Stereostructure Activity Relationships of Catecholamines on Human Platelet Function (43071)

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Abstract. **The concentration-dependent effects of clonidine, isomers of epinephrine, norepinephrine (NE), isoproterenol, cobefrin and α-methyldopamine, and related desoxy analogs (epinine, dopamine, *N*-isopropyldopamine) were examined on human platelets. The rank order of aggregatory potency (pD_2 values) was R(−)-epinephrine (6.3) > R(−)-NE (5.9) > (±)-*erythro*-cobefrin (5.3) > S(+)-epinephrine (4.7) = S(+)-NE (4.7) = clonidine (4.7) = dopamine (4.6) > epinine (4.4) > S(+)-α-methyldopamine (4.3) = R(−)-α-methyldopamine (4.3) > (±)-*threo*-cobefrin (3.7). The isoproterenol isomers and *N*-isopropyldopamine were inactive as agonists. In 9 of 16 platelet-rich plasma preparations, R(−)-epinephrine, R(−)-NE, and (±)*erythro*-cobefrin were agonists and the remaining analogs blocked R(−)-NE-induced aggregation with a rank order of inhibitory potencies (pK_B values) of clonidine (6.2) > S(+)-α-methyldopamine (5.0) > dopamine (4.6) = R(−)-α-methyldopamine (4.4) ≥ S(+)-NE (4.3) > *N*-isopropyldopamine (4.1) > S(+)-isoproterenol (3.7) = R(−)-isoproterenol (3.5). Each compound was also able to reverse prostaglandin E_1 (PGE_1) (0.1 μM)-induced blockade of the maximal aggregation response to ADP. At high concentrations, R(−)-isoproterenol was more potent than either the S(+)-isomer or desoxy analog, *N*-isopropyldopamine, in the reversal of PGE_1 inhibition of ADP aggregation. Phentolamine blocked these α_2-adrenoceptor-mediated actions against PGE_1 on ADP aggregation. The rank order of potency for the reversal of PGE_1-mediated inhibition of ADP aggregation by these catecholamines was similar to that observed for platelet aggregation. Our results indicate that (i) the stereochemical requirements for the interaction of catecholamines with platelet α_2-adrenoceptors are in agreement with the Easson-Stedman hypothesis and other α-adrenoceptor tissues; (ii) catecholamines lacking a benzylic hydroxyl group in the R-configuration and/or possessing an *N*-isopropyl group were α_2-adrenoceptor antagonists; (iii) clonidine gave quantitatively different responses compared with catecholamines for interaction with α_2-adrenoceptors; and (iv) inhibition of platelet adenylate cyclase is correlated to the inhibition of epinephrine-induced aggregation response for this series of compounds.**
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The classification of α-adrenoceptors into subclasses of α_1 and α_2 types has been aided by studying the stereochemical requirements for interaction of catecholamines with these receptor systems (1–3). In recent years, the evaluation of platelet α-adrenergic receptor sites has led to proposals of the presence of a predominant α_2- or α_2-A-subtype population (4, 5). The Easson-Stedman hypothesis (6) has represented a useful framework to assess stereochemical requirements for the interaction of catecholamines in α_1- and α_2-receptor systems. For catecholamines, three functional groups (a benzene ring with two phenolic hydroxyl groups [catechol function]; a benzylic hydroxyl group; and an amino nitrogen atom) are important for a three-point attachment leading to the activa-

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tion of adrenergic receptors (2, 6). Moreover, the orientation of the benzylic hydroxyl group of catecholamines in the R(−)-configuration confers optimal activity, whereas the corresponding S(+)-isomer and desoxy analogs (lacking a benzylic hydroxyl group) are less potent and equally active. Thus, the expected stereochemical relationship and rank order of direct activity on α-adrenoceptors for catecholamines are R(−)-isomer > S(+)-isomer = desoxy form.

Although extensive studies of the stereochemical interactions of phenethylamines, including norepinephrine (NE) and epinephrine in other α-adrenoceptor systems have been reported (2, 3, 7), only a few reports of structure-activity relationships of catecholamines in human platelets have appeared (8–11). α-Adrenoceptor activation in platelets by phenethylamines is dependent upon the presence of one or two phenolic hydroxyl groups on the benzene ring, a benzylic hydroxyl group, and either an unsubstituted or methyl-substituted amino nitrogen (3, 4, 10). Furthermore, earlier work has shown that the interactions of catecholamines with human platelets is stereoselective (R[−]-isomer > S[+]-isomer) (9, 11) and that the rank order of aggregation potency is epinephrine > NE ≫ isoproterenol (4). Additional studies have shown that the isomers of catecholamines displace radioligands from platelet α_2-receptors (4, 11–14), inhibit platelet adenylate cyclase activity (4, 11, 14, 15), and stimulate the sodium/proton exchange (16) in the same stereoselective fashion (R[−]-isomer > S[+]-isomer).

The objectives of the present report are to more extensively characterize the stereochemical specificity of the human platelet α_2-adrenoceptor system to catecholamines and to examine the effect of selected catecholamines on α-adrenoceptor-mediated inhibition of platelet adenylate cyclase activity. Clonidine was included as a representative of the imidazoline class which is known to interact with platelet α_2-adrenoceptors (4, 11, 13, 24). A preliminary report of this work has appeared (17).

Materials and Methods

Drugs and Chemicals. Drug sources were ADP, prostaglandin E_1, R(−)-norepinephrine hydrochloride, R(−)-isoproterenol bitartrate, and dopamine hydrochloride (Sigma Chemical Co., St. Louis, MO); S(+)-isoproterenol bitartrate, *N*-isopropyldopamine bitartrate, and S(+)-norepinephrine bitartrate (Sterling-Winthrop, Rensselaer, NY); clonidine (Boehringer-Ingelheim, West Germany); and phentolamine (Ciba-Geigy, Summit, NJ). The isomers and desoxy analogs of epinephrine, (±)-*erythro*- and (±)-*threo*-cobefrin, and the S(+)-and R(−)-isomers of α-methyldopamine were kindly supplied by Popat N. Patil, Professor of the Division of Pharmacology (The Ohio State University, Columbus, OH).

Blood Collection and Platelet Preparation. Human venous blood was collected by venipuncture into 3.8% trisodium citrate (9:1, v/v) from 15 volunteers who reported to be free of aspirin-containing medication for at least 10 days. Citrated blood was centrifugated at 120*g* for 15 min at room temperature to obtain platelet-rich plasma (PRP). Platelet-poor plasma was obtained by centrifugation of the PRP at 1100*g* for 10 min. Platelet concentrations in PRP were determined by phase contrast microscopy and adjusted with platelet-poor plasma to between 250,000 and 350,000/mm^3.

Aggregation Studies. Platelet aggregation was performed according to the turbidometric method of Born (18) as modified by Mustard *et al.* (19) using a Chronolog dual channel aggregometer (Chrono-Log Corp., Havertown, PA). For each sample a cuvette containing a 0.45-ml PRP and a magnetic bar was placed in the aggregometer with a stirring speed of 1100 rpm. Varying concentrations of each drug were added to PRP to make a total volume of 0.5 ml, and the resulting aggregation responses were monitored. Solvent vehicle was used in control samples, and drugs that did not produce aggregation were preincubated for 1 min before addition of the inducer. In most experiments, aggregation responses were monitored throughout a 6-min period.

Prostaglandin E_1 (PGE_1) is a potent activator of platelet adenylate cyclase and inhibits platelet aggregation by ADP (4, 20). Clonidine and selected catecholamines did not produce platelet aggregation in 9 of 16 platelet preparations. Activation of α_2-receptors by partial agonists leads to an inhibition of platelet adenylate cyclase which opposed the action of PGE_1 (4, 15). To assess whether these compounds interact with α_2-receptors in platelets, each drug was added 1 min prior to PGE_1, and the aggregation response to ADP was followed for an additional 6 min. In these experiments, phentolamine (1 μM) was also preincubated with platelets to establish the specificity of the drug action against PGE_1. Phentolamine (1 μM) alone did not modify the aggregation response to ADP in the presence or absence of PGE_1.

Evaluation of Data. Effective concentration 50 (EC_{50}) values were determined graphically from percentage of aggregation *versus* log molar drug concentration plots and were expressed as those concentrations required to produce 50% of the maximal transmittance. Data were also expressed as pD_2 values (negative log EC_{50}) for each drug. The dissociation constant of a competitive antagonist at a concentration ratio of 2 (pK_B value) of drug-induced platelet aggregation was determined from the method described by Furchgott and Bursztyn (21).

Results

In these studies, human platelet-rich preparations gave responses to all selected catecholamines (6 of 16

preparations) (described herein as responsive platelet preparations). Representative aggregation tracings of the concentration-dependent effects of R(−)- and S(+)-epinephrine, epinine and (±)-*erythro*-cobefrin are given in Figure 1, and the concentration-response relationships of all compounds tested in these preparations are presented in Figure 2. In the remaining platelet preparations (nine donors) clonidine and catecholamines, with the exception of R(−)-NE, R(−)-epinephrine, and (±)-*erythro*-cobefrin, were unable to produce aggregation responses (described herein as nonresponsive platelet preparations). Drugs were studied both for their inhibition of R(−)-NE-induced platelet aggregation and reversal of PGE_1-mediated inhibition of ADP-induced aggregation.

Stereochemical Relationships in Responsive Platelet Preparations. *Benzylic or hydroxyl group substitution.* Hydroxyl group substitution at the β-carbon atom produces an asymmetric center on the ethylamine side chain of catecholamines (epinephrine and NE) and gives rise to two possible stereochemical configurations (Table I). The isomers and corresponding desoxy derivatives (epinine and dopamine, respectively) of epinephrine and NE produced platelet aggregation in a concentration-dependent manner (Fig. 2). The pD_2 values of R(−)-NE, S(+)-NE, and corresponding desoxy derivative (dopamine) were 5.9, 4.7, and 4.6, respectively, and the corresponding pD_2 values were 6.3, 4.7, and 4.4 for R(−)-epinephrine, S(+)-epinephrine, and epinine, respectively (Table I). These data demonstrate that R(−)-isomers of NE and epinephrine are from 30 to 95-fold more potent than their corresponding S-enantiomers and desoxy derivatives. *N*-isopropyldopamine and isoproterenol isomers were unable to produce platelet aggregation at the highest concentration (1 m*M*) used.

α-Methyl carbon substitution. α-Carbon substitution of NE as α-methylnorepinephrine (cobefrin) also produces another asymmetric center, giving four possible stereochemical configurations. Only the (±)-*erythro* and (±)-*threo* racemates of cobefrin were available and they were found to exhibit a concentration-dependent aggregation (Fig. 2 and Table I). pD_2 Values obtained for (±)-*erythro*- versus (±)-*threo*-cobefrin were 5.3 and 3.7, respectively. This 78-fold difference in aggregation potency between *erythro*- versus *threo*-cobefrin is similar to the observed stereoselectivity ratio for R(−)- and S(+)-isomers of NE and epinephrine. Substitution with an α-methyl group in dopamine also produces two stereoisomers (S[+]- and R[−]-α-methyldopamine). pD_2 Values of S(+)- and R(−)-α-methyldopamine were equivalent (4.3) and were somewhat less potent than dopamine (Table I).

Substitution at the amino nitrogen atom. The nitrogen atom of catecholamines is important for binding to α-adrenergic receptors. *N*-methyl substitution of NE, as in epinephrine, produces an increase in α_2-adrenergic agonist activity. R(−)-epinephrine was a more potent agonist than R(−)-NE on platelet aggregation whereas it appeared that epinine was less active than dopamine (Table I). *N*-isopropyl substitution dramatically reduced agonist activity, since neither the isoproterenol

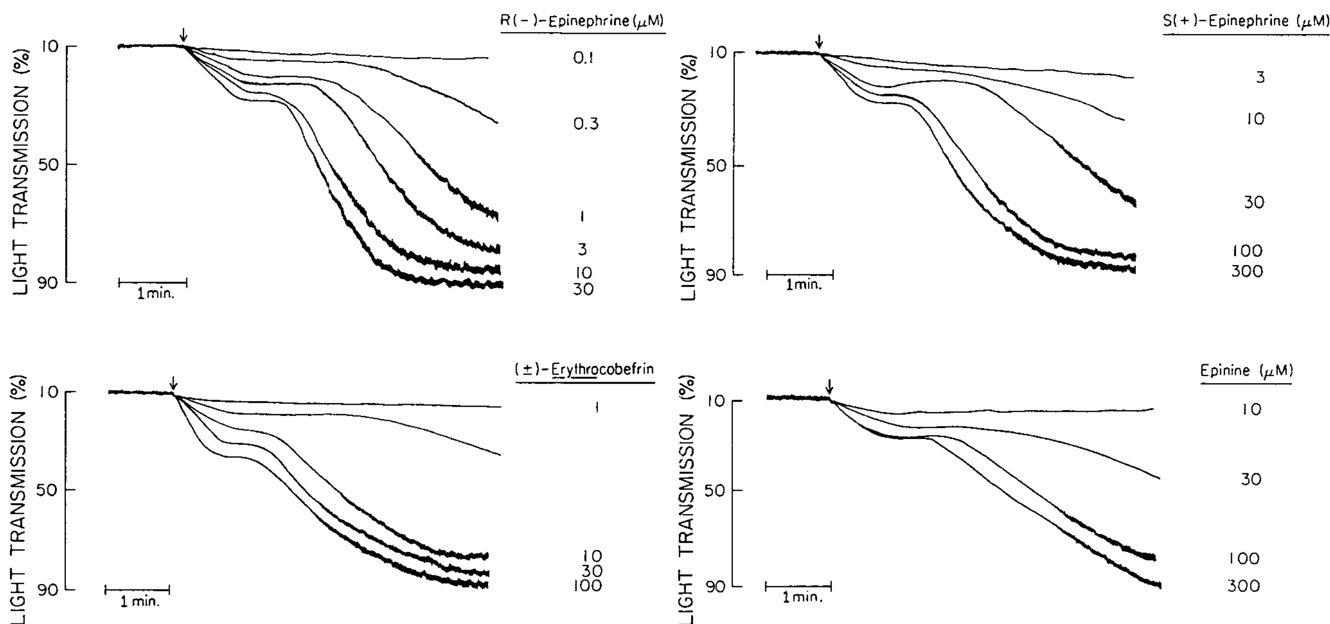


Figure 1. Concentration-dependent increases in human platelet aggregation induced by R(−)-epinephrine, S(+)-epinephrine, (±)-*erythro*-cobefrin, and epinine. Aggregation responses were monitored by changes in light transmittance over a 6-min period. Drugs were added after a 1-min incubation in the aggregometer. Tracings are representative of concentration-response data obtained in three other platelet-rich plasma preparations.

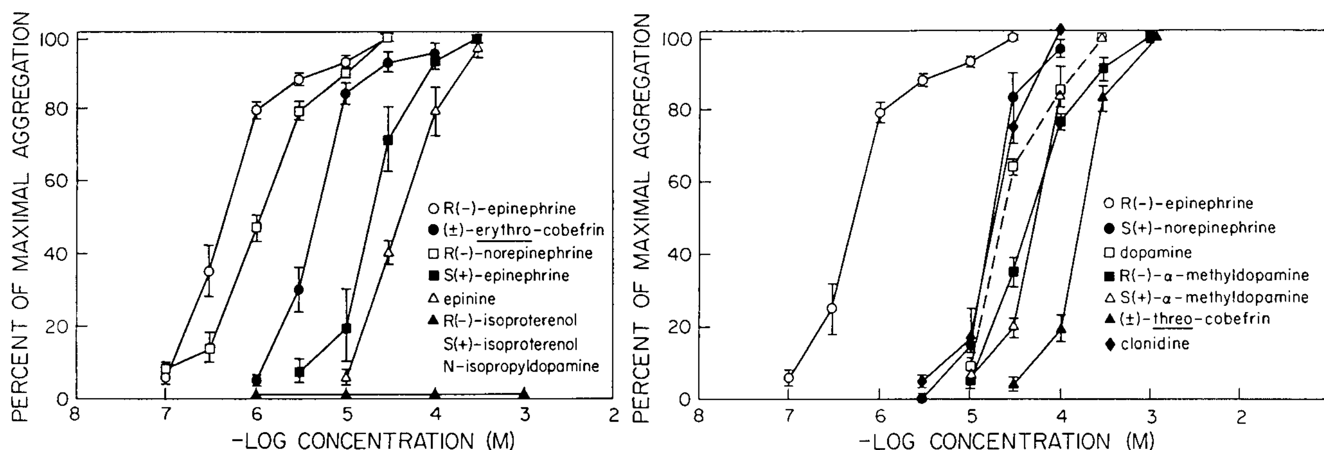
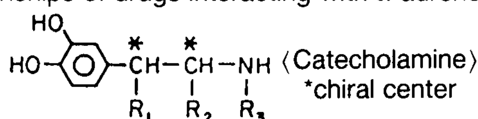


Figure 2. Concentration-response relationships of catecholamines and clonidine as agonists in human platelets. Data represent the mean ± SE of $n \geq 3$. See insert in figure for key of compounds used.

Table I. Structure activity relationships of drugs interacting with α-adrenergic receptors in human platelets



Compound	R_1	R_2	R_3	pD_2[a]	EC_{50} (μM)	pK_B[b]	K_B (μM)
Epinephrine	OH	H	CH_3				
R(−)-isomer				6.34	0.46	—	—
S(+)-isomer				4.74	18.2	ND[c]	ND
Epinine	H	H	CH_3	4.39	40.7	ND	ND
Norepinephrine	OH	H	H				
R(−)-isomer				5.88	1.3	—	—
S(+)-isomer				4.72	19.1	4.27	53.7
Dopamine	H	H	H	4.65	22.4	4.61	24.5
Isoproterenol	OH	H	$CH(CH_3)_2$				
R(−)-isomer				NA[d]	—	3.69	204
S(+)-isomer				NA	—	3.51	309
N-Isopropyldopamine	H	H	$CH(CH_3)_2$	NA	—	4.14	72.5
α-Methylnorepinephrine (cobefrin)	OH	CH_3	H				
(±)-*erythro*-isomer				5.33	4.7	—	—
(±)-*threo*-isomer				3.74	182.0	3.74	182.0
α-Methyldopamine	H	CH_3	H				
S(+)-isomer				4.34	45.7	5.00	10.0
R(−)-isomer				4.26	55.0	4.44	36.3
Clonidine	α_2-Adrenergic agonist			4.72	19.1	6.18	0.66
Phentolamine	α-Adrenergic antagonist			—	—	8.04 8.09[e]	0.0091 0.0081

[a] $pD_2 = -\log$ effective concentration-50 $= -\log EC_{50}$. Mean of n = 3–6 preparations.
[b] $pK_B = -\log[D]/CR\text{-}1$ where [D] = molar drug concentration ratio shift in EC_{50} of R(−)-epinephrine. Mean of n = 3–4 preparations.
[c] ND, not determined.
[d] NA, not active at concentrations up to 1 m*M*.
[e] Value obtained against R(−)-NE.

isomers nor *N*-isopropyldopamine produced platelet aggregation in concentrations up to 1 m*M* (Fig. 2).

Stereostructure Relationships in Nonresponsive Platelet Preparations. *Characterization of inhibitory activities of catecholamines.* R(−)-epinephrine, R(−)-NE, and (±)-*erythro*-cobefrin produced agonist activity in each of the human platelet preparations whereas other catecholamine analogs exhibited either agonist or antagonist profiles of activity. For compounds which did not produce aggregation, their ability to block aggregation induced by R(−)-NE was examined (Fig. 3). In the latter studies, these compounds blocked the

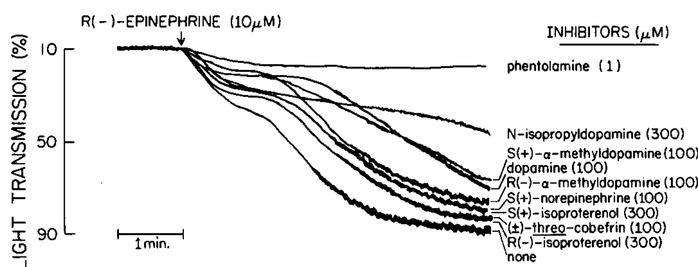
aggregation responses to R(−)-epinephrine and shifted the concentration-response curve of R(−)-epinephrine to the right (Fig. 3), indicating that these drugs are competitive inhibitors of the aggregation responses to this agonist. Experimentally determined pK_B values for each compound are given in Table I. The rank order of inhibitory potencies (K_B values, μM) of the catecholamines was S(+)-α-methyldopamine (10) > dopamine (25) > R(−)-α-methyldopamine (36) > S(+)-NE (54) > *N*-isopropyldopamine (73) > (±)-*threo*-cobefrin (182) > R-(−)-isoproterenol (204) > S(+)-isoproterenol (309). The antagonist potencies for these catecholamines were nearly the same as pD_2 values obtained from responsive preparations, implying that they interact with similar receptor binding sites in these preparations.

Clonidine also acted as an inhibitor of epinephrine-induced activation with nonresponsive preparations with pK_B values of 6.18, while it also behaved as the inducer of platelet aggregation in responsive preparations with a pD_2 value of 4.72 (Table I). Thus, unlike catecholamines, clonidine shows a differential affinity as an antagonist and agonist in these two preparations (Table I). In addition, the α-adrenoceptor antagonist phentolamine blocked the aggregatory responses to R(−)-epinephrine and R(−)-NE with pK_B values of 8.04 and 8.09, respectively. These results indicate that this α-adrenergic receptor antagonist interacts with the same adrenoceptor binding sites as these catecholamines.

Effect of catecholamines on reversal of PGE_1 inhibition of ADP aggregation. PGE_1 is known to increase cytosolic cAMP levels and, thus, inhibits platelet aggregation by inducers of human platelet activation, e.g., the activation of platelets by ADP is inhibited by PGE_1 (20). α_2-Adrenergic receptor activation is known to inhibit the adenylate cyclase system, presumably through an inhibitory guanine nucleotide-binding protein (4, 13–15). We hypothesized that if these drugs showed inhibitory activity in these nonresponsive preparations as partial agonists, then catecholamines should reverse the inhibition imposed by PGE_1 on ADP-induced platelet activation. As expected, several catecholamines and clonidine, which behaved as inhibitors of R(−)-epinephrine induced platelet activation reversed PGE_1 effects on ADP aggregation (Figs. 4 and 5), indicating that they are acting as partial agonists of α_2-receptors in platelets. We also examined the comparative inhibitory effects of isoproterenol isomers and *N*-isopropyldopamine and found that only high concentrations ($\geq$100 μM) reversed PGE_1 effects on ADP aggregation, giving an order of potency of R(−)-isoproterenol > S(+)-isoproterenol = *N*-isopropyl-dopamine (Fig. 4). The α-antagonist, phentolamine, blocked these drug-mediated effects, indicating that clonidine and these catecholamines are acting as partial agonists at α_2-receptors in platelets (Fig. 4 and Table I). The rank order of the reversal of PGE_1 inhibition by the drugs (100 μM) was S(+)-α-methyldopamine > dopamine > R(−)-α-methyldopamine > clonidine > R(−)-isoproterenol > (±)-*threo*-cobefrin > S(+)-NE ≥ S(+)-isoproterenol = *N*-isopropyldopamine (Figs. 4 and 5). This rank order pattern of activity was similar to that observed for their blockade of R(−)-epinephrine-induced aggregation.

Discussion

α-Adrenergic receptors of the human platelet are unique in that they are exclusively of the α_2 type or α_2-A subtype (4, 5). In a previous study, we also reported (11) that the α_2-mediated responses to isomers of catecholamines are qualitatively different in platelets which were referred to as responsive or nonresponsive platelet preparations. In this regard, our studies have examined the structural requirements of catecholamines for platelet aggregation and of antagonism of catecholamine-induced aggregation in human platelets. The Easson-Stedman theory (6) was proposed in an attempt to explain the stereoisomeric potency differences of catecholamines on adrenergic receptors. This theory hy-



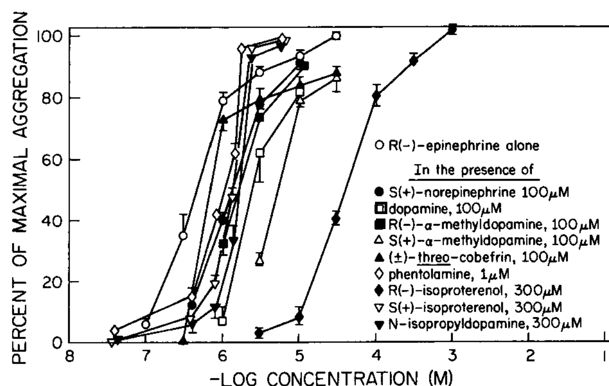


Figure 3. Effect of selected catecholamines (100 μM) and phentolamine (1 μM) as inhibitors of R(−)-epinephrine (10 μM)-induced platelet aggregation (left panel) and on the concentration-aggregation response relationship of R(−)-epinephrine (right panel). Data represent the mean ± SE of $n \geq 3$. See insert in figure for key of compounds used.

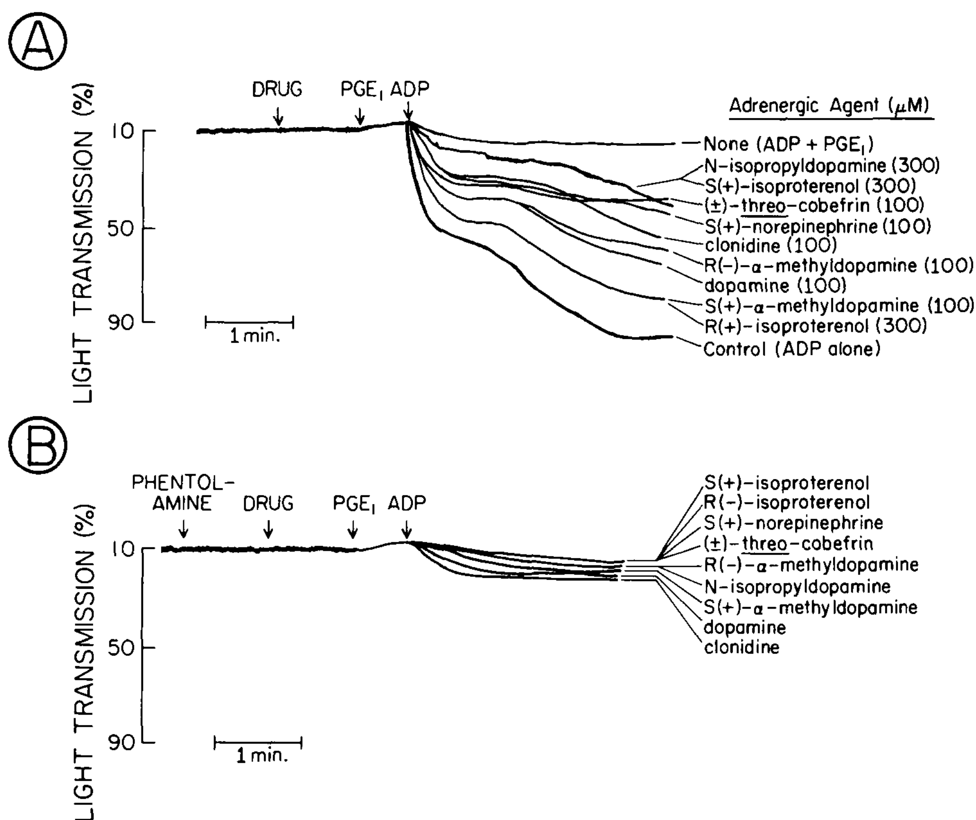


Figure 4. Comparative effects of clonidine and selected catecholamines on PGE_1 (0.1 μM)-mediated inhibition of ADP (3 μM) aggregation in nonresponsive platelet preparations. (A) Drug-induced reversal of PGE_1-mediated inhibition of ADP aggregation. Drug and PGE_1 were added at 1.5 min and 0.5 min, respectively, prior to ADP (3 μM). (B) Effect of phentolamine on the drug-induced reversal of PGE_1-mediated inhibition of ADP aggregation. Phentolamine, drug, and PGE_1 were added at 2.5, 1.5, and 0.5 min, respectively, prior to ADP (3 μM). Concentrations of each agent are given in the figure, and data are representative of ≥3 preparations.

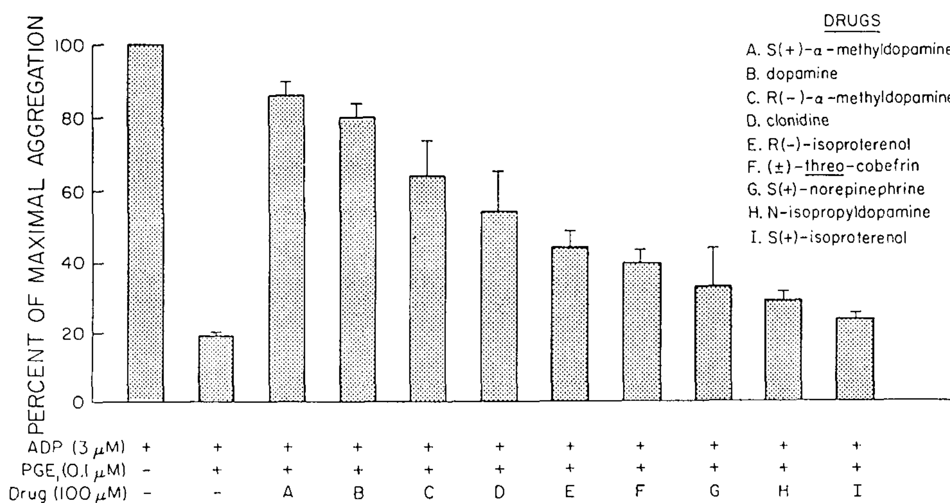


Figure 5. Reversal of PGE_1-mediated inhibition of ADP-induced aggregation by selected catecholamines and clonidine in nonresponsive platelet-rich preparations. See insert in figure for key of compounds used. Data represent the mean ± SE of $n \geq 3$.

pothesized an involvement of three points of attachment (catechol, benzylic hydroxyl, and amino nitrogen groups) during binding of the catecholamine to the adrenergic receptor. According to this theory, R(−)-isomers interact with the receptor by the most favorable three-point attachment and are the most potent form. In contrast, S(+)-isomer and desoxy derivatives interact only by a two-point attachment to the receptor and are equipotent with each other and less potent than the R(−)-isomer. Our data demonstrate that the platelet α-adrenergic receptor system adheres to this proposal since R(−)-NE and R(−)-epinephrine are more potent than their corresponding enantiomers and desoxy derivatives, and S(+)-isomers and desoxy derivatives were

nearly equipotent with each other (Table I). The stereoselectivity potency ratio of 15-fold and 40-fold for the isomers of NE and epinephrine are in good agreement with their reported activity differences in other α_1- and α_2-adrenoceptor tissues (2, 3, 7).

Our findings also indicate that the presence of a chiral center at the α-methyl carbon atom produces either a small potency difference (3-fold) of S-(+)-α-methyldopamine > R(−)-α-methyldopamine or no change in the interaction of catecholamines with α_2-receptors in platelets. In this regard, α-methylnorepinephrine (cobefrin) also showed a high degree of stereoselectivity ([±]-*erythro*-cobefrin ≫ [±]-*threo*-cobefrin) which was identical to that of the isomers of epinephrine (40-fold). (±)-*Erythro*-cobefrin is a *racemic* compound which retains one isomeric form (1R,2S-isomer) that has an S-configuration at the α-carbon atom and the optimally active R-configuration for the benzylic hydroxyl group at the β-carbon atom (2, 7). In other systems, results have indicated that 1R,2S-*erythro* form is more potent than 1S,2R-*erythro* isomer and the isomers or racemate of *threo*-cobefrin (2, 3, 22). In agreement with these findings, we found that the *erythro*-conformation of cobefrin is more potent for activation of platelet α_2-adrenoceptors than *threo*-cobefrin. Since 1S,2R-*erythro*-cobefrin had no agonist activity in other adrenoceptor systems (3, 22), it can be suggested that actual pD_2 value of the 1R,2S(−)-isomer of *erythro*-cobefrin is greater than 5.3. The partial agonist activity seen with *threo*-cobefrin may be due to the 1R,2R-isomeric form which retains the appropriate configuration of the benzylic hydroxyl group. Although α-methyl substitution of dopamine did not increase agonist activity at platelet α-adrenergic receptor, the S-configuration showed a small increase in affinity compared with R(−)-α-methyldopamine as an antagonist of epinephrine-induced aggregation. The reason for increased affinity of S(+)-α-methyldopamine is most likely related to the preferred orientation of the α-methyl group in the S-configuration.

Previous structure activity (4, 10) studies with catecholamines in platelets show that epinephrine is more potent than NE as an inducer, and that substitution with a larger *N*-alkyl group as in isoproterenol reduces potency or abolishes platelet aggregatory activity. Our data show that R(−)-epinephrine was more active than R(−)-NE and the isoproterenol isomers and desoxy analog (*N*-isopropyldopamine) were inactive as agonists in platelets. Thus, among *N*-alkyl-substituted catecholamines only *N*-methyl substitution of NE increases intrinsic activity of the drug molecule for this system, otherwise, substitution of the amino nitrogen atom with an isopropyl group dramatically decreases intrinsic activity and potency of these compounds on these platelet α-adrenergic receptors. This is a consistent observation seen for the interaction of catecholamines with α-adrenergic tissues (2–4).

Our laboratory (11) and others (4, 23, 24) have reported that human platelets exhibit differences in responsiveness to α-adrenoceptor agonists of the phenethylamine and imidazoline classes. Jakobs (23) indicated that only natural agonists such as NE and epinephrine produced full agonist responses in platelets, whereas phenylephrine and synthetic imidazolines are either weakly active or act as antagonists in human platelets. In this study, we observed that the imidazoline, clonidine, was a weak agonist, and had a higher affinity for the blockade of R(−)-epinephrine-mediated aggregation (Table I). Moreover, the absence of platelet aggregation with selected catecholamines (e.g., isoproterenol and dopamine) has been previously reported (10). The underlying mechanism for nonresponsiveness or responsiveness of α-adrenoceptor agonists in human platelets is unknown, although it has been suggested that a plasma factor may be involved (10). Nevertheless, based upon our data and that of others (3, 10, 11), it appears that the presence of a catechol group (benzene ring with two phenolic hydroxyl groups), a benzylic hydroxyl group in the R-configuration, and an unsubstituted or methyl-substituted nitrogen atom are important requirements for full agonist activity of catecholamines in platelets. This is supported by the findings that only R(−)-NE, R(−)-epinephrine, and (±)-*erythro*-cobefrin possessed maximal aggregatory responses in all platelet preparations used.

Our studies showed that catecholamines lacking the benzylic hydroxyl group or possessing the S(+)-configuration, and clonidine, exhibited considerably reduced agonist activities or behaved as antagonists of catecholamine-induced platelet aggregation (Table I). Experiments were designed to determine whether catecholamines and clonidine could act as partial agonists in nonresponsive platelet preparations. ADP-induced aggregation was almost totally inhibited by PGE_1 and this inhibition was reversed up to 80% by these drugs that had shown inhibitory activity to epinephrine-induced aggregation in the nonresponsive platelet preparations. The catecholamine-mediated reversals of PGE_1 inhibition of ADP aggregation were blocked by phentolamine. Clonidine effects were identical to the results with catecholamines. The data indicate that the reversal of PGE_1 inhibition is receptor mediated and that these drugs are not pure antagonists but are acting as partial agonists of α-adrenoceptors in these platelet preparations.

It is well known that stereochemical requirements of imidazolines and catecholamines in α_1- and α_2-adrenoceptors differ markedly (3, 7, 25, 26). Unlike the catecholamines used in these studies, clonidine showed considerable differences in potency as an agonist *versus* antagonist properties in platelets (Table I). It has pre-

viously been reported that the inhibitory potency of clonidine against catecholamine-induced aggregation correlates to the corresponding inhibitory activity on platelet adenylate cyclase (24). Our experiments also revealed that blockade of PGE_1 effect on ADP aggregation by clonidine and selected catecholamines were favorably correlated to their antagonism of R(−)-epinephrine-induced aggregation (Fig. 5 and Table I). Thus, the mechanism of these compounds as partial agonists may be related to cAMP lowering effects (4). In contrast, the inhibition of platelet adenylate cyclase is not linked to aggregation responses for full agonists (4, 16, 27). Alternatively, stimulation of the sodium/proton exchange system may explain the aggregatory action of catecholamines (16, 27). To explain these differences between these two classes of α-adrenoceptor agents, in stereochemical requirements and profiles of activity in platelets, it has been proposed that imidazolines may act via different binding sites or activating mechanism for the α-adrenoceptor or transduction process (3, 25, 28). It is clear that the mechanism must also await our understanding of the basis for responsive and nonresponsive platelets. A characterization of the mechanisms of agonist and antagonist binding to α_2-adrenoceptors and coupling with guanine nucleotide-binding proteins may provide some clue to elucidating these differences in platelet responsiveness (29). Our laboratory is continuing to investigate the structural requirements and criteria for agonist *versus* antagonist activity for these two classes of α-adrenoceptor agonists.

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