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ALPHA-ADRENOCEPTOR-MEDIATED ACTIONS OF OPTICAL ISOMERS AND DESOXY ANALOGS OF CATECHOLIMIDAZOLINE AND NOREPINEPHRINE IN HUMAN PLATELETS: *IN VITRO*

CHANG-HO AHN, AKIHIKO HAMADA, DUANE D. MILLER and DENNIS R. FELLER*
Divisions of Pharmacology and Medicinal Chemistry, College of Pharmacy, The Ohio State University, Columbus, OH 43210, U.S.A.

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Abstract—Adrenoceptor-mediated effects of the enantiomers of optically active imidazoline, 2-(3,4,α-trihydroxybenzyl imidazoline (catecholimidazoline; CI), and norepinephrine (NE), and the corresponding desoxy derivatives, 2-(3,4-dihydroxybenzyl)imidazoline (desoxy-CI) and dopamine, have been investigated in human platelets. Differences between responsiveness of platelets from donor to donor were observed in the presence of the isomers and the desoxy analogs of NE and CI. In certain platelet preparations, all compounds gave concentration-dependent stimulatory responses, whereas in other preparations, only $R(-)$-NE and $R(-)$-CI were inducers of platelet aggregation and serotonin release. The rank order of stimulatory potencies (EC_{50}; μM) for CI and NE was $R(-)$-NE (1.3) > $R(-)$-CI (7.5) > $S(+)$-NE (19) = $S(+)$-CI (20) = dopamine (22) > desoxy-CI (>35). Unlike $R(-)$-CI, both $S(+)$-CI and desoxy-CI were either agonists or antagonists of human platelet function. In preparations unresponsive to the $S(+)$-isomers or desoxy analogs, the potencies (EC_{50}) for $R(-)$-NE and $R(-)$-CI were 1.7 and 7.7 μM respectively. The corresponding inactive CIs [$S(+)$-CI and desoxy-CI] were inhibitors of both primary and secondary phases of aggregation and serotonin release responses to $R(-)$-CI and $R(-)$-NE, respectively. In contrast, the aggregation responses to ADP, arachidonic acid or U46619 were not blocked by $S(+)$-CI or desoxy CI. The rank order of inhibitory potencies for selected α-adrenoceptor agents against $R(-)$-NE was phentolamine > clonidine > desoxy-CI > $S(+)$-CI. Moreover, the relative inhibitory potencies of phentolamine and desoxy-CI against aggregation responses to $R(-)$-NE and $R(-)$-CI, respectively, were the same. These results suggest that (1) the enantiomers and desoxy derivatives of CI and NE mediate their effects in human platelets by an interaction with α-adrenoceptors; (2) catecholamines and imidazolines interact with the same α-adrenoceptors in human platelets; (3) the stereochemical requirements of both chemical classes for stimulatory activity in human platelets adhere to the Easson–Stedman hypothesis in this α_2-adrenoceptor system; and (4) desoxy-CI possessed the highest potency as an antagonist of α-adrenoceptors which suggests that the hydroxy group at the benzylic carbon atom of these imidazolines may not be required for maximal binding to adrenoceptors in platelets.

The Easson–Stedman hypothesis [1] represented a useful framework to explain the chemical requirements for the interaction of catecholamines such as epinephrine and norepinephrine in α_1-, α_2-, β_1- and β_2-adrenoceptor systems [2]. The interaction of the catechol moiety, nitrogen atom, and orientation of the β-hydroxy group on the ethylamino side chain in the $R(-)$-configuration were important features for the proposed three-point attachment of catecholamines to putative α- and β-adrenoceptor sites. For the corresponding $S(+)$-isomer or desoxy derivatives, the β-hydroxy group is incorrectly oriented or absent. These compounds were assumed to undergo a two-point attachment and were considered equally effective for receptor occupation and pharmacological activity in adrenergic tissues. Thus, the stereochemical relationship and relative order of direct activity of catecholamines on adrenergic receptors are $R(-)$-isomer > $S(+)$-isomer = desoxy form [2].

To further test the compatibility of the Easson–Stedman hypothesis for the interaction of structurally related catecholamines with adrenoceptor systems, a set of optically active catecholimidazolines which possess the phenethylamine backbone were synthesized and examined for α_1 and α_2-adrenoceptor activities [3, 4] (Fig. 1). This previous work showed differences in the potency profile of the isomers and desoxy analogs of norepinephrine (NE) and 2-(3,4-α-trihydroxybenzyl)imidazoline (CI) between α_1- (guinea pig aorta) and α_2- (field stimulated guinea pig ileum) adrenoceptor systems. For catecholamines, the rank order of potency was $R(-)$-NE > $S(+)$-NE ≈ dopamine (desoxy analog) in these α_1- and α_2-adrenoceptor systems. In contrast, the corresponding affinity profiles of the CIs were desoxy-CI ≥ $R(-)$-CI > $S(+)$-CI in both α_1- and α_2-adrenoceptor systems, *in vivo* and *in vitro*. In α_2-adrenoceptor systems, desoxy-CI was a partial agonist [3, 4]. These results indicate that (1) the rank order of potencies of CIs is clearly different from

* Send reprint requests to: D. R. Feller, Ph.D., Division of Pharmacology, College of Pharmacy, 500 West 12th Ave., The Ohio State University, Columbus, OH 43210.

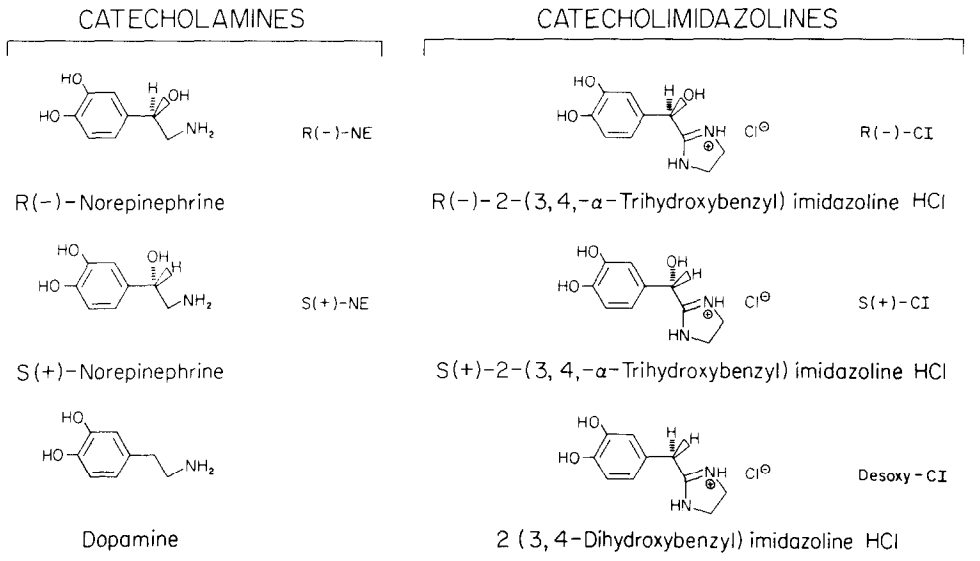

Fig. 1. Chemical structures of $R(-)$ and $S(+)$-isomers and corresponding desoxy analogs of norepinephrine (NE) and catecholimidazoline (CI). The corresponding chemical name and abbreviations for the CIs are presented.

that predicted by the Easson–Stedman theory [1], and (2) the structure–activity relationships for catecholamines and CIs in these adrenoceptor systems are different.

The comparative potencies of the interaction of epinephrine and NE with α-adrenoceptor sites in human platelets have been studied [5–7]. In more recent studies with the use of synthetic α-adrenoceptor agents, and radioligand displacement techniques, the subtype of receptors for NE (or epinephrine) in human platelets has been primarily characterized as an α_2-adrenoceptor population [8–11]. Thus, human platelets represent an α_2-adrenoceptor population which is not neuronally innervated, and offer an alternative system for the study of these two important chemical classes of α-agonists. Our experiments were undertaken to evaluate the applicability of the Easson–Stedman hypothesis for catecholimidazolines on human platelet function and to examine the comparative interaction of catecholamines and catecholimidazolines with α_2-adrenoceptor sites in platelets.

METHODS

Chemicals. [^{14}C]Serotonin (sp. act. = 58 mCi/mmole) was purchased from the Amersham Corp. (Arlington Heights, IL). Drug sources were: ADP, arachidonic acid, $R(-)$-norepinephrine hydrochloride and dopamine hydrochloride (Sigma Chemical Company, St. Louis, MO); $S(+)$-norepinephrine bitartrate (Sterling-Winthrop, Rensselaer, NY); clonidine (Boehringer-Ingelheim, West Germany); phentolamine (Ciba-Geigy, Summit, NJ); and U46619 [15S-hydroxy-9α, 11α-epoxymethanoprosta-5Z, 13E-dienoic acid; UpJohn Diagnostics, Kalamazoo, MI]. The isomers and desoxy analog of 2-(3,4-α-hydroxybenzyl) imidazoline, as hydrochloride salts, were prepared in our laboratories [3].

Blood collection and platelet preparation. Human venous blood was collected by venipuncture into 3.8% trisodium citrate (9:1, v/v) from fifteen volunteers who reported to be free of aspirin-containing medication for at least 10 days. Citrated blood was centrifuged 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 between 250,000 and 350,000 per mm^3.

Aggregation studies. Platelet aggregation was performed according to the turbidometric method of Born [12] as modified by Mustard *et al.* [13] using a Chrono-log dual channel aggregometer (Chrono-Log Corp., Havertown, PA). For each sample a cuvette containing 0.45 ml PRP and a magnetic bar was placed in the aggregometer with a stirring speed of 1100 rpm. Various 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 which 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.

Serotonin release. [^{14}C]Serotonin (5-HT; 0.05 μCi/ml PRP) was incubated with PRP for 30 min prior to the aggregation studies and used to monitor the secretion of platelet contents from the dense granules. Aliquots of PRP prelabeled with 5-HT were used directly in these studies. Secretion of [^{14}C]-5-HT from platelets was measured in the supernatant fraction of PRP preparations before and after challenge with drugs. Samples were centrifuged at 1200 g for 1 min in an Eppendorf microfuge (Brinkmann, Westbury, NY), and an aliquot of the supernatant fraction was counted in a scintillation mixture on a Beckman liquid scintillation spectrometer

(Beckman, model LS 6800, Palo Alto, CA) using external standardization to monitor the extent of quench. Secretion data were calculated as the relative percentage of [^{14}C] in platelets released by the appropriate inducer.

Evaluation of data. Effective concentration-50 (EC_{50}) values were determined graphically from percent 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 logarithm of the affinity constant estimated as the EC_{50} value) for each drug [14]. Data were analyzed using Student's *t*-test at the 5% level of significance [15]. The negative logarithm of the concentration of a competitive antagonist at a concentration ratio of 2 (pA_2 value) of drug-induced platelet aggregation was determined from the method described by Arunlakshana and Schild [16].

RESULTS

Comparison of the effects of isomers and desoxy analogs of norepinephrine (NE) and catecholimidazoline (CI) on human platelet function. Our preliminary experiments indicated that the responsiveness of PRP preparations to the stereoisomers and desoxy analogs of NE and CI was dependent upon the donor used. Nine of fifteen PRP preparations gave concentration-dependent responses to the isomers and desoxy analogs of NE and CI. In these PRP preparations, the *R*(−)-isomers were more active than the corresponding *S*(+)-isomers or desoxy analogs (Fig. 2, A and B). Desoxy-CI gave a response which was 9% of the maximal light transmittance produced by the isomers of CI. The rank order of stimulatory potencies (EC_{50} values, μM) for these compounds was *R*(−)-NE (1.3) > *R*(−)-CI (7.5) > *S*(+)-NE (19) = *S*(+)-CI (20) = dopamine (22) > desoxy-CI (>35). In addition, *R*(−)-NE and *R*(−)-CI were 14.5- and 2.7-fold more active than the corresponding *S*(+)-isomers respectively.

In six of fifteen PRP preparations, only the *R*(−)-isomers of CI and NE gave aggregatory and secretory responses. No aggregation responses were seen in the presence of a 1.0 mM concentration of the *S*(−)-isomers or desoxy forms. These results were also reproducible in PRP preparations taken from the same donor at different times. By contrast to the *R*(−)-isomer, neither the *S*(+)-isomers nor desoxy (dopamine and desoxy-CI) analogs of these compounds gave an aggregatory response in these PRP preparations within 5 min in the concentration range of 0.3 to 1000 μM (Fig. 2, C and D). Concentration-dependent increases in serotonin release and in the primary and secondary phases of aggregation were seen with *R*(−)-NE and *R*(−)-CI in the concentration range of 0.1 to 500 μM (Fig. 3). Both compounds produced a maximal serotonin release of about 40% at the highest concentration used, and *R*(−)-NE was more potent as an inducer of these aggregatory and secretory responses than *R*(−)-CI.

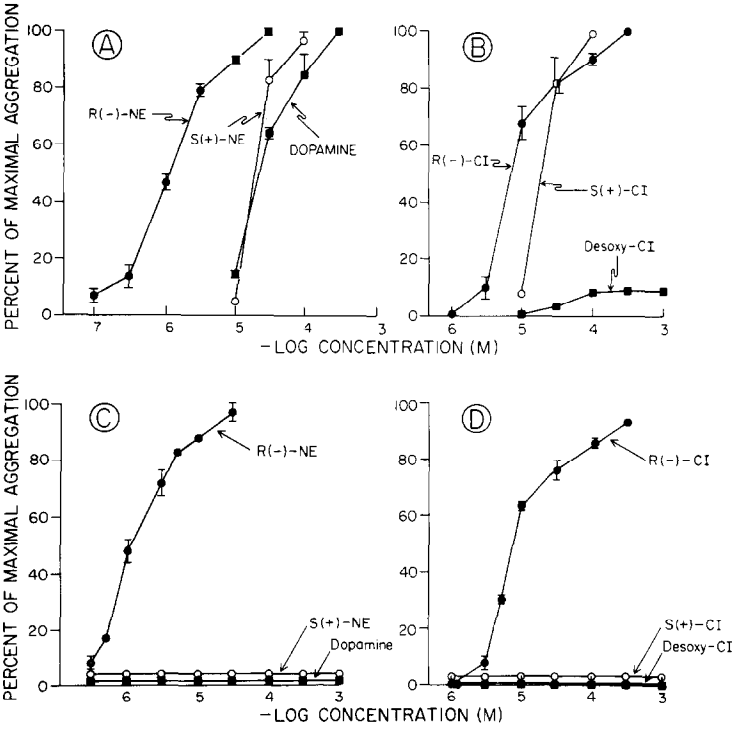

Fig. 2. Comparative concentration–response relationships of the stereoisomers and desoxy analogs of norepinephrine (NE; panels A and C) and catecholimidazoline (CI; panels B and D) in responsive (panels A and B) and nonresponsive (panels C and D) preparations. Each value represents the percent of the maximal aggregation response seen with *R*(−)-NE. Data are expressed as the mean ± S.E.M. of three donors.

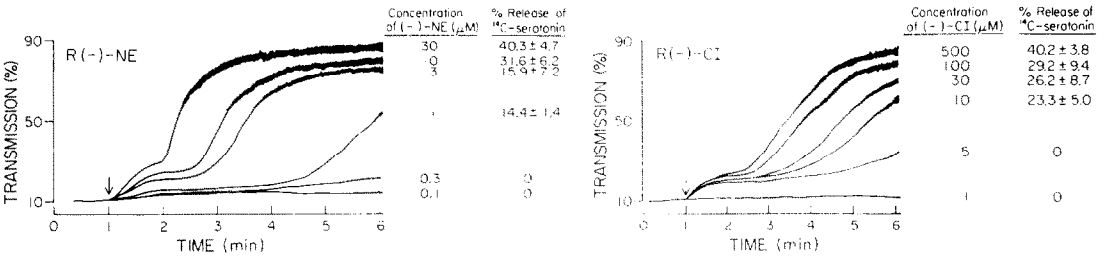

Fig. 3. Concentration-dependent stimulation of aggregation and serotonin release by *R*(−)-NE (left panel) and *R*(−)-CI (right panel) in human platelet-rich plasma (PRP). *R*(−)-NE and *R*(−)-CI were added to platelets 1 min before addition of inducer. The percent of serotonin release was measured as described in Methods. Superimposed tracings of platelet aggregation are representative of three experiments. Each value on secretion of [^{14}C]serotonin is the mean ± S.E.M. of at least three donors.

The calculated EC_{50} values for platelet aggregation were found to be 1.7 and 7.7 μM for *R*(−)-NE and *R*(−)-CI, respectively, showing that a 4.5-fold difference in potency exists between these two compounds (see Fig. 2, C and D). This value is similar to the 5.7-fold difference seen for *R*(−)-NE and *R*(−)-CI in those PRP preparations responsive to the *S*(+)-isomers and desoxy analogs. Moreover, the stimulatory potencies of the *R*(−)-isomers of NE and CI in both types of PRP preparations were the same ($P > 0.05$).

Comparison of the effects of S(+)- *and desoxy-CI as inhibitors of* R(−)-*NE- and* R(−)-*CI-induced platelet function.* The remaining experiments were designed to examine the inhibitory activities of the *S*(+)-isomers and desoxy analogs of CI and NE in those PRP preparations responsive only to the *R*(−)-isomers of CI and NE. Both *S*(+)-CI and desoxy-CI were able to block the primary and secondary phases of aggregation response to *R*(−)-NE and *R*(−)-CI in a concentration-dependent manner (Fig. 4). As shown, a shift in the time course and maximal transmittance of the aggregation responses to each inducer was seen for each compound at concentrations of 0.3 to 100 μM. In addition, desoxy-CI was a more potent inhibitor than *S*(+)-CI of aggregatory responses to *R*(−)-NE and *R*(−)-CI. Similarly, desoxy-CI completely blocked serotonin release by *R*(−)-NE (1 μM and 3 μM) and *R*(−)-CI (100 μM) in human platelets (Fig. 5, A and B). The

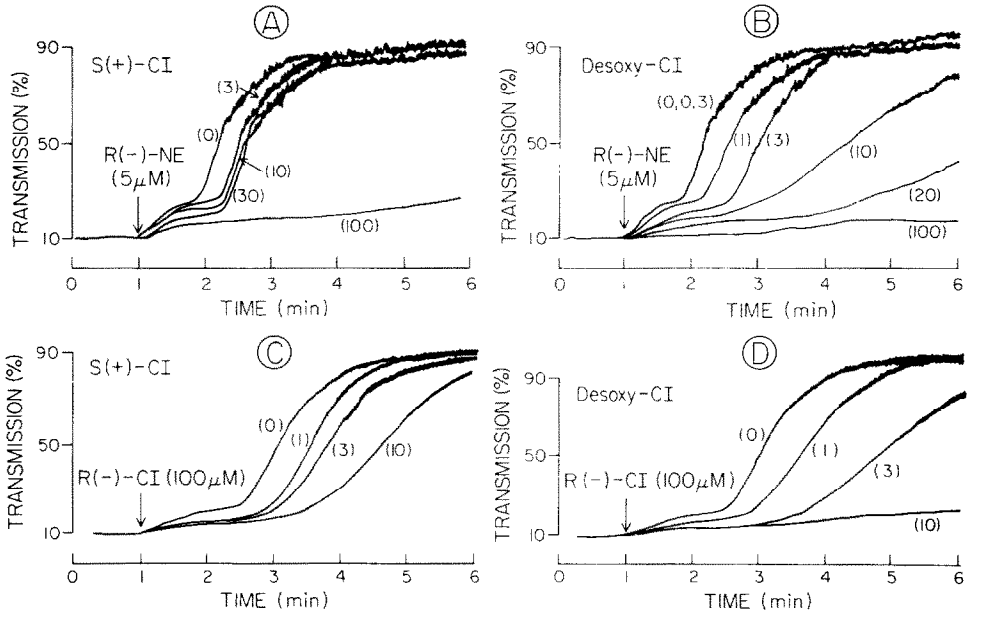

Fig. 4. Concentration-dependent inhibition of *R*(−)-NE- and *R*(−)-CI-induced human platelet aggregation by *S*(+)-CI and desoxy-CI. Key: (A) various concentrations of *S*(+)-CI against *R*(−)-NE, 5 μM; (B) various concentrations of desoxy-CI against *R*(−)-NE, 5 μM; (C) various concentrations of *S*(+)-CI against *R*(−)-CI, 100 μM; and (D) various concentrations of desoxy-CI against *R*(−)-CI, 100 μM. Various concentrations of *S*(+)-CI or desoxy-CI were added to platelets for 1 min before addition of inducer. These concentrations of *S*(+)- or desoxy-CI did not give any aggregatory response alone. Superimposed tracings of the aggregation responses to various concentrations of inhibitor are representative of data from three donors.

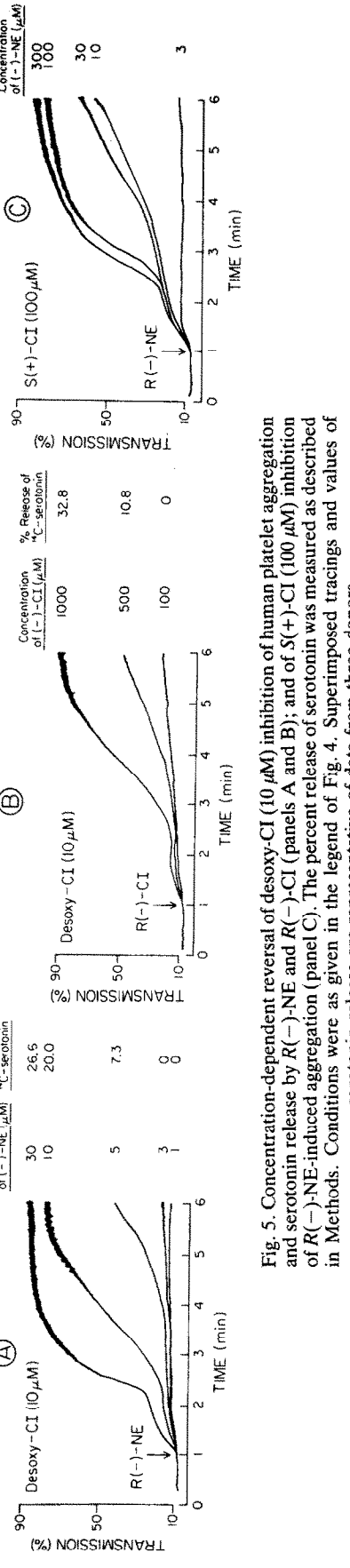

Fig. 5. Concentration-dependent reversal of desoxy-CI (10 μM) inhibition of human platelet aggregation and serotonin release by $R(-)$-NE and $R(-)$-CI (panels A and B); and of $S(+)$-CI (100 μM) inhibition of $R(-)$-NE-induced aggregation (panel C). The percent release of serotonin was measured as described in Methods. Conditions were as given in the legend of Fig. 4. Superimposed tracings and values of serotonin release are representative of data from three donors.

inhibitory effects of desoxy-CI on platelet function were reversed in a concentration-dependent manner by $R(-)$-NE and $R(-)$-CI at concentrations which greatly exceeded the EC_{50} values for these inducers. In other experiments, the blockade of $R(-)$-NE-induced aggregation by $S(+)$-CI was also reversed by high concentrations (3–300 μM) of $R(-)$-NE (Fig. 5C). The data indicate that desoxy-CI is a more potent inhibitor than $S(+)$-CI of aggregatory and secretory responses to these inducers.

To further assess the specificity of the anti-aggregatory actions of $S(+)$-CI and desoxy-CI, their effects on arachidonic acid (200 μM)-, U46619 (2 μM)- and ADP (5 μM)-induced platelet aggregation were examined. The aggregatory responses to these inducers were not blocked by the presence of 100 μM $S(+)$-CI or desoxy-CI (data not presented). This concentration of $S(+)$- or desoxy-CI produced a complete inhibition of the biphasic aggregation responses to $R(-)$-NE or $R(-)$-CI in these preparations. These data support the proposal that the antiaggregatory effects of these agents are selective for the antagonism of α-adrenoceptor-mediated platelet activation.

Comparative inhibitory potencies of CIs and selected α-adrenoceptor agents (phentolamine and clonidine) on R*(−)-NE and* R*(−)-CI-induced aggregation.* In PRP preparations, clonidine was either a weak inducer or did not cause aggregation in the concentration range of 0.01 to 1.0 mM. Both clonidine and phentolamine were tested as antagonists of aggregation responses to $R(-)$-NE and $R(-)$-CI in human platelets. As shown in Fig. 6, clonidine and phentolamine, like desoxy- and $S(+)$-CI, shifted the time–response relationship of aggregation to the right and were inhibitors of both the primary and secondary phases of aggregation to $R(-)$-NE and $R(-)$-CI. The data also indicate that phentolamine is much more effective as an inhibitor of platelet function than clonidine. Antagonism of $R(-)$-NE and $R(-)$-CI-induced aggregation by phentolamine and clonidine was reversed by these inducers in a concentration-dependent manner (data not presented).

Comparative potencies of selected compounds as inhibitors of R*(−)-NE- and* R*(−)-CI-induced platelet aggregation.* The comparative potencies of selected α-adrenoceptor agents, and the isomers and desoxy analogs of NE and CI are summarized in Table 1. In platelets responsive to the isomers and desoxy analogs of NE and CI, the rank order of stimulatory potency was $R(-)$-isomer > $S(+)$-isomer = desoxy analog. Both $R(-)$-NE and $R(-)$-CI were more potent than clonidine in these platelet preparations.

For platelet preparations that did not aggregate to the $S(+)$-isomer or desoxy analog of CI, their ability to block platelet activation by the $R(-)$-isomers of NE and CI was tested and compared with phentolamine and clonidine. Phentolamine, clonidine and CIs [desoxy-CI and S-(+)-CI] produced a parallel shift to the right in the concentration–response relationship of aggregation for $R(-)$-NE. The comparative pK_B values for phentolamine, clonidine, $S(+)$-CI and desoxy-CI against aggregation respones to $R(-)$-NE and $R(-)$-CI are summarized in Table 1. As presented, the rank order of inhibitory poten-

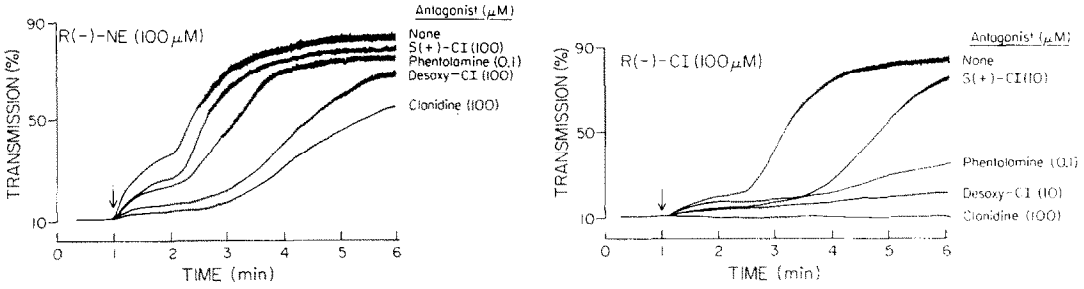

Fig. 6. Comparative inhibitory effects of $S(-)$-CI, desoxy-CI, phentolamine and clonidine against $R(-)$-NE (left panel) and $R(-)$-CI- (right panel) induced human platelet aggregation. Concentrations of each antagonist are given in the parentheses. Data of superimposed tracings are representative of at least three donors.

cies (K_B values; μM for these compounds against $R(-)$-NE induced aggregation was phentolamine (0.009) > clonidine (0.66) > desoxy-CI (2.2) > $S(+)$-CI (11.5). The α-adrenoceptor antagonist phentolamine showed a nearly identical potency for the inhibition of $R(-)$-CI-induced ($pK_B = 8.18$) aggregation as that for $R(-)$-NE ($pK_B = 8.05$). Further, the pA_2 value of 5.66 for desoxy-CI against $R(-)$-NE-induced aggregation was similar to the average pK_B (6.13) against $R(-)$-CI-induced aggregation (Table 1). In the experiments, the inhibitory potency (pK_B values) of various concentrations of desoxy- or $S(+)$-CI against aggregation responses to $R(-)$-CI was unchanged, suggesting that these compounds were competitive antagonists.

Table 1. pD_2 and pK_B values of selected α-adrenoceptor agents and isomers or desoxy analogs of norepinephrine (NE) and catecholimidazolines (CI) on human platelet aggregation

Drug (M)	pD_2^*	$pK_B^\dagger$
(I) Agonist activity		
$R(-)$-NE	5.88 ± 0.12	
$S(+)$-NE	4.72 ± 0.14	
Dopamine	4.65 ± 0.23	
$R(-)$-CI	5.20 ± 0.09	
$S(+)$-CI	4.69 ± 0.16	
Desoxy-CI	4.40 ± 0.13	
Clonidine	4.47 ± 0.28	
(II) Antagonist activity		
(A) Against $R(-)$-NE		
Phentolamine (10^{-7})		8.05 ± 0.08
Desoxy-CI (10^{-5})		5.57 ± 0.03‡
Desoxy-CI (5×10^{-5})		5.56 ± 0.09‡
Desoxy-CI (10^{-4})		5.73 ± 0.06‡
$S(+)$-CI (10^{-5})		5.16 ± 0.11
$S(+)$-CI (10^{-4})		4.72 ± 0.24
Clonidine (10^{-5})		6.18 ± 0.05
(B) Against $R(-)$-CI		
Phentolamine (10^{-7})		8.18 ± 0.04
Desoxy-CI (10^{-6})		6.25 ± 0.14
Desoxy-CI (10^{-5})		6.01 ± 0.18

Values are expressed as the mean ± S.E.M. (N = 3–9).

* pD_2 equals $-\log EC_{50}$.

† $pK_B = -\log \frac{\text{antagonist (M)}}{\text{concentration ratio} - 1}$

‡ pA_2 value ± S.E.M. of desoxy-CI against $R(-)$-NE was calculated as 5.66 ± 0.05.

DISCUSSION

Imidazolines are an important class of drugs which interact with α-adrenoceptors, and only recently have studies [3, 4] been carried out with optical isomers to test the validity of the Easson–Stedman hypothesis [1]. Optical isomers and corresponding desoxy forms of norepinephrine (NE) and 2-(3,4-α-trihydroxybenzyl) imidazoline (CI) were examined for their comparative interaction with α-adrenoceptors in human platelets. In certain human platelet preparations, all compounds gave a concentration-dependent aggregation response with a rank order of potency of $R(-)$-isomer > $S(+)$-isomer > desoxy analog within each chemical series. Only desoxy-CI was a partial stimulant of human platelet function, and this finding is in agreement with reports of this compound in other α_2-adrenoceptor systems [3, 4]. The stereoselective potency differences for the optical isomers of NE and CI were 14.5- and 3.3-fold, respectively (Table 1), confirming the importance of the benzylic hydroxy group for efficacy of NE and CI in this pharmacological system. These structure–activity relationships of CI in human platelets are also consistent with the Easson–Stedman hypothesis [1] for the interaction of catecholamines with adrenoceptor systems, and differ from results of the optical isomers and desoxy analogs of CI [3, 4] and other imidazolines [17, 18] in α_1- and α_2-adrenoceptor systems.

Several lines of functional evidence obtained in our studies show that the optically active CIs interact as agonists and/or antagonists of α-adrenoceptors in human platelets. They are: (1) the α-antagonist, phentolamine, blocked aggregatory responses to $R(-)$-NE and $R(-)$-CI with a nearly identical inhibitory potency; (2) both $S(+)$- and desoxy-CI blocked the aggregatory responses to $R(-)$-NE and $R(-)$-CI in a concentration-dependent and competitive manner; (3) the α_2-agonist, clonidine, behaved in an identical fashion to $S(+)$- and desoxy-CI in responsive versus nonresponsive platelet preparations; and (4) the stereoselective agonist potency difference of the optical isomers of CI [$R(-) > S(+)$] for stimulation of platelet aggregation was similar to those

reported for their interaction in other α-adrenoceptor systems [3, 17].

The observation that only the *R*(−)-isomers of NE and CI were able to produce aggregation in certain preparations suggests that differences may exist for the role of the benzylic hydroxy group for interaction with platelet α-adrenoceptors. In these platelet preparations, no stimulatory activity was seen with compounds either lacking this hydroxy group (dopamine or desoxy-CI) or possessing the hydroxy group in an *S*(+)-orientation. These results clearly show that the responses of platelets to isomers and desoxy analogs of CI and NE were donor dependent. In addition, our findings with clonidine are similar to previous reports of responsiveness or nonresponsiveness of human platelets to this α_2-adrenoceptor agonist [11]. We also observed a differential responsiveness of human platelet preparations to clonidine and the absence or presence of aggregation responses in human platelets to clonidine, and the *S*(+)-isomers and desoxy analogs of NE and CI were qualitatively consistent. Clonidine was also a more potent inhibitor of *R*(−)-NE-induced aggregation than either desoxy- or *S*(+)-CI (Table 1). These data support the view that the platelet responses to clonidine, and both chemical classes of drugs (catecholamines and CIs) are mediated by a similar mechanism involving α-adrenoceptors. Furthermore, the differential potencies of clonidine, and the optical isomers or desoxy forms of CI or NE on responsive or nonresponsive human platelet preparation need clarification and will be a subject of further study in our laboratory.

We also evaluated whether the *S*(+)-isomers and desoxy analogs of CI and NE were effective as antagonists of platelet activation by both *R*(−)-CI and *R*(−)-NE. Our results showed that these analogs were able to block both primary and secondary waves of aggregation and secretory responses to *R*(−)-NE and *R*(−)-CI, whereas responses to other inducers including biphasic aggregation to ADP, or aggregation to arachidonic acid and U46619 (a thromboxane A_2 agonist) were not modified by *S*(+)- or desoxy-CI. These data demonstrate that *S*(+)-CI or desoxy-CI inhibit *R*(−)-NE- and *R*(−)-CI-induced platelet activation at a site prior to arachidonic acid release. This inhibition by *S*(+)-CI and desoxy-CI is reversed in the presence of increasing concentrations of *R*(−)-NE or *R*(−)-CI, and is suggestive of a competitive-type of interaction with α-adrenoceptor sites in platelets. The corresponding inhibitory potencies of desoxy-CI against aggregatory responses to *R*(−)-NE and *R*(−)-CI were nearly identical, and these results further suggest that CIs and catecholamines interact at common α-adrenoceptor sites in human platelets. Like *S*(+)-CI and desoxy-CI, the α_2-adrenoceptor agonist, clonidine, also blocked the aggregatory responses to *R*(−)-CI and *R*(−)-NE in human platelets. Thus, the stimulatory and inhibitory actions of the CIs in human platelets are highly selective and presumably mediated by an interaction with the same α-adrenoceptors as NE.

Our studies also indicate that the potency of desoxy-CI is greater than either *S*(+)-CI or *R*(−)-CI in platelet preparations responsive only to *R*(−)-NE and *R*(−)-CI, even though this compound was functionally less active in platelet preparations which responded to all compounds. When the comparative inhibitory actions of *S*(+)-CI and desoxy-CI were examined in platelet preparations responsive only to *R*(−)-NE and *R*(−)CI, the rank order of potency for the blockade of *R*(−)-NE-induced aggregation was desoxy-CI ($pK_B = 5.66$) > *S*(+)-CI (pK_B) = 4.94). Also, the pD_2 value of *R*(−)-CI in these preparations was 5.2. This rank order of potency for CIs of desoxy-CI > *R*(−)-CI > *S*(+)-CI is identical to that reported by Ruffolo *et al.* [4, 17–19] and Miller *et al.* [3]. In our studies, the isomeric potency differences between isomers of CI were about 3-fold and are in good agreement with data of these isomers in other α_1- and α_2-adrenoceptor systems [3, 17]. Unlike *R*(−)-CI, we have also observed that *S*(+)-CI and desoxy-CI were either partial or full agonists, or antagonists of *R*(−)-NE-induced aggregation in platelets. Thus, *S*(+)-CI and desoxy-CI, along with clonidine, exhibited properties as partial agonists in human platelets. In contrast, Ruffolo *et al.* [4, 17, 18] reported only agonist activity for each of these CIs in other α-adrenoceptor tissues with a rank order of stimulatory activity as desoxy-CI ≥ *R*(−)-CI > *S*(+)-CI. Based upon these comparative structure–activity relationships, it is proposed that the interaction of CIs with α-adrenoceptor sites in human platelets may be different from those CIs on α_1- and α_2-adrenoceptor systems as reported by Miller *et al.* [3] and Ruffolo *et al.* [4, 17, 18]. Additional work with selective radioligands for α-adrenoceptors and further characterization of partial agonism with these compounds will be needed to fully resolve the qualitative and quantitative differences noted in the structure–activity relationships of these two chemical classes in α-adrenoceptor systems.

α-Adrenoceptor sites in human platelets have been characterized largely as α_2, and to a lesser degree as a mixture of α_1/α_2-subtypes [8–11]. In previous reports with these CIs, Ruffolo *et al.* [4, 18] indicated that imidazolines and catecholamines (e.g. NE) may interact with different sites on the α-adrenoceptor with different types of α-adrenoceptors or with one or two common sites on the α-adrenoceptor. Based upon our function studies with the use of *S*(+)-CI, desoxy-CI, clonidine and phentolamine as inhibitors of platelet activation by *R*(−)-NE and *R*(−)-CI, one may propose that these two chemical classes of compound interact with similar, if not identical, α-adrenoceptor sites. Moreover, the similarity in the functional effects of the optical isomers and desoxy analogs of CI and NE in human platelets suggests that there may be a commonality of the site of interaction for these compounds with a single class or with a mixed α_1–α_2-adrenoceptor population in platelets.

Our work also indicates, for the first time, that appropriate chemical modification of the imidazoline nucleus has lead to a compound possessing full α-adrenoceptor stimulatory activity in human platelets [e.g. *R*(−)-CI]. Earlier studies with imidazoline analogs, including clonidine, in platelets have shown these compounds to be either partial stimulants or inactive in platelets [10, 11]. With the availability of additional analogs, we will be able to gain further

insight into the stereochemical requirements of the catechol ring, basic amino nitrogen and benzylic hydroxy group for the interaction of CIs with α-adrenoceptor sites in platelets.

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