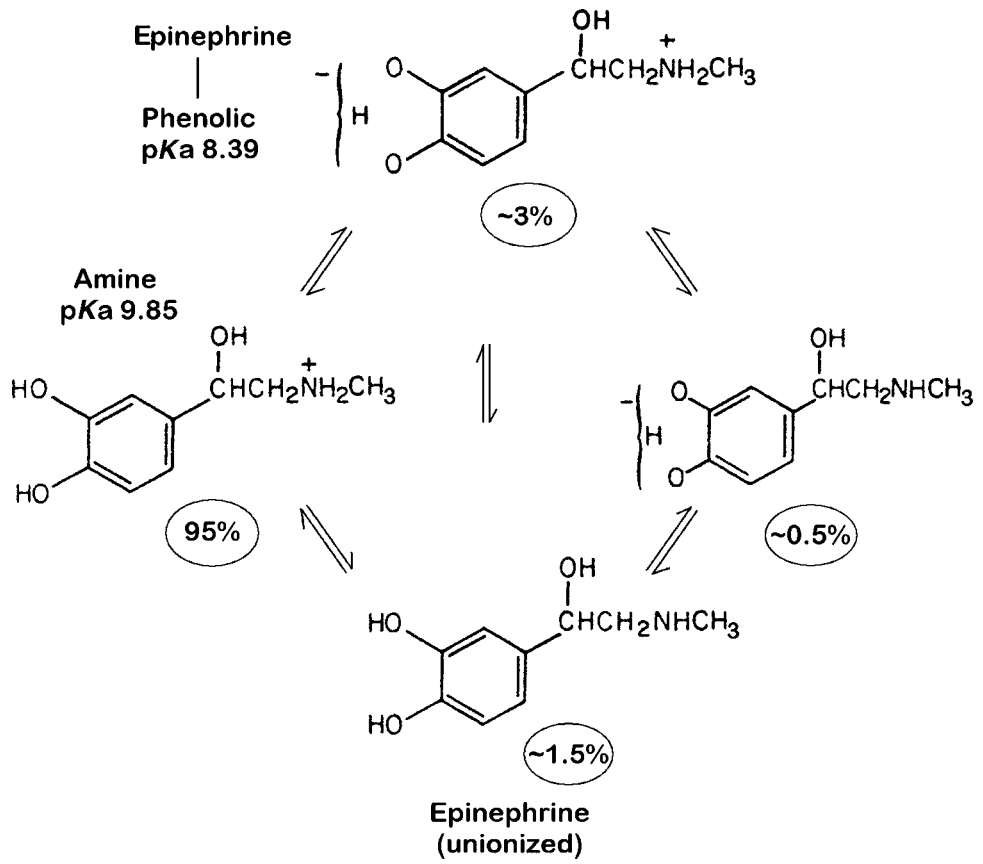


Fig. 3. Epinephrine with two p*K*a values can exist in four different species in solution at the physiologic pH. Data represented from the report by Ganellin.[27]

tency at α-adrenoceptor with little β-adrenoceptor activating property. 2-F-(±)norepinephrine maintained the β-adrenoceptor activity with little α-adrenoceptor activity. The fluoro substitution alters the ionization characteristics of the meta- and parahydroxyl groups of the catecholamine, norepinephrine. This latter agonist is equally potent in activating α- and β-adrenoceptors. Liposolubility is increased by the fluorine substitution and it also uncovers the importance of ionization of specific catechol groups in differentiating the efficacy at the adrenoceptor.[31] Enantiomers of 6-F, and 2-F-(±)norepinephrine were resolved and adrenoceptor binding assays of enantiomers with α_1–α_2, β_1 and β_2 adrenoceptors were performed. Depending on the position of F-substitution, *R*-form had a marked effect in promoting adrenoceptor affinity. The effects of F-substitution for the *S*-forms were less predictable.[32] Catecholimidazolines and desoxy catecholimidazolines were substituted with F at the 2, 5, and 6 positions of the catechol ring were investigated. Either in vivo or in vitro, the vascular activity mediated by α-adrenoceptors was significantly increased in 5-F-catecholimidazoline when compared with other substitutions. Because catecholimidazolines are poor activators of β-adrenoceptor, the F-substitution provided inconclusive improvements of the efficacy at the cardiac receptor.[33–35] The efficacy predictions from the F substitution of norepinephrine cannot be extended to the catecholimidazolines containing apparently similar functional groups.

Liposolubility, ionization, and solution conformation of catecholamines and imidazoline sympathomimetic drugs provided the most valuable information about the activation of the receptor in the biophase. The log P_c values of oxymetazoline, naphazoline, tetrahydrozoline, and tolazoline are 4.8, 3.8, 3.5, and 2.6, respectively. These imidazolines are highly liposoluble as compared with water soluble catecholamines with log $P_c \leq 1$. Chemical structures of catecholamines and some imidazoline type of agonists are presented in Figure 4. The imidazolines will diffuse to the membrane receptor biophase much faster than a catecholamine, like norepinephrine. It is proposed that one of the charged nitrogens of the imidazoline ring interacts with a specific site on the α-adrenoceptor. Which of the two nitrogens is preferred by the site at the receptor? The conformational flexibility of the imidazoline ring is such that there may be equal probability of either nitrogen for the interaction with the receptor site. The aromatic functional group of imidazolines appear to be a sequentially secondary site for the activation of the α-adrenoceptor. Thus, for the initial activation of the α-adrenoceptor by tolazoline-like molecules, a two-site interaction is expected. In all probability, a charged nitrogen interacts first with the receptor, and this initial interaction flips the aromatic group

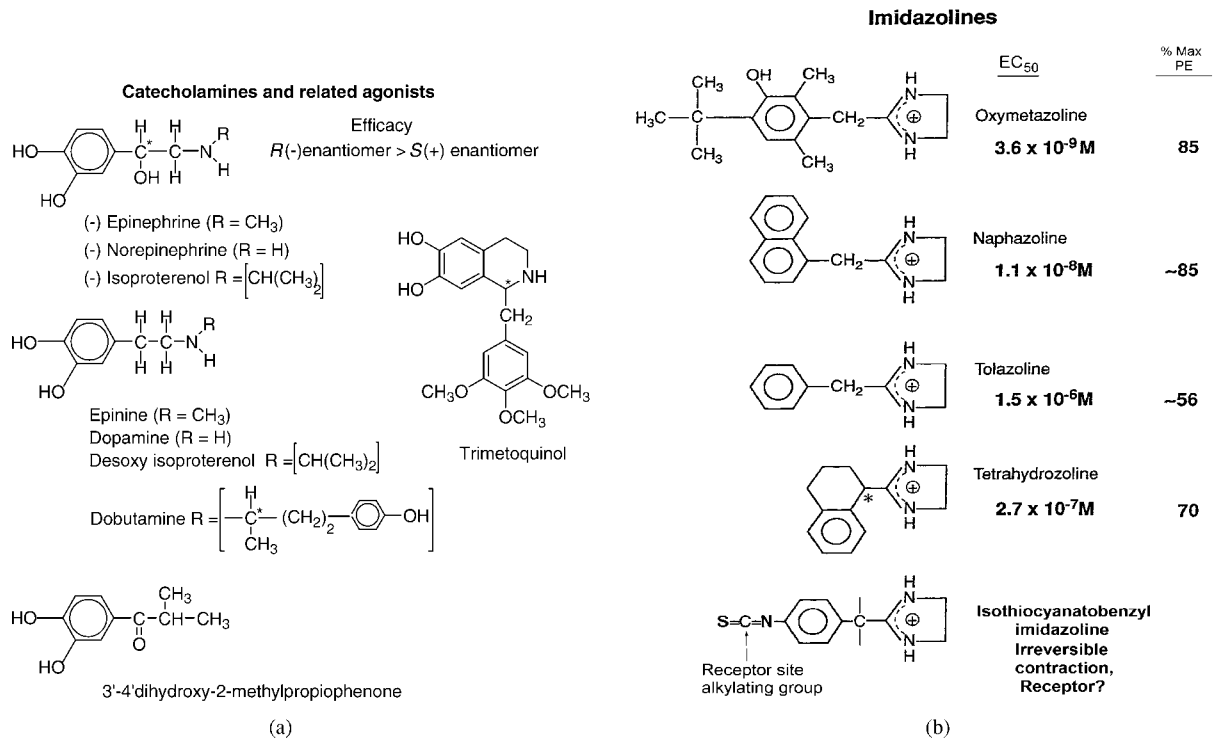


Fig. 4. (**a**, **b**) Representative chemical structures of catecholamines and imidazolines and related molecules. Chirality indicated by *. Note the partial agonist activity of imidazolines relative to phenylephrine (PE) on the vascular system.[36] Isothiocyanatobenzyl imidazoline (SCN-tolazoline) appears as an "irreversible agonist" on the vascular system.

of the agonist to the second site. The partial agonist activity[36] may be related to partial conformational change of the receptor due to interaction "hesitancy" of a nitrogen of the imidazoline ring. Based on steric-structure activity studies with catecholamines and imidazolines, it is postulated that the sequence of functional groups of (−)-norepinephrine for interaction with the α-adrenoceptor appears as a charged amino group first, catechol-phenyl second, and benzylic hydroxyl the last to produce the maximum effective conformational change for high efficacy.[37,38] The low efficacy of (+)-norephinephrine and dopamine may be due to the lack of the interaction of the benzylic hydroxyl with the receptor. It must be emphasized that the priority of sequence of group designation around asymmetric center of catecholamines by the well-known Cahn, Ingold, and Prelog rule may not follow the group sequence for the activation of the receptor by a given series of agonists.

In elucidating the potency of catecholimidazolines, Miller et al.[39] observed the most fascinating relative order of potency of agonists for the activation of α-adrenoceptor: (−)catecholimidazoline = desoxycatecholimidazoline >(+) − catecholimidazoline. The molecular geometry for activation of the α-adrenoceptor was defined. The pattern of the activation of the receptor by imidazolines was different than that of catecholamines. In catecholamidines (CA) where two free NH_2 groups are linked to the carbon, the rank order of potency for the activation of the α-adrenoceptor was desoxy CA > (−) CA > (+) CA.[7] The benzylic hydroxyl group is of little value in the efficacy (Fig. 5). As compared with catecholamines, catecholimidazolines are very weak (EC_{50} > 100 μM) activators of β-adrenoceptor. This property also extends to other noncatechol imidazolines. On the basis of these observations a clear structural distinction between catecholamines and imidazolines is evident in activating α and β adrenoceptors.

Thermodynamic analysis of drug–receptor interactions provided some insight into the efficacy parameter. Large efficacy differences in the mechanical and pharmacological effects of enantiomers of epinephrine indicate that the higher efficacy of (−)-form related to the enthalpy parameter associated with the benzylic hydroxyl group must be highly efficient. The benzylic hydroxyl group of the (−)-enantiomer appears to act like an arrow from the bow in search of the correct target site on the receptor, so that specific conformational change occurs. To maintain the evolutionary efficiency, the energy utilization for the interaction must be small. Rice et al. calculated Gibb's free energy ΔG° from the dissociation constants, the values −8.1 and −6.2 kcal/mol for (−)- and (+)-enantiomer of epinephrine, respectively.[40] This indicates that interactions of enantiomers is largely entropy driven, there is a small contribution from enthalpy.

EFFICACY, DESENSITIZATION AND CROSS-DESENSITIZATION

Repeated application of a sympathomimetic agonist at short intervals reduces its pharmacological effects. The

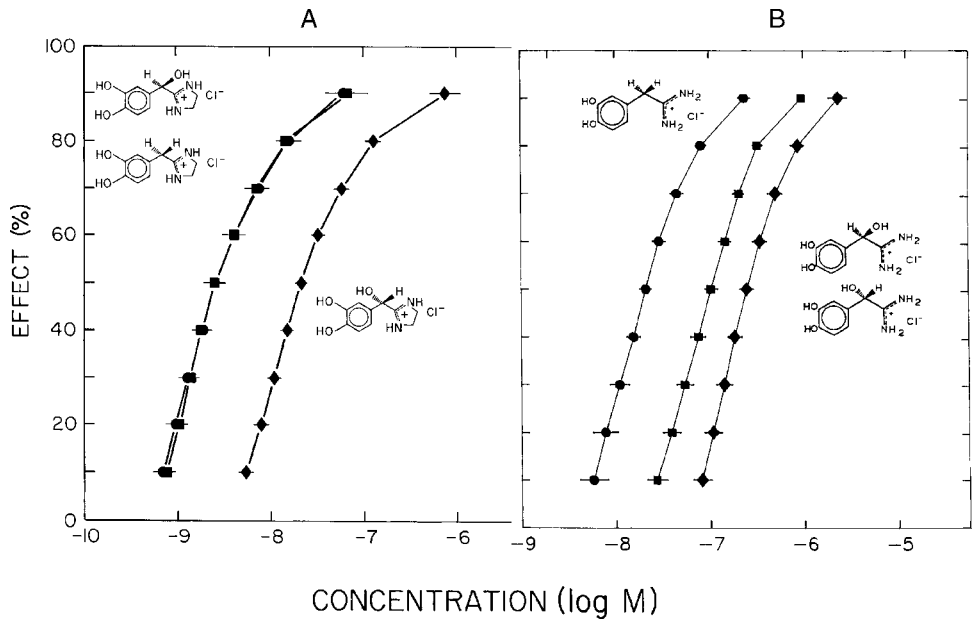


Fig. 5. Concentration response curves of catecholimidazolines-5A and catecholamidines-5B their desoxy analogs obtained on rat aorta containing α-adrenoceptor. The rank order of activity is desoxy form (■) = (−) enantiomer (●) > (+)enantiomer (◆). In catecholamidine (CA) series 5B, the rank order of potency is desoxy CA (●) > (−) CA (■) > (+) CA (◆).[7] In both types of molecules the pattern of α-adrenoceptor mediated activity differs from that of the Easson–Stedman postulate.

receptor-related mechanisms are not entirely clear. Receptor conformation, second messenger and/or related regulatory ionic mechanisms may be altered. Biophase drug disposition mechanisms may also be altered. On the isolated rat vas deferens, oxymetazoline, (10 μM) application at 20-min intervals, almost abolished the α-adrenoceptor-mediated contractile response by the seventh application. During the desensitized state, the contractile response to other α-adrenoceptor agonists like (−)-phenylephrine, (−)- and (+)-norepinephrine, dopamine, epinine, (±)-methoxamine, and (±)-α-methyl norepinephrine is preserved.[23] The contractile response to other imidazolines was abolished during the oxymetazoline desensitized state. (see Fig. 6). This interesting, puzzling observation indicates dual possibilities for the control of efficacy of two types of sympathomimetic substances. The receptor system desensitized to oxymetazoline can be activated by (−)-norepinephrine by interaction with different sites of the receptor. Two types of agonists may activate different subtypes of adrenoceptors. Both molecules may activate the same type of receptor, which may connect to different G-protein transduction pathways for the expression of pharmacological efficacy.

Miller and associates[41–43] synthesized a fascinating affinity label SCN-substituted tolazoline. At a narrow range of relatively high concentrations, the affinity reagent produces long lasting vascular contraction. A classical α-adrenoceptor blocker does not reduce the response significantly. On guinea pigs, stomach smooth muscle SCN-tolazoline will also produce a contractile response. The tissue, however, develops both self-blockade and cross-blockade to other agonists. We refer to such a molecule as "an irreversible agonist" for which the receptor, as well as the mechanism of induction of efficacy is not settled. It was assumed that efficacy of all classical agonists was related to the rapid association and dissociation of the agonist with the specific receptor. The SCN-substituted imidazoline presumably does not easily dissociate from the receptor because the SCN-group interacts irreversibly. It must also be emphasized that total stimulus output after activation of unknown cellular receptor mechanisms may produce the contractile response by several mechanisms. Furthermore, repeated doses of oxymetazoline produced an

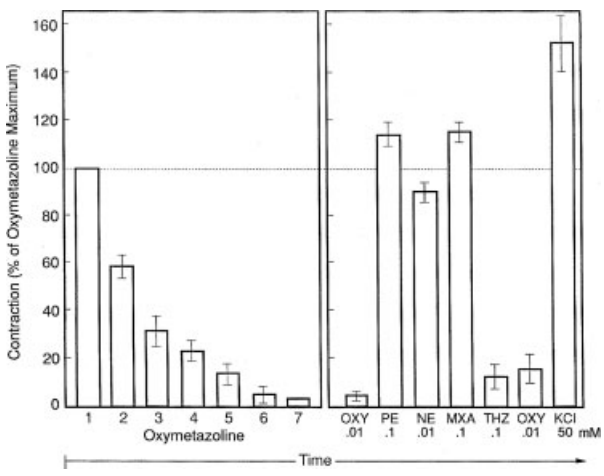


Fig. 6. Rat vas deferens desensitized to the repeated doses of oxymetazoline (oxy) (10 μM) at 20-min intervals maintains its response to other α-adrenoceptor agonists like phenylephrine (PE), norepinephrine (NE), and methoxamine (MXA). The response to KCl maintained whereas that of tetrahydrozoline (THZ) is abolished (redrawn from Ref. 23).

expected reduced vascular response. During this phase of desensitization, SCN-substituted tolazoline maintained its normal contractile efficacy. The mechanism of efficacy of the two types of molecules must be different (Patil, P.N. unpublished report).

ENANTIOMERIC AGONIST COMBINATIONS IN RELATION TO EFFICACY

Racemates contain equal amounts of each enantiomer. Thus, as compared with the biologic activity of a single potent enantiomer, the activity of the racemate will be half. If the other enantiomer is "inert" nothing could be stated about its efficacy. There are a few examples in which the so-called "inactive" form may be "inert."[44] For several reasons, it appears doubtful if (+)-cobefrin (erythro α-methyl norepinephrine) is active at all. Its apparent activity may be totally due to contamination with the active form. The weak vascular α adrenergic activators (−)- and (+)-isoproterenol have nearly equal EC_{50} values but different maximal effects. Desoxyisoproterenol behaves similar to (+)-isoproterenol, confirming the predictions of the Easson–Stedman theory.[45] From the analysis of the dose–response curve, it is implied that both (−)- and (+)-isoproterenol must also interact with the receptor but with different efficacy.

The intrinsic activities of dobutamine isomers present the most fascinating variation in pairs of catecholamine series of drugs where the asymmetric center is at the N-substituted carbon.[46] The (−)-enantiomer is a potent α-adrenergic agonist and also a weaker β-adrenoceptor agonist when compared with the (+) enantiomer. The latter enantiomer, however, is a stronger β-adrenoceptor activator and lacks α-adrenoceptor agonist activity. But, it blocks α-adrenoceptor mediated activity of the (−)-form. Maximal inotropic effects of the racemate are greater than that of either enantiomer. The rationality based on the cardiac receptor studies in vitro indicates that a racemate may be better than a single enantiomer. These enantiomers provide an important tool to analyze receptor mediated complexities in efficacies.

PHARMACOLOGIC QUANTITATION OF EFFICACIES OF ENANTIOMERS

α-Adrenoceptors

An accurate comparison of the receptor-related efficacies of agonists after fractional inactivation of spare receptors by an irreversible blocker can be made on isolated tissues containing functionally intact systems.[13] On the rabbit aorta, Kim et al.[47] determined the dissociation constants of (−)- and (+)-epinephrine for α-adrenoceptor activation as 0.3 and 10.8 μM, respectively. The intrinsic efficacy of (+)-epinephrine relative to the potent enantiomer was 0.44. The low efficacy of the (+)-form or the desoxy form was confirmed in a receptor protection experiment. It is important to note that due to spare receptors, maximum contractions of both enantiomers appear equal in normal tissue. The latter observation can be misinterpreted as the

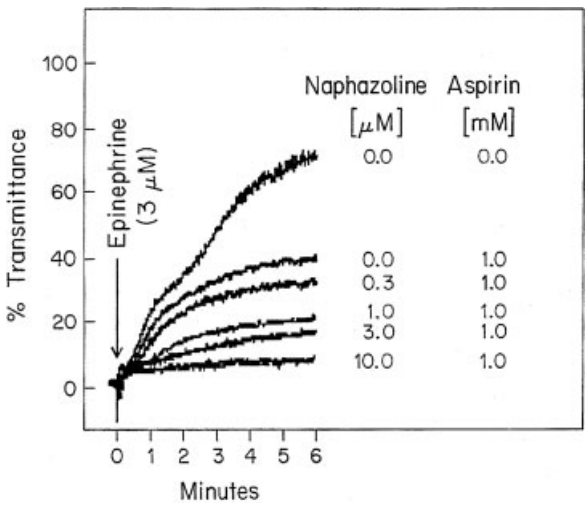


Fig. 7. Two phases of platelet aggregation by (−)-epinephrine. Inhibition of secondary phase by aspirin and the primary phase by naphazoline (data from Feller and coworkers[35] and personal communication, 1995).

enantiomers having equal intrinsic activity. Such an interpretation in relation to the conformational changes of receptors can be confusing. Rice et al.[40,48] extended investigations about intrinsic activities of enantiomers of catecholamines various organs of rat. Expected differences in efficacy between epinephrine enantiomers were seen in spleen, portal vein, and aorta. The values were nearly equal in the vas deferens where spare receptor numbers, as well as receptor mediated coupling efficiency, appear to be very high.

Drugs, which are partial agonists in the normal functional organs, readily exhibit differences in the maximum response or efficacy. Enantiomers of tetrahydrozoline and hydroxytolazoline show marked differences in the α-adrenoceptor-mediated pharmacologic activity in blood vessels.[49,50] Intrinsic efficacy differences must arise at the adrenoceptor. In comparing enantiomers for efficacies in organ systems with multiple receptors, there is no guarantee that both enantiomers will prefer only a single type of receptor. S(+)-hydroxytolazoline produces two types of contractile responses. Only one component is blocked by the α-adrenoceptor antagonist prazosin.[50]

Feller and co-investigators[35,51–54] completed the most comprehensive studies on α_2-adrenoceptor-mediated platelet aggregation by a series of enantiomeric agonists. The primary and secondary phases of platelet aggregation are induced by the catecholamine or the catecholimidazoline. The second phase is prostaglandin-pathway dependent because it is selectively blocked by acetylsalicylic acid treatment. Naphazoline, which is a partial agonist on the vascular α_1-adrenoceptor system, inhibits the primary phase of aggregation of epinephrine in the acetylsalicylic acid pre-treated platelets (Fig. 7). Adenosine diphosphate, thromboxane A_2 and serotonin-dependent pathways of platelet aggregation are also recognized. In platelets from some human donors, both enantiomers of norepinephrine produced concentration dependent aggregation, whereas

TABLE 1. Human platelet aggregatory and anti-aggregatory properties of enantiomers of catecholamines and their desoxy analogs[52,63]

Catecholamine	EC_{50}[a] (μM)	K_B (μM)
(−)-Epinephrine	0.46	–
(+)-Epinephrine	18.2	NT
Epinine (desoxy epinephrine)	40.7	NT
(−)-Norepinephrine	1.3	–
(+)-Norepinephrine	19.1	54[b]
Dopamine (desoxy norepinephrine)	22.4	25[b]
(−)-Isoproterenol	–[c]	204
(+)-Isoproterenol	–[c]	309
Desoxy-isoproterenol	–[c]	73

n, 3–6; NT, not tested.
[a]Maximal aggregation was equal for all drugs. K_B determined against (−)epinephrine-induced aggregation.
[b]Platelets from some donors do not exhibit aggregation[52]; therefore, anti-aggregatory effects (K_B) were quantitated.
[c]Not active at concentrations up to 1 mM.

the response to (+)-norepinephrine was absent in preparations obtained from other donors. The reason for the complete lack of aggregation or efficacy of a lesser active enantiomer is not known. Perhaps, the initial component, which triggers the stereoselective aggregation is missing. (+)-Norepinephrine, however, blocks the aggregatory effect of the (−)-norepinephrine. The blocking activity of the enantiomer measured as K_B of 54 μM is statistically equal to the EC_{50} for the aggregatory effect of ∼20 μM. Dopamine, which lacks the benzylic hydroxyl group, shows identical behavior to (+)-norepinephrine (Table 1). The pattern of the activity conforms to that of the Easson–Stedman postulate.

In the platelet preparation desoxycatecholimidazoline appears to be a partial agonist. In the vascular preparation, however, maximal effects, as well as EC_{50} of the desoxycatecholimidazoline are equal to that of (−)-catecholimidazoline.[37] The vascular contractile response to the catechol derivative is not affected by acetylsalicylic acid treatment. The human platelet preparation appears to be the most useful system to elucidate the molecular mechanisms of efficacy so that specific antiplatelet aggregatory drugs for therapeutic benefit can be developed.

The maximal effects of (−) and (+)-epinephrine were investigated on rabbit aorta with functional antagonistic α- and β-adrenoceptors. The percent maximum contraction was equal for each form. The enantiomeric activity difference at EC_{50} or ED_{50} was 1.8 log units. When the vascular tone was induced by histamine, the maximal β-adrenoceptor-mediated relaxation of each enantiomer was ∼50% of the initial contraction by the spasmogen. Because of the limited receptor and/or the functional reserve of the β-adrenoceptor, unequal efficacy related differences of the enantiomers were expected in the vascular system. The enantiomeric activity difference for the relaxation at EC_{50} was 1.74 log units, close to 1.8 log units obtained for α-adrenoceptor-mediated contraction (Patil, unpublished data). Thus, elucidating the role of affinity and efficacy parameters in relation to the receptor mediated functional control of the responses by enantiomeric substances still presents a challenge.

β-Adrenoceptors

Initial molecular events associated with the activation of the β-adrenoceptor by catecholamines are well studied.[55–59] DeSantis et al.[60] measured the rise in cAMP after (−) and (+)-norepinephrine in fat cells. The enantiomeric activity difference for lipolysis measured at EC_{50} was 300. The ratio for rise in second messenger cAMP was also 300. Similar co-relations were obtained for the activation of β-adrenoceptor by enantiomers of isoproterenol.[57,59] These important quantitative observations indicated that initial activation of β-adrenoceptor-mediated efficacies of the enantiomers were stereoselective. No decrease in the maximum cardiac response to a β-adrenergic agonist is observed after intensive treatment with an irreversible β-adrenoceptor blocker.[61] The rat heart contains a very high receptor reserve with an extremely efficient coupling mechanism. Even the residual cardiac receptors are capable of producing maximal effects of an agonist.

In well-designed experiments Birnbaum et al.[62] measured the increase in atrial rate as well as cAMP rise after (−)-, or (+)-isoproterenol. The concentration–effect curve for the production of mechanical effects was at least 100-fold to the left of that for the production of cAMP. The chronotropic response was greatly amplified. At the EC_{50}, the enantiomeric differences for both parameters were ∼2500 fold. The maximal chronotropic effects, as well as cAMP production, were also equal for the enantiomers. Because of spare receptors and/or related spare function, no differences in the efficacy for enantiomers were observed. In rat uteri, maximally stimulated levels of cAMP were 52, ± 3.8 and 40.5, ± 4.5 pmol/mg of protein by (−)- and (+)-isoproterenol, respectively. The difference in the production of second messenger by the enantiomers in part reflects an unequal efficacy in the transduction cascade. Dithiothreoitol, which selectively reduces sulfhydryl groups of the receptors presumably altering conformational changes of the β-adrenoceptor, clearly reduces atrial rate after (+)-norepinephrine over that of the (−)-norepinephrine.[60]

Steric structure activity studies for the series of agonists for the β-adrenoceptor-mediated glycerol release from the rat fat cells indicated that (−)-isoproterenol was 300 times more potent than the (+)-isoproterenol. The intrinsic activity, as indicated by the maximal glycerol release by each enantiomer, was equal. Desoxyisoproterenol, however, had low intrinsic activity of 0.57. (−)-Tetrahydroisoquinoline was about 1000 times more potent than the (+)-form with intrinsic activity of 0.71.[55] In guinea pig tracheal smooth muscle, β-adrenoceptor mediated muscle relaxant activity of (−)-trimetoquinol was ∼20% greater than the (+)-enantiomer. Maximum cyclic AMP produced by the latter enantiomer was nearly half that produced by the (−)-form. Both enantiomers, however, produced equal maximal rate accelerating effect on the guinea pig atria. The onset of action of these agonists was relatively slow for the functional response. As much as 10–15 min were

required to reach the equilibrium in the atria at a given concentration of the agonist. A 1000-fold concentration range of the agonist was needed to complete the dose–response curve in the heart. In spite of such technical difficulties, about 18% difference was observed in the maximal effect of the potent *S*(−)-tetrahydropapaveroline over that of the *R*(+)-form. In all probability, differences in the maximal effect reflect the differences in the intrinsic efficacy at the β-adrenoceptor.

SIGNIFICANCE OF STEREOSELECTIVE DRUG BINDING

The stereoselective quantitative pharmacologic effects of catecholamines on α and β-adrenoceptor in various organs were well defined. Even the intracellular increase in second messengers associated with the mechanical pharmacologic effect was well correlated with activation of the β-adrenoceptors. The adrenoceptor containing membranes from the organs were separated. The availability of high specific activity radiolabeled high affinity β-adrenoceptor antagonist provided a powerful tool to investigate the specific interactions of catecholamines in competition with the labeled blocker. Structurally related enantiomers of agonists and antagonists exhibited marked stereoselectivity in competition for the equilibrium binding with the labeled blocker.[64–66] For the agonists the degree of stereoselectivity roughly paralleled to that for the rise in cAMP, the indicator of the pharmacologic response. The linear relationship between the agonist affinity in terms of binding in homogenates to that of the efficacy parameter in the organ remained a difficult task.

De Robertis and coworkers extracted α-adrenoceptors from bovine spleen. The proteolipids containing the receptor were incorporated into an artificial membrane. In this preparation, norepinephrine produced stereospecific dose-dependent membrane conductance changes and this effect was antagonized by the α-adrenoceptor blocker phentolamine. The concentrations of the agonist and antagonist to produce this effect were relatively high 10^{-5}–10^{-4} M, well above EC_{50} of the agonist in the organ containing α-adrenoceptor.[67] A subcellular fraction from rat vas deferens was isolated, where labeled dihydroazapetine (an α-adrenoceptor blocker) exhibited saturable binding. The affinity constants of five different imidazolines showed excellent positive correlations to their α-adrenoceptor activating property in the isolated organ. Oxymetazoline was the most potent in the series. Paradoxically, the binding of (−)-methoxamine, (−)-norepinephrine and (−)-phenylephrine was increased over the (+)-enantiomers. Different modes of interaction of two types of agonists with the α-adrenoceptor were postulated.[68] These observations prompted us to design new pharmacologic experiments in the rat vas deferens. In an oxymetazoline-desensitized organ, catecholamines and methoxanine still maintained the contractile effect or the receptor-related efficacy. The mode of binding and/or related events of two types of agonists must be different.[23] When the competition of norepinephrine enantiomers and dopamine was investigated with labeled clonidine, an imidazoline type of agonist, or WB 4101, a benzodioxan analogue with α-adrenoceptor blocking properties, the inhibition of binding followed an interesting pattern. For the competition with the agonist clonidine the order of potency was (−)-norepinephrine > dopamine > (+)-norepinephrine, whereas that against WB 4101 was as predicted from the Easson–Stedman hypothesis where (−)-norepinephrine > (+)-norephinephrine = dopamine.[69] All these binding studies on membranes containing α-adrenoceptors indicate that relationship between the binding constant and efficacy of the agonist in intact organ system is a nonlinear complex process. The primary cellular event after activation includes increase in inositol phosphate and Ca^{++} which is needed for efficacy.[70]

The receptor-interacting conformation of catecholamines was elucidated to understand the efficacy of potent agonists in the system. Conformationally, restricted *cis* and *trans*-2-(3,4-dihydroxyphenyl)-cyclobutylamines were synthesized to investigate the dopamine receptor-interacting conformation of the agonist. The trans-form had 20 times the affinity of the cis-form. The efficacy promoting functional groups of several adrenergic and dopaminergic agonists are in the trans-interactive conformation.[28]

AGONIST SPECIFIC INTERACTING SITE AT THE ADRENOCEPTORS AND ITS EFFICACY IMPLICATIONS

Since 1948, Ahlquist's classification of α and β adrenoceptors is well accepted. On the basis of the relative pharmacologic potencies of agonists, β-adrenoceptor subtypes β_1 (heart) β_2 (lungs) were defined.[71] The neuronal prejunctional α-adrenoceptor, which inhibits the release of the transmitter and the postjunctional receptor were subclassified as α_2 and α_1-adrenoceptor subtypes, respectively.[72] Between 1986 and 1994, Dixon, Strader, Lefkowitz and Perez et al., cloned and characterized the pharmacological properties of the major subtypes of adrenoceptors.[73]

For the seven transmembrane domain activated receptors, many components of the cellular organization undergo synergistic interaction before a mechanical response is observed: G-proteins, extracellular and intracellular Ca^{++}, inositol triphosphate, cAMP, and prostaglandins to name a few. In homogenates or cloned receptors, the agonist-activated cascade of transduction is likely to be distorted to provide an accurate efficacy parameter of an agonist observed in an organ. Even in intact cells, in the absence of the synaptic transmitter cloned receptors may not acquire the same conformation as in intact vertebrate organs. These investigations, however elegant, should be viewed with caution in understanding efficacy of sympathomimetic amines. At a recent meeting Milligan reported that G-protein-coupled receptors may form dimers or oligomers which may regulate receptor function and related efficacy of agonists.[74] In this review, receptor monomer simplicity is maintained to explain efficacy.

Some time ago, Furchgott et al.[75] observed in vitro the vascular relaxation in response to light. The observation has been reinvestigated in relation to physiologic adapta-

tion. The superficial blood vessels in skin react to light with relaxation whereas deep muscle blood vessels do not. The mechanism of light activated transduction leading to vascular relaxation is unknown. The apparent similarity of the light sensitive 7TMG protein receptor of the eye and that of the vascular system response should not be ignored. The mechanisms of efficacy or alterations by photons in the two systems may be different.

All these observations in various organ systems provided the foundation for a new inquiry with better methods to understand transduction by agonists at receptor sites. Information from organ systems provides the best tool to guide additional studies.

β-Adrenoceptor

Strader et al.[76] elucidated the β-adrenoceptor agonist and the antagonist-interacting sites within the seven transmembrane G-protein linked architecture of the receptor. Site-directed mutagenesis experiments revealed that Asp^{113} of the transmembrane helix III is critical for interaction of the $^{+}NH_3$ cation of norepinephrine or other catecholamines. Asp^{79} in helix II, if substituted with Ala in the β_2-adrenoceptor, resulted in a decrease in affinity and efficacy without significant effect on the binding of antagonists. Ser^{204} and Ser^{207} in the transmembrane helix V are postulated to form hydrogen bonds with the catechol group of the agonist. If these Ser residues are substituted with Ala, the affinity of catecholamine agonists is decreased 10 fold with 50% decrease in efficacy. Phe^{290} in the VIth transmembrane helix presumably stabilized the aromatic ring of the catecholamine. Wieland et al.[77] recognized Asn^{293} of the TM VI as the probable site for the interaction of the benzylic OH group of the more potent enantiomer of the catecholamine. A fluorescein maleimide probe attached at Cys^{265} of the intracellular loop of the β_2-adrenoceptor was used to investigate conformational changes after activation of the receptor by structurally related agonists.[78] It was deduced that (−)-norepinephrine causes:

1. Rapid changes in domain V due to interaction of the catechol groups with two of the three $Ser^{203,204,207}$ residues.
2. The chiral OH then interacts with Asn^{293} of the domain VI.
3. The charged amino group ($—^{+}NH_3$) then interacts with the Asp^{113} of the domain III. This change appears to be slow.

According to the studies of Lohse[79] the second phase coincides with a conformational change of the receptor. Based on the steric structure–action studies on isolated organs, similar sequential activation of the β-adrenoceptor was proposed.[37]

α-Adrenoceptor

Piascik and Perez[80] reviewed α-adrenoceptor interaction topography for the agonists. Ser^{188} and Ser^{192} are involved in the interaction of the catechol group of the agonist. These investigators identified Phe^{308} and Phe^{312} of the TM-VII domain, as the most important sites for α_1-adrenoceptor blockers and partial agonist imidazolines.

Perez and co-investigators indicated the presence of a salt bridge constraint in the α_{1b}-adrenoceptor.[81,82] At the critical site, the carboxyl group of Asp^{125} of the III TM domain and the $^{+}NH_3$ cation of the Lys^{331} of the VII TM domain form a bridge. Sympathomimetic activity of catecholamines or imidazolines results from disruption of the bridge by the charged nitrogen of the agonist. Depending on the molecular nature of the agonist, specific conformational change of the receptor presumably occurs after initial activation at this site. Second messenger changes also occur as the result of the disruption of the salt bridge.

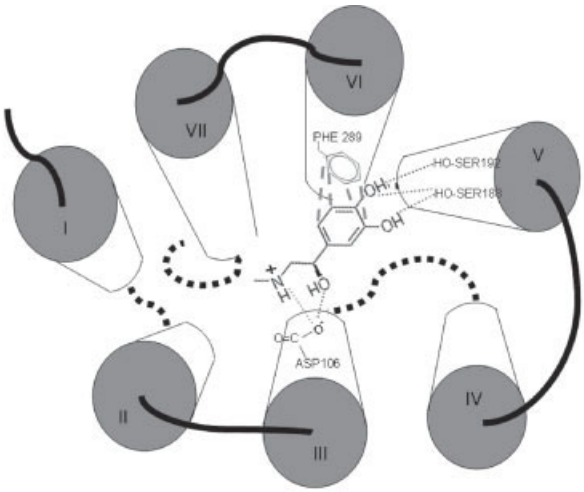
The α_{2A}-adrenoceptor has a cysteine residue at position 201 (corresponding to position 203 on the β_2-adrenoceptor). Molecular modeling and site-directed mutagenesis shows that this cysteine, as well as Ser^{200} and Ser^{204}, plays an important role in the interaction of the catechol oxygens with TM helix V of the α_{2A}-adrenoceptor.[83,84] The aromatic ring is postulated to interact with TM helix VI through Tyr^{394} and Phe^{391} as well as TM III through Val.[114] Consistent with this premise, the mutation of Tyr^{394} to Phe results in a 60-fold reduction in the affinity of (−)-norepinephrine (P. Hieble, unpublished data).

Benzylic OH Group

The site of interaction between the chiral benzylic hydroxyl group of catecholamines with α-adrenoceptors is not established. Ser^{165} in TM IV was initially suggested as a favorable location for the potential stereoselective interaction between a hydroxyl group and the β_2-adrenoceptor.[76] The investigators were unable to express the β_2-adrenoceptor mutant where Ser^{165} was replaced by alanine to test this hypothesis. However, subsequent studies showed this mutation to have no effect on the affinity of the β_2-adrenoceptor for isoproterenol.[77] Similarly, this mutation had no effect on the affinity of the α_{2A}-adrenoceptor for norepinephrine or epinephrine.[85] Molecular modeling and/or site directed mutagenesis has suggested possible interaction with amino acids on TM II, TM VI, and TM VII.[84] Evaluation of the binding affinity of a series of agonists with the α_{2A}-adrenoceptor, combined with molecular modeling, suggests that the benzylic hydroxyl group interacts with the same aspartic acid Asp^{113}. This interaction is postulated to occur via hydrogen bonding between a hydroxyl group and one of the side-chain oxygen atoms. Molecular modeling of the agonist-receptor complex shows that this interaction cannot occur for the (+)-enantiomer.

Molecular Modeling of Adrenoceptors

In our own modeling efforts, both homology models of α_{1A} and β_1 adrenoceptors were built with the MODELLER 9v1 program by using bovine rhodopsin (PDB ID 1F88) as the template. (−)-Epinephrine, (+)-epinephrine and oxymetazoline were docked into the agonist binding sites by using AutoDock v4β.[86,87] The Lamarckian genetic algorithm (LGA) for ligand conformational searching was selected because it has enhanced performance relative to



(-)Epinephrine docking at α_{1A}adrenoceptor

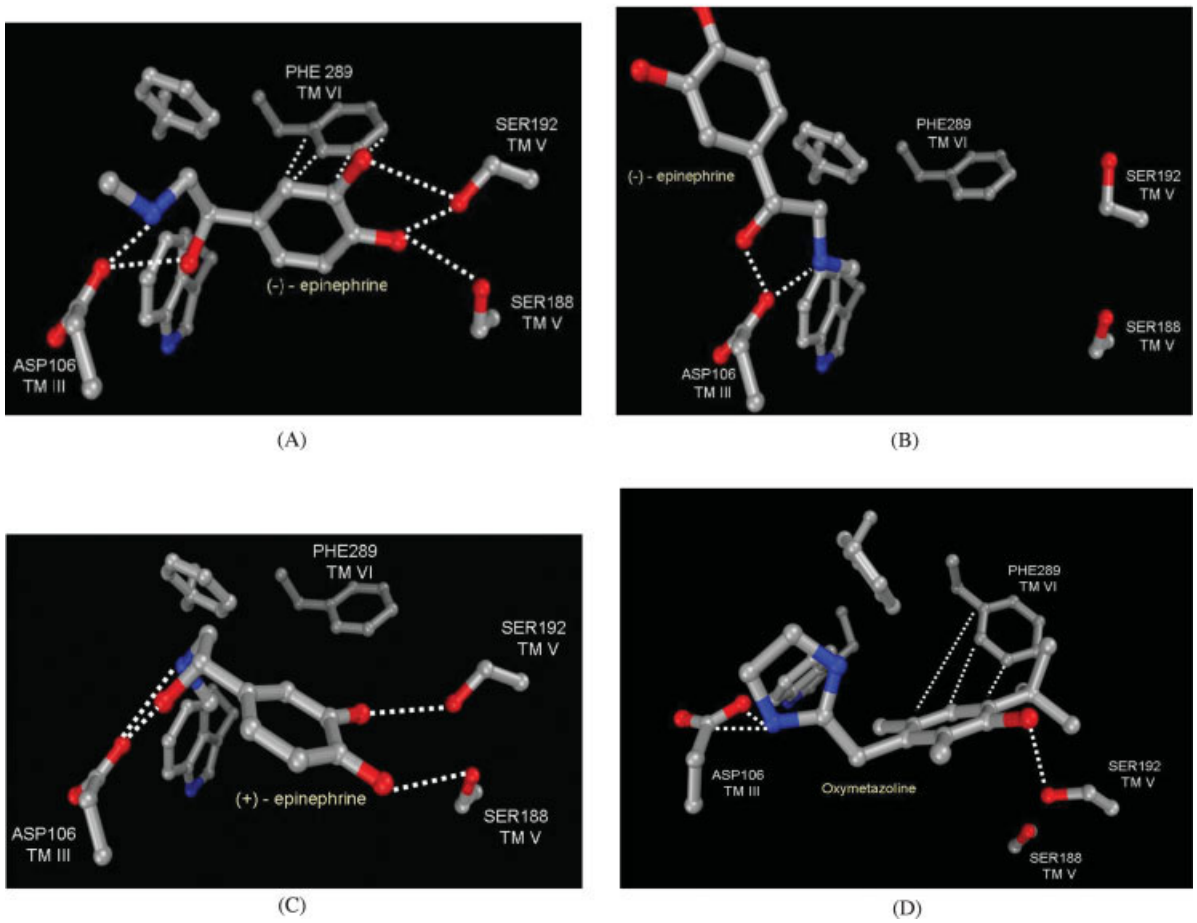


Fig. 8. Modeled binding modes of (−)-epinephrine, (+)-epinephrine and oxymetazoline to α_{1A} adrenoceptor. (**A**) Initial anchoring of (−)-epinephrine with Asp^{106}; (**B**, **C**, **D**) (−)-epinephrine, (+)-epinephrine, and oxymetazoline stable binding modes. Note in Figures 8b and 8c $-^{+}NH_3$ and chiral −OH of the enantiomers interact with Asp^{106} of the adrenoceptor. But in (+)-epinephrine, interaction with PHE^{289} of TM VI domain is lacking. The modeling of epinine remains to be examined.

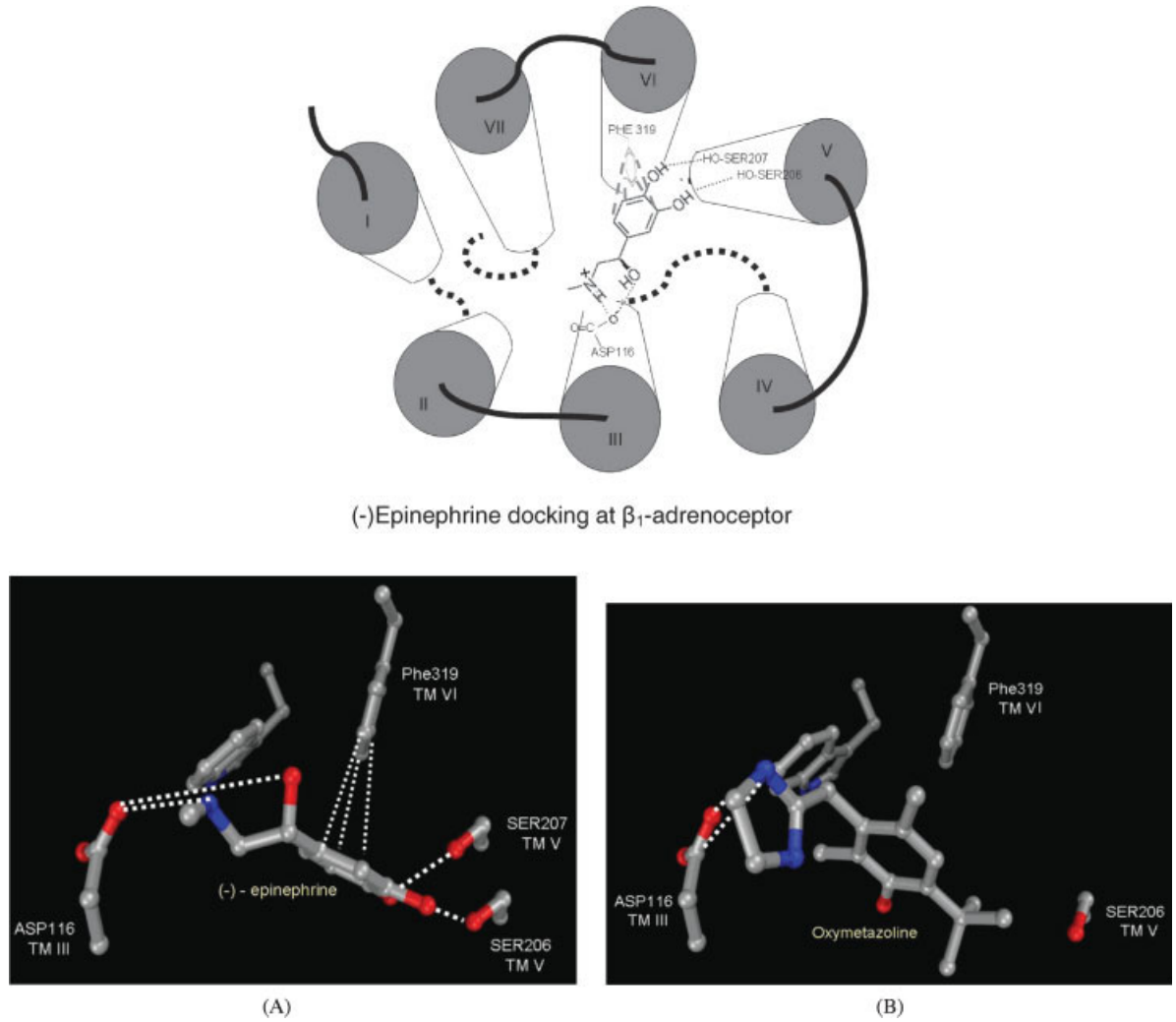


Fig. 9. Modeled binding modes of (−)-epinephrine (**A**) and oxymetazoline (**B**) to β_1 adrenoceptor. The imidazoline fails to interact significantly with the Ser residues to activate the receptor.

simulated annealing or the generic genetic algorithm. Besides using fully flexible, we allowed the side chains of ligand contacting residues to be flexible also, in order to simulate the binding process and the induced-fit effect. Amino acids in TM III, V and VI domains of α_{1A} and β_1-adrenoceptor were numbered as for human protein receptors.

In the docking simulation of (−)-epinephrine to the α_{1A} adrenoceptor, two binding modes were observed. Figures 8 and 8A show the initial anchoring of (−)-epinephrine to the receptor by electrostatic and hydrogen bonding interactions between the positively charged amine group of (−)-epinephrine and Asp^{106} at TM III. Figure 8B shows the final binding mode of (−)-epinephrine. Besides the well-preserved interactions between the amine of (−)-epinephrine and Asp^{106}, the para-hydroxyl group of (−)-epinephrine forms hydrogen bonds with both Ser^{188} and Ser^{192} on TM V (three H-bonds). The phenyl ring of (−)-epinephrine has aromatic interactions with Phe^{289} and the Ser^{188} hydroxyl side chain flips upon final binding. Surprisingly, the chiral hydroxyl group appears to form hydrogen bond with Asp^{106} also. So the binding starts with the amine anchoring to Asp^{106} and the chiral hydroxyl group, and then proceeds to form hydrogen bonds with Ser^{188} and Ser^{192} and an aromatic interaction with Phe^{289}. From initial anchoring to final binding, the gain on free energy of binding is only 0.7 Kcal/mol. It appears that the final binding mode is to trigger the global conformational change of the receptor for activation, which is associated more with efficacy than affinity. Figure 8C shows the binding of (+)-epinephrine to the α_{1A} adrenoceptor. Compared to (−)-epinephrine, its binding energy is 1.3 Kcal/mol weaker, which is comparable to the experimental difference of 1.9 Kcal/mol. Even though the absolute binding of both agonists was proposed to be mainly entropy-driven, the difference here is mainly enthalpic because (+)-epinephrine loses the aromatic interaction with Phe^{289} and para-hydroxyl group forms one hydrogen bond with Ser^{192} (two H-bonds instead of three of the catechol groups). So (+)-epinephrine is weaker than (−)-epinephrine in both

affinity and efficacy. The modeling of epinine will be explored.

Figure 8D shows oxymetazoline binding to α_{1A} adrenoceptor. The imidazoline ring shows strong electrostatic and hydrogen bonding interactions with Asp^{106}. Aromatic and hydrophobic interactions play important roles in this case.

For the β_1 adrenoceptor, again both (−)-epinephrine and (+)-epinephrine bind similarly except the chiral hydroxyl group of the latter does not interact. This observation with (+)-epinephrine is in contrast to that for the α_{1A}-adrenoceptor. Figures 9 and 9A show the binding mode of (−)-epinephrine. The amine group shows both electrostatic and hydrogen bonding interactions with Asp^{116} of TM III. The meta-hydroxyl group forms a hydrogen bond mainly with Ser^{206} instead of Ser^{207}/Ser^{210} on TM V. However, an alternate binding mode does show the para-hydroxyl group forming hydrogen bond with Ser^{204} (unpublished data and Ref. 76). This hydrogen bonding seems very important. For epinephrine, the docked mode is consistent and conserved; whereas the oxymetazoline docking mode is not consistent and the phenyl ring flips around due to lost anchoring with Ser^{206}. Therefore, oxymetazoline is not able to trigger global conformation change and does not activate β_1 adrenoceptors.

CONCLUDING REMARKS

In discussing evolutionary changes such as specificity, regulation and cooperativity in enzymes, Koshland[88] indicated that these large molecules became programmed to allow one substrate to exhibit negative cooperativity whereas a second substrate may show positive cooperativity. The so-called "transition state" of the enzyme–substrate interaction is viewed as a near attack conformation.[89] Parallel events may occur at the active sites of the adrenoceptor and its subtypes, for activation by the chiral agonists. The crystal structure of the β_2-adrenoceptor was reported.[90] Our model was similar in both overall structure and active site residue orientations.

There is no doubt that resolution of a single active enantiomer of old and new drugs can result in clinical and therapeutic benefit in humans. The detailed study of their efficacy differences is equally fascinating for understanding the initial molecular events in the activation of the receptor. The efficacy at the receptor is ultimately expressed at the clinical level in patients.

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