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RELATIONSHIPS BETWEEN THE CHEMICAL CONSTITUTION OF AGGREGATION INHIBITORS AND HUMAN BLOOD PLATELET RESPONSE PROFILE

ANDREW LASSLO *, RONALD P. QUINTANA **, RANDY W. JOHNSON, JEN L. NAYLOR and MARION DUGDALE

Department of Medicinal Chemistry and Department of Medicine, University of Tennessee Center for the Health Sciences, Memphis, TN 38163 (U.S.A.)

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Gradually altered synthetic entities were employed as molecular probes, and arachidonic acid, ADP, human α-thrombin and the Ca^{2+} ionophore A23187 as aggregation-inducing agents, in a comprehensive study on the response profile of human blood platelets with an emphasis on the effects of exogenous and increased intracellular Ca^{2+}. Corroborating further previous conclusions, some representative carbamoylpiperidine derivatives, at concentrations effecting substantial inhibition of ADP-induced aggregation, failed to retain that effect when 5.0 mM Ca^{2+} was introduced into the otherwise identical test medium; reference compounds chlorpromazine and propranolol registered corresponding inhibitory patterns. At increased concentrations the compounds' inhibitory potency was regenerated even in the presence of 5 mM Ca^{2+}. In fact, in sufficiently high concentrations, the compounds were even capable of inhibiting aggregation elicited by 15 μM of the ionophore A23187; so did chlorpromazine and propranolol. Another set of congeners revealed the striking sensitivity of ionophore A23187-induced human blood platelet aggregation to the surface active potencies of inhibitor molecules. The loss in inhibitory potency was directly related to the lesser hydrophobic character of the molecule.

Introduction

During the past few years, exploratory studies in our laboratories have disclosed significant relationships between the molecular constitution and physicochemical properties of a number of synthetic entities, and their effect on blood platelet function [1–12]. In this paper, we report the effect of several of our compounds upon a broader response profile of human blood platelets, (i) employing arachidonic acid, adenosine diphosphate (ADP), human α-thrombin and the Ca^{2+} ionophore A23187 as aggregation-inducing agents, and (ii) evaluating the influence of external and internal Ca^{2+}. As in our previous investigations, dominant emphasis was placed on the systematic and gradual change in the chemical structure of the evaluated entities to allow meaningful interpretation of platelet response patterns. Some of the work reported in this paper constitutes a part of a dissertation submitted by Randy W. Johnson to the Graduate School of Medical Sciences, at the University of Tennessee, in partial fulfillment of the requirements for the Ph.D. degree in medicinal chemistry.

Materials and Methods

The compounds listed below have been conceived, designed and synthesized in our laboratories, and have been discussed in our preceding

* To whom correspondence should be addressed.
** Present address: Optical Products Division, Alcon Laboratories, Inc., Fort Worth.

papers [1–5,9–15]: α,α′-bis[3-(*N*,*N*-diethylcarbamoyl)piperidino]-*p*-xylene dihydrobromide (I); 1,6-bis[3-(*N*,*N*-diethylcarbamoyl)piperidino]hexane dihydrobromide (II); 1-decyl-3-(*N*,*N*-diethylcarbamoyl)piperidine hydrobromide (III); 1-hexyl-3-(*N*,*N*-diethylcarbamoyl)piperidine hydrobromide (IV); *N*,*N*′-bis(1-decylnipecotoyl)piperazine dihydriodide (V); 1,2-bis[*N*-(1-hexylnipecotoyl)-*N*-methylamino]ethane dihydriodide (VI); 1,6-bis[*N*-(1-hexylnipecotoyl)-*N*-butylamino]hexane dihydriodide (VII); 1,6-bis[*N*-(1-decylnipecotoyl)-*N*-methylamino]hexane dihydriodide (VIII); 1,2-bis[*N*-(1-decylnipecotoyl)-*N*-methylamino]ethane dihydriodide (IX); 1,6-bis[*N*-(1-decylnipecotoyl)-*N*-butylamino]hexane dihydriodide (X); 1-hexyl-6-hydroxy-1,2,3,4-tetrahydroquinoline hydriodide (XII); 1-decyl-6-hydroxy-1,2,3,4-tetrahydroquinoline hydriodide (XIII); 1,10-bis(6-hydroxy-1,2,3,4-tetrahydroquinolino)decane dihydriodide (XIV); α,α′-bis(6-hydroxy-1,2,3,4-tetrahydroquinolino)-*p*-xylene dihydrobromide (XV); α,α′-bis(1,2,3,4-tetrahydro*iso*quinolino)-*p*-xylene dihydrobromide (XVI); 1,6-bis[*N*-(1-methylnipecotoyl)amino]hexane dihydriodide (XVII).

N,*N*′-Bis(1-hexylnipecotoyl)piperazine dihydriodide (XI) was prepared, via its quaternary analog, employing the synthetic sequence described earlier in procedures C and B_4 of our preceding paper [1]. After recrystallization from absolute alcohol, the quaternary compound melted at 252.0–253.0°C (uncorr.) (yield 20.3%). Anal *. Calc'd. for $C_{28}H_{42}N_4O_2I_2$: C, 46.68%; H, 5.88%; N, 7.78%; I, 35.23%. Found: C, 46.74%; H, 5.81%; N, 7.78%; I, 35.37%. Recrystalized from absolute alcohol, the target compound (XI) melted at 245.5–247.0°C (uncorr.) (yield 39.7%). Anal. Calc'd. $C_{28}H_{54}N_4O_2I_2$: C, 45.91%; H, 7.43%; N, 7.65%; I, 34.65%. Found: C, 45.72%; H, 7.49%; N, 7.58%; I, 34.89%.

1,6-Bis[*N*-(1-methylnipecotoyl)-*N*-methylamino]hexane dihydriodide (XVIII) was also obtained by means of procedures C and B_4 reported in the referenced paper [1]. The absolute-alcohol-washed quaternary intermediate melted at 141.0–142.0°C (uncorr.) (yield 96.6%). Anal. Calc'd. for $C_{22}H_{32}N_4O_2I_2$: C, 41.40%; H, 5.05%; N, 8.78%; I, 39.76%. Found: C, 41.29%; H, 5.12%; N, 8.66%; I, 39.69%. After removal of residual moisture by azeotroping with absolute alcohol, the target compound (XVIII) melted at 115.0–116.0°C (uncorr.) (yield 92.2%). Anal. Calc'd. for $C_{22}H_{44}N_4O_2I_2$: C, 40.63%; H, 6.82%; N, 8.61%; I, 39.02%. Found: C, 40.40%; H, 6.95%; N, 8.58%; I, 39.11%.

Chlorpromazine hydrochloride (Cat. No. C-8138/Lot No. 71F-7704), dipyridamole (Cat. No. D-9766/Lot No. 121F-0070), nifedipine (Cat. No. N-7634/Lot No. 102F-0859), propranolol hydrochloride (Cat. No. P-0884/Lot No. 80F-0190) and tetracaine hydrochloride (Cat. No. T-7508/Lot No. 41F-0218) were obtained from the Sigma Chemical Co. of St. Louis, MO, and 8-(diethylamino)octyl 3,4,5-trimethoxybenzoate (TMB-8) (Cat. No. 86,180-4/Lot No. 03222 C-J) and aspirin (Cat. No. 13,292-6/Lot No. 092777) from the Aldrich Chemical Co. of Milwaukee, WI. Trifluoperazine dihydrochloride (SKF, No. 5019A_2/IS0 No. 202126/Code 5-5TFD) was a gift from the Smith Kline and French Laboratories of Philadelphia, PA and sulfinpyrazone (SN-82047) was donated by the Ciba-Geigy Corporation. All were acquired in reagent grade.

The sodium salt of adenosine 5′-(trihydrogen diphosphate) (ADP) and its preparation for use as an aggregation-inducing agent was described earlier [1,5]. The highly purified human α-thrombin and its application were also characterized in one of these papers [1]. Calcium chloride dihydrate, acquired in reagent grade from the Fisher Scientific Co. of St. Louis, MO, was employed in studying effects of external Ca^{2+}; a 5.0 M solution in redistilled water was prepared fresh each week and stored at 0°C until use. The calcium ionophore A23187 (calimycin) was procured from Sigma, St. Louis, MO; a 10 mM stock solution in absolute alcohol was prepared and stored at −80°C until use. The sodium salt of arachidonic acid was obtained in > 99% purity from Nu-Chek Prep., Elysian, MN; the contents of the sealed tube were distributed into 0.01632-g portions, carefully weighed into the nitrogen atmosphere of polypropylene tubes, and stored at −80°C, for the eventual preparation of 10 mM solutions and subsequent working dilutions (1–9 mM) in modified Tyrode's buffer [5].

* All analyses were carried out by the Galbraith Laboratories, Knoxville, TN, U.S.A.

Aggregometric determinations were performed at least in duplicate, with plasma acquired from different donors, employing the turbidimetric procedure; the methodology and technique have been described in the previously referenced papers [1,5]. In these experiments the minimal concentration of ADP eliciting maximal biphasic aggregation averaged 4.2 ± 1.8 μM for 129 plasma samples. A final concentration of 1.0 unit of human α-thrombin/ml of platelet-rich plasma was used here, also, to induce platelet aggregation in each instance (60 plasma samples). In stimulating platelets with arachidonic acid, the protocol delineated for ADP [1,5] was followed; the lowest concentration initiating prompt maximal aggregation (within 30 ± 20 s) averaged 0.7 ± 0.17 mM sodium arachidonate for 75 plasma samples. Employing the ionophore, again the ADP protocol [1,5] was adapted but a final ionophore concentration of 15 μM was used instead in all runs (107 plasma samples); since it required 0.75 μl [8] of the 10 mM stock solution (in absolute alcohol) to effect that concentration, evaluated compounds were introduced into the test system in up to 20 μl of redistilled water and the determinations checked against corresponding controls with the respective additional μl of redistilled water. The protocol for the evaluation of the effect of external Ca^{2+} on the aggregation-inhibitory potency of our compounds also followed, basically, that employed in our conventional procedure for the study of inhibitors in ADP-induced aggregation [1,5]; in this instance, however, exactly 1 min prior to introducing the evaluated compound in its solution, 0.5 μl of the 5.0 M solution of calcium chloride (in redistilled water) was injected into the platelet rich plasma test medium yielding a final 5 mM concentration of Ca^{2+}; here, the minimal concentration of ADP eliciting maximal biphasic aggregation averaged 4.7 ± 2.1 for 82 plasma samples.

Results and discussion

We have discussed previously platelet aggregation-inhibitory specific amine functions in our series of compounds, in which ancillary molecular components optimized hydrophobicity for their penetration of the platelet plasma membrane's lipid bilayer [1,2,5]. In summary, these tertiary alicyclic amine functions are subject to broad variances in protonation, contingent upon the pH of their immediate vicinity and upon the specific compounds' pK_a values; this, in turn, enables them (in suitably structured molecules) to assume appropriate hydrophobic character for the penetration of the platelet membrane's lipid bilayer without interfer-

TABLE I

BLOOD PLATELET FUNCTION RESPONSE PROFILE OF CARBAMOYLPIPERIDINE DERIVATIVES AND SOME REFERENCE COMPOUNDS

n.d., not determined.

Compound		% Inhibition (potentiation) effected in aggregation induced by			
No.	Concentration (μM)	Arachidonic acid	ADP	ADP with 5.0 mM Ca^{2+}	Ca^{2+} ionophore A23187
I	10	1 ± 7	46 ± 2	10 ± 4	(3 ± 4)
II	25	5 ± 5	31 ± 2	8 ± 2	(2 ± 7)
III	50	(5 ± 7)	23 ± 6 [a]	8 ± 4	(7 ± 2)
IV	100	4 ± 5	24 ± 4 [a]	9 ± 1	(2 ± 3)
Propranolol-HCl	50	4 ± 3	31 ± 3	7 ± 4	2 ± 2
Chlorpromazine-HCl	50	5 ± 6	43 ± 1	11 ± 1	1 ± 7
TMB-8-HCl	50	5 ± 2	8 ± 0	16 ± 0	4 ± 1
Aspirin	50	100 ± 0	24 ± 2 [b]	20 ± 1	(5 ± 8)
Aspirin	20	100 ± 0	n.d.	n.d.	n.d.

[a] From Quintana et al. [5].
[b] From Quintana et al. [2].

ing, subsequently, with their transformation into corresponding cations [5]. The penetrated amines can then be envisioned to generate their cationic species in quantities capable of (i) interacting with and reducing the response-sensitivity of anionic phospholipids, (ii) stabilizing, thereby, membrane complexes of the dense tubular system and of other storage sites sequestering calcium in platelets and, consequently, (iii) impeding or blocking mobilization of additional Ca^{2+} into the cytosol by conventional stimuli [3,4,9,10]. This leads to an elevation in the threshold required for triggering or sustaining platelet aggregation, and only stimuli of considerably greater intensity could actuate the process [10].

This rationale is further corroborated by data reported in this paper. Our carbamoylpiperidine compounds I–IV listed in Table I are among those [5] embraced by the mechanism of action outlined above. Their inhibitory potency of ADP-induced aggregation and its relationship to their chemical structure [5], to their surface-active characteristics [3,4], and to their interaction with anionic phospholipids [4] was reported earlier. In the most recent studies, we were particularly interested in examining the impact of externally added and intracellularly released Ca^{2+} with respect to our compounds' potency in inhibiting conventional ADP-induced human blood platelet aggregation. As anticipated, all four representative compounds of our carbamoylpiperidine series [5] failed to retain their inhibitory effect in ADP-stimulated platelets at the indicated concentrations when 5.0 mM Ca^{2+} was introduced into the otherwise identical test medium and, correspondingly, neither registered any inhibition of the Ca^{2+} ionophore A23187-induced aggregation (Table I). The data on propranolol, chlorpromazine, TMB-8 and aspirin have been included for reference purposes. Assessments concerning free Ca^{2+} concentration in the cytosol of the resting platelet range from 0.1 μM [16,17] to 6 μM [18]. Feinberg and LeBreton [18] attribute ADP-activation of platelets to a stimulus transfer implicating Na^{+} and leading to redistribution of bound intracellular calcium into cytosolic Ca^{2+}. White [19] believes that ionophore A23187-mediated Ca^{2+} relocation into platelet cytoplasm is, alone, sufficient to effect morphological processes identical to those elicited by platelets stimulated with release-inducing agents and, according to Feinstein and Walenga [20], "it could be assumed that the magnitude and nature of the

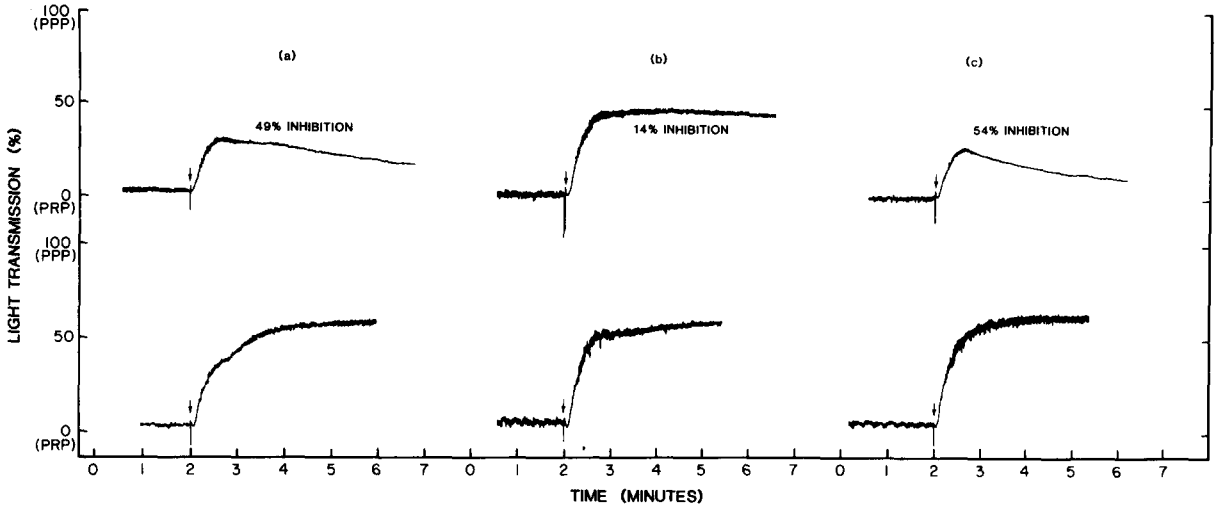

Fig. 1. The effect of exogenous Ca^{2+} on the inhibitory potency of compound I in ADP-induced aggregation; (a) 10 μM of compound I in conventional ADP-induced aggregation, (b) 10 μM of compound I in ADP-induced aggregation with 5 mM Ca^{2+} added exactly 1 min prior to introducing the compound, (c) 25 μM of compound I in ADP-induced aggregation with 5 mM Ca^{2+} added exactly 1 min prior to introducing the compound. Arrows indicate addition of 4 μM ADP. Tracings at the bottom are controls with matching ethanol content (0.5 μl 95% EtOH). Blood donors in these experiments were R.W.J. (a), T.J.W. (b) and C.R.G. (c). (Refer to experimental details in text.)

response to the ionophore is a function of the amount of internal Ca^{2+} that is mobilized." Consequently, it should not be surprising that a substantial increase in Ca^{2+} concentration, corresponding to stimuli of considerably greater than conventional intensity, could reduce or nullify the aggregation-inhibitory potency of our compounds registered at Ca^{2+} concentrations prevailing under conventional ADP-induced aggregation. Reference compounds chlorpromazine and propranolol evidenced similar effects under parallel conditions (Table I). Neither should it be surprising that higher concentrations of the same compounds regenerate their inhibitory effects at increased Ca^{2+} levels (Fig. 1). Accordingly, at 50 μM concentrations compounds I and II effected $77 \pm 6\%$ and $34 \pm 2\%$ inhibition in ADP-induced aggregation in the presence of 5.0 mM Ca^{2+}; and at 5000 μM concentrations they elicited, respectively, $42 \pm 2\%$ and $17 \pm 1\%$ inhibition of aggregation generated by 15 μM of ionophore A23187 (Table IV). Reference compounds chlorpromazine and propranolol also inhibited the ionophore-induced aggregation at higher concentrations (Table IV). It is especially noteworthy that our carbamoylpiperidine derivatives retained their relative strengths within the context of structure-activity interpretations delineated earlier [2,5]. Here, too, the molecule in which the platelet aggregation-inhibitory specific amino functions are spaced optimally (at approx. 8 Å) for interaction with platelet aggregation-inhibitory specific target sites is substantially stronger [9]. This congener, the α,α'-bis[3-(*N*,*N*-diethylcarbamoyl)piperidino]-*p*-xylene (I), the most potent of this series, is a relatively close analog of the corresponding but weaker bis(diethylcarbamoylpiperidino)hexane (II); in I a phenylene moiety is replacing four of II's six methylene units linking the same two carbamoylpiperidine components [5]. The two ring nitrogens within the rigid xylylene-linked congener (I) are locked into an interatomic distance of 7.8–8.3 Å with a flexibility variance of 0.5 Å, compared to the 4.4–9.1 Å range between the corresponding nitrogen atoms in the hexane-linked derivative (II) in which the flexibility variance is practically tenfold greater (4.7 Å) due to the hexamethylene chain's buckling capacity [5]. Overall, it is quite apparent that the concentration gradients of free Ca^{2+} and of the inhibitor are heavily instrumental in determining platelet function response.

The concentration gradient appears to be an equally important factor in the inhibition of arachidonic-acid-induced aggregation and, interestingly, the most potent compound in this series (I) was capable of exerting 100% blockage when its concentration was increased to a sufficiently high level (50 μM); similarly, at sufficiently high levels, chlorpromazine (250 μM) and propranolol (250 μM) registered 100% inhibition, as well.

TABLE II

BLOOD PLATELET FUNCTION RESPONSE PROFILE OF BIS(1-ALKYLNIPECOTOYLAMINO)ALKANE AND BIS(1-ALKYLNIPECOTOYL)PIPERAZINE DERIVATIVES

Compound		% Inhibition (potentiation) effected in aggregation induced by			
No.	Concentration (μM)	Arachidonic acid	ADP	ADP with 5.0 mM Ca^{2+}	Ca^{2+} ionophore A23187
V	8	4 ± 2	48 ± 2	38 ± 7	3 ± 4
VI	15	1 ± 1	41 ± 1	35 ± 3	(9 ± 2)
VII	20	(2 ± 7)	46 ± 6	32 ± 3	4 ± 1
VIII	25	2 ± 2	45 ± 4	41 ± 5	8 ± 8
IX	25	(3 ± 3)	57 ± 1	39 ± 2	3 ± 0
X	50	4 ± 4	39 ± 2 [a]	24 ± 2 [b]	(9 ± 1)
XI	50	3 ± 3	46 ± 2	36 ± 3	(8 ± 7)

[a] From Lasslo et al. [1].

[b] Among the five volunteers on whose platelets these experiments were carried out, two averaged (in two runs each) 10 ± 8 values. These findings appear to parallel responder/non-responder-type relationships. Corresponding effects were not observed, in this instance, with the aggregating agent acting through the release of internal Ca^{2+}. (See also Tables III and IV.)

Some of the antiplatelet properties of our bis(alkylnipecotoyl)piperazine and related ethylenediamine and hexamethylenediamine congeners (V–XI) cited in Table II have been also discussed extensively in a previous paper [1]. These entities, with a set of common structural denominators different from those in Table I, were comparatively less affected in their inhibitory potency of ADP-induced aggregation by externally added Ca^{2+}; like those in Table I, they failed to block aggregation mediated by intracellularly released Ca^{2+} at the concentrations listed in Table II. In another similar trend, at higher concentrations (50 μM), the effect of the more potent of these inhibitors was not reduced by exogenous 5.0 mM Ca^{2+} (e.g., V, 96 $\pm$ 2%; VI, 76 $\pm$ 5%; VII, 87 $\pm$ 2%; IX, 88 $\pm$ 4%). However, the observation which merits particular attention is the unique potency of compound V in inhibiting (76 $\pm$ 4% at 50 μM; see Fig. 2) aggregation generated by intracellularly released Ca^{2+} which appears to be among the strongest antagonisms of ionophore A23187-induced human blood platelet aggregation (Table IV) hitherto reported. Even its considerably weak congener (XI) is substantially stronger than TMB-8, and parallels in potency to propranolol and chlorpromazine (Table IV). In addition to the postulates advanced at the beginning of this discussion, one can not fail to discern features in the chemical constitution of this series of compounds (Fig. 1 in Ref. 1), common in ethylenediamine derivatives bearing carbonyl functions and in related entities, which could be instrumental in exerting chelating effects. The pivotal action, however, is associated with the tertiary amines [9,10] structured around these compounds' two ring nitrogens. We have been emphasizing the significance of hydrophobicity in molecules affecting platelet function [1,3–5,9]; and here, again, is an excellent illustration of the pivotal influence exerted by the hydrophobic vector. The massively greater potency of compound V compared to that of its congener XI in inhibiting the

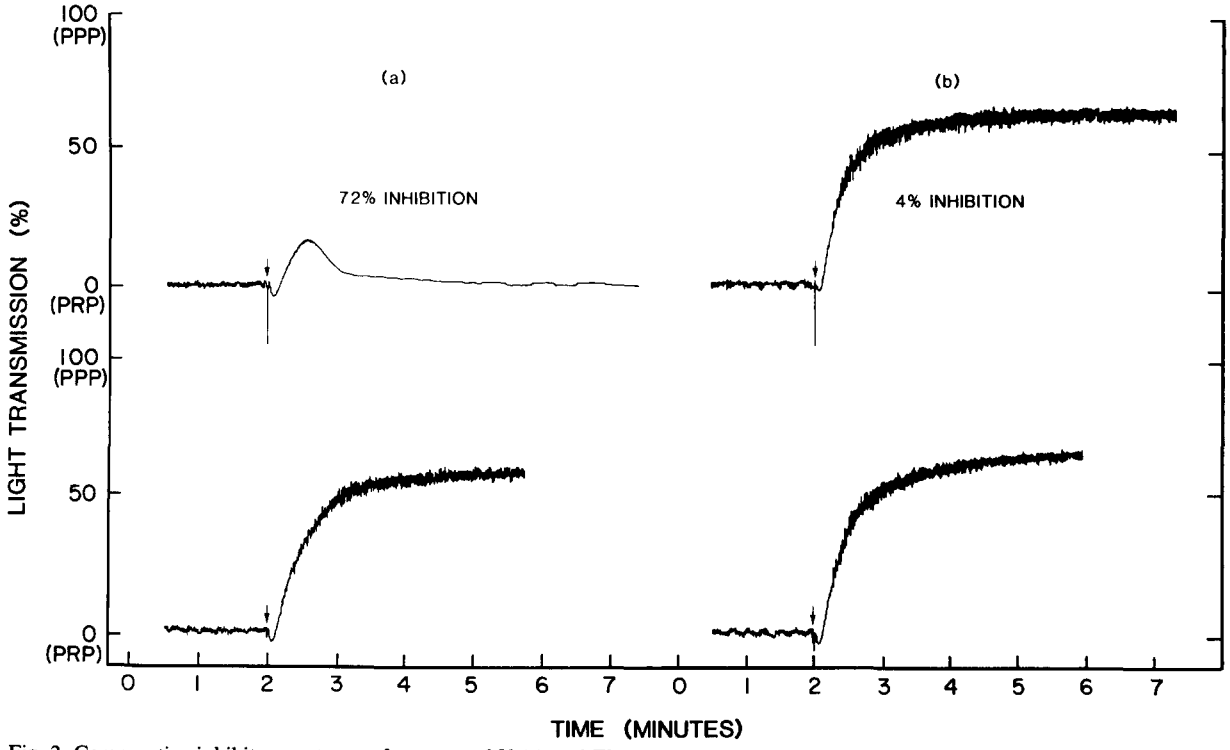

Fig. 2. Comparative inhibitory potency of compound V (a) and TMB-8 (b), at 50 μM concentrations, on ionophore A23187-induced aggregation. Arrows indicate addition of 15 μM Ca^{2+} ionophore A23187 in 0.75 μl of absolute ethanol. Tracings at the bottom are controls with the additional 20 μl of water. Blood donors in these experiments were D.E.C. (a) and D.R.B. (b). (Refer to experimental details in text.)

TABLE III

BLOOD PLATELET FUNCTION RESPONSE PROFILE OF 1,2,3,4-TETRAHYDROQUINOLINE AND -ISOQUINOLINE DERIVATIVES

Compound		% Inhibition (potentiation) effected in aggregation induced by			
No.	Concentration (μM)	Arachidonic acid	ADP	ADP with 5.0 mM Ca^{2+}	Ca^{2+} Ionophore A23187
XII	50	100±0	37±1 [a]	18±6	10±5 [b]
XIII	50	100±0	33±2 [a]	32±8	(6±2)
XIV	50	100±0	26±0 [a]	19±1	(2±3)
XV	50	100±0	22±3 [a]	30±4	(4±2)
XVI	50	3±2	(4±5) [a]	(2±6)	(4±5)

[a] From Lasslo et al. [1].

[b] Among the four volunteers on whose platelets these experiments were carried out, two averaged (in two runs each) 40±9 values. These findings appear to parallel responder/non-responder-type relationships. (See also Tables II and IV.)

ionophore-induced aggregation (Table IV) must be attributed to the four additional methylene units in each of the former's two *N*-alkyl substituents, the only difference between the two molecular structures; and it is obvious that the two *N*-hexyl radicals in XI can not exert a hydrophobic influence comparable to that of the two *N*-decyl radicals in V (cf. Refs. 2, 5). Again, the more potent derivatives in this series (e.g., V, VII, IX) effected 100% inhibition in arachidonic-acid-induced aggregation when their concentration was raised to 50 μM.

Our tetrahydroquinolinoalkanes (XII–XVI) listed in Table III, too, were the subject of earlier platelet aggregation-inhibitory studies [1,9]. In this instance, exogenous Ca^{2+} did not reduce generally the inhibitory potency of these derivatives in ADP-induced aggregation; and, at the concentrations specified in Table III, none of them affected aggregation generated by internally released Ca^{2+}. The exception involving the tangible decrease in the inhibition of the hydroxytetrahydroquinolinohexane (XII), in the presence of 5.0 mM exogenous Ca^{2+}, could be explained (i) by its lesser hydrophobicity compared to that of the corresponding decane analog (XIII) in which the potential for effective hydrophobic bonding is enhanced by the additional four methylene units [3,5], and (ii) by its limitation to a single aggregation-inhibitory specific amine function compared to two such functions in each of compounds XIV and XV [1,3,5]. The inactivity of compound XVI is not unexpected, since we have concluded earlier [1,9] that the tertiary amines in the bicyclic moieties are blocked from interacting with aggregation-inhibitory specific target sites by the considerable bulk of the rigid aromatic structures fused to the piperidine rings. It has been envisioned, therefore, that the action exerted by this series of compounds could be effected through

TABLE IV

OVERVIEW OF % INHIBITION (OR POTENTIATION) EXERTED BY SOME OF OUR DERIVATIVES AND SEVERAL REFERENCE COMPOUNDS ON Ca^{2+} IONOPHORE A23187-INDUCED AGGREGATION

n.d., not determined.

Compound	Concentration (μM)			
	50	250	500	5000
V	76±4	n.d.	n.d.	n.d.
XI	(8±7)	90±1	n.d.	n.d.
Chlorpromazine-HCl	(1±7)	85±3	96±4	n.d.
Propranolol-HCl	4±3	88±8	93±1	n.d.
TMB-8-HCl	4±1	31±2	81±4	n.d.
XV	(4±2)	7±7	74±4	n.d.
XII	40±9 [a]	n.d.	49±2	n.d.
I	(19±1)	n.d.	4±4	42±2
II	n.d.	n.d.	n.d.	17±1
Aspirin	(5±8)	n.d.	n.d.	1±0

[a] Among the four volunteers on whose platelets these experiments were carried out, two averaged (in two runs each) 10±5 values. These findings appear to parallel responder/non-responder-type relationships. (See also Tables II and III, and refer to text.)

their phenolic hydroxyl functions by enhancing membrane fluidity as suggested by Steiner [21] for vitamin E; compound XVI is void of such functional groups. Moreover, as earlier data hinted [1,9], the compounds are quite effective in inhibiting arachidonic-acid-induced aggregation.

Responder/non-responder type relationships were observed for compound X (see Table II, footnote b) and for compound XII (see Table III, footnote b). It is interesting that, among all the compounds discussed in this paper, only these two registered such characteristics. The fact that 50 μM concentrations of XII (an otherwise not particularly potent compound) exerted $40 \pm 9\%$ inhibition of ionophore A23187-induced aggregation does merit attention, even if this effect was limited only to some donors' platelets.

To provide a meaningful perspective of influences exerted on ionophore A23187-induced aggregation by some of our derivatives and several reference compounds, an overview is presented in Table IV. It is obvious that the inhibitory potencies of compounds V and XI exceed that of TMB-8, propranolol and chloropromazine, and that compound V has a strikingly strong blocking effect (vide supra). There is a level at which compounds I and II also inhibit aggregation induced by internally released Ca^{2+}, even though at much higher concentrations. It should be noted, however, that aspirin does not reflect any inhibitory tendency even at 5000 μM concentration.

For the reader's convenience, Table V summarizes inhibitory potencies in ADP- and thrombin-induced aggregations, including those published earlier, and lists the effects of a number of reference compounds determined under identical conditions. In studying the data generated by compounds V–XI, at 50 μM concentrations, we noted a remarkable, hitherto unpublished, correlation

TABLE V

OVERVIEW OF INFLUENCES EXERTED ON ADP- AND THROMBIN-INDUCED AGGREGATION BY SOME OF OUR DERIVATIVES AND SEVERAL REFERENCE COMPOUNDS

Compound	Concn. (μM)	% Inhibition (potentiation) effected in aggregation induced by	
		ADP	Thrombin
I	50	66 ± 1 [a]	82 ± 2
II	50	51 ± 3 [a]	31 ± 6
III	50	23 ± 6 [a]	13 ± 3
IV	100	24 ± 4 [a]	4 ± 0
V	50	94 ± 2 [b]	89 ± 1 [b]
VI	50	61 ± 2 [b]	53 ± 2 [b]
VII	50	77 ± 6 [b]	71 ± 2 [b]
VIII	50	86 ± 4 [b]	88 ± 4 [b]
IX	50	83 ± 1 [b]	68 ± 2 [b]
X	50	39 ± 2 [b]	20 ± 4 [b]
XI	50	46 ± 2	25 ± 4
XII	50	37 ± 1 [b]	19 ± 1
XIII	50	33 ± 2 [b]	12 ± 3
XIV	50	26 ± 0 [b]	(5 ± 5)
XV	50	22 ± 3 [b]	(5 ± 3)
XVI	50	(4 ± 5) [b]	0 ± 0
Propranolol-HCl	50	31 ± 3	2 ± 6
Chlorpromazine-HCl	50	43 ± 1	11 ± 3
TMB-8-HCl	50	8 ± 0	11 ± 1
Aspirin	50	24 ± 2 [c]	13 ± 4 [b]
Trifluoperazine-2HCl	50	52 ± 4	9 ± 9
Tetracaine-HCl	50	6 ± 3	11 ± 3
Dipyridamole	100	8 ± 1	3 ± 3
Nifedipine	50	7 ± 7	2 ± 4
Sulfinpyrazone	100	(2 ± 2)	8 ± 5

[a] From Quintana et al. [5].
[b] From Lasslo et al. [1].
[c] From Quintana et al. [21].

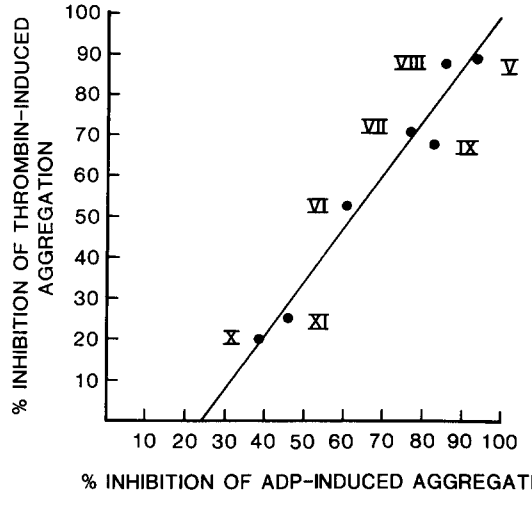

Fig. 3. Correlation between the inhibitory effects of bis(1-alkylnipecotoylamino)alkane and bis(1-alkylnipecotoylamino)piperazine derivatives on ADP- and thrombin-induced aggregation. All evaluations were carried out at 50 μM compound concentrations. (Refer to Table V for specific values.)

between the results obtained with these two aggregation-inducing agents (Fig. 3); employing the method of least squares [22], the Pearson product-moment correlation coefficient was computed according to Kirk [23] at $r = 0.981$!

In one of our previous papers [1] we pointed out that semi- or unsubstituted amides are generally less active than fully substituted ones since the amide-H is likely to be involved in hydrogen bonding among the compound's molecules, adversely affecting platelet aggregation-inhibitory potency; consequently, such N-H functions could impede or eliminate the sought biologic action. Compound XVIII was prepared to further corroborate this premise: In it, both amide-H functions of compound XVII ($17 \pm 2\%$ inhibition at 500 μM) have been replaced with amide-CH_3 groups; this was the only modification, and it increased the inhibitory potency of XVIII ($58 \pm 3\%$ at 500 μM) by more than 200% in ADP-induced human blood platelet aggregation. While one hastens to add that XVIII is far from being a strong inhibitor, especially with respect to its other congeners discussed in this paper, it does confirm our previously advanced interpretation [1] that amide-H functions are capable of interfering with the effectiveness of compounds designed for blocking ADP-induced human blood platelet aggregation.

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