membrane. Alternatively, the different patterns could be due to physical differences in individual virions such as those that determine the ability of a virion to cause hemolysis (8).

In summary, Sendai virus can fuse with liposomes containing only PC, cholesterol, and gangliosides. This suggests that gangliosides may have a role in membrane fusion in addition to their receptor function. In liposomes of this simple composition, the course of disassembly is variable.

This work was supported by grant AI-15540 from the National Institutes of Health and grant PCM 78-08931 from the National Science Foundation.

Received for publication 22 May 1981.

REFERENCES

1. Haywood, A. M. 1974. Fusion of Sendai viruses with model membranes. *J. Mol. Biol.* 87:625–628.
2. Haywood, A. M. 1975. "Phagocytosis" of Sendai virus by model membranes. *J. Gen. Virol.* 29:63–68.
3. Haywood, A. M., and B. P. Boyer. 1981. Initiation of fusion and disassembly of Sendai virus membranes into liposomes. *Biochim. Biophys. Acta.* 646:31–35.
4. Haywood, A. M. 1974. Characteristics of Sendai virus receptors in a model membrane. *J. Mol. Biol.* 83:427–436.
5. Bunow, M. R., and B. Bunow. 1979. Phase behavior of ganglioside-lecithin mixtures. *Biophys. J.* 27:325–337.
6. Maggio, B., F. A. Cumar, and R. Caputto. 1978. Induction of membrane fusion by polysialogangliosides. *Fed. Eur. Biochem. Soc. Lett.* 90:149–152.
7. Howe, C., and C. Morgan. 1969. Interactions between Sendai virus and human erythrocytes. *J. Virol.* 3:70–81.
8. Homma, M., K. Shimizu, Y. K. Shimizu, and N. Ishida. 1976. On the study of Sendai virus hemolysis. *Virology.* 71:41–47.

HUMAN BLOOD PLATELET AGGREGATION-INHIBITORY TARGET SITES ASSUMED TO INVOLVE MEMBRANE PHOSPHOLIPIDS

R. P. QUINTANA, A. LASSLO, AND M. DUGDALE,
Departments of Medicinal Chemistry and Medicine, University of Tennessee Center for the Health Sciences, Memphis, Tennessee 38163 U.S.A.

In an extensive study identifying structural features associated with the inhibition of adhesion-release-aggregation chain reactions in human blood platelets, we employed a series of carbamoylpiperidine derivatives with systematic and gradual changes in their molecular constitution, allowing meaningful interpretation of biological response variances. Current indications are that the entities conceptualized and synthesized in our laboratories (1–6) inhibit platelet aggregation (culminating at 5 μM concentrations) by penetrating the lipid bilayer of the platelet membrane and by interacting, as cations, with negatively charged phospholipids (e.g., phosphatidylserine and phosphatidylinositol) within the bilayer's inner segment (7). Following such penetration, those cations could be interfering with phospholipase-A_2 activation by counteracting stimulus-induced mobilization of Ca^{++} ions and Ca^{++}-dependent phospholipase-A_2 activity (8), thereby rendering platelets less susceptible to aggregation reactions. Our studies, employing our mono- and bis(carbamoylpiperidino)alkanes and -aralkanes as molecular probes, suggest platelet aggregation-inhibitory target sites spaced at 8 Å, and yield exacting information on the influence of hydrophobicity, planarity and geometric isomerism, as well as on the significance of interatomic distances between, and the charge levels of, the amino functions primarily instrumental in consummating interactions leading to the inhibition of human blood platelet aggregation.

To confirm these interpretations, the effects of our carbamoylpiperidine derivatives are being evaluated at surface pressures estimated to occur in actual platelet membranes (34 mN m^{-1})(9), on monomolecular films of phosphatidylserine whose location in the inner segment of the platelet membrane's lipid bilayer is acknowledged, on those of phosphatidylcholine, which is known to be a constituent of the bilayer's outer leaflet, and on related pure and mixed monolayer systems; e.g., cholesterol/phospholipid in ratios of 0.5, paralleling those observed in platelet membranes (10).

MATERIALS AND METHODS

A Payton aggregometer (Payton Associates Inc., Buffalo, NY) with a dual channel recorder (PF10H0-D) and a Coulter counter ZBI (Coulter Electronics Inc., Hialeah, FL) were employed in our platelet aggregation determinations (11); the inhibitory potency of our compounds was classified: 0 ≤ 10%, 1+ = 11%–19%, 2+ = 20%–29%, 3+ = 30%–39%, 4+ = 40%–49%, 5+ = 50%–59%, 6+ ≥ 60%. Interfacial and surface tension values were determined (12) on a Kahlsico duNouy instrument TE03 (Kahl Scientific Instrument Corp., El Cajon, CA). Surface pressure and potential measurements (13) are being carried out in a recent version of our previously conceptualized (14) Wilhelmy-type surface balance system, equipped with a dual pen Omniscribe B-5000 (Houston Instrument Div., Bausch and Lomb Inc., Austin, TX).

Please address correspondence to Dr. Lasslo.

BIOPHYS. J. © Biophysical Society · 0006-3495/82/01/130/04 $1.00
Volume 37 January 1982 130–133

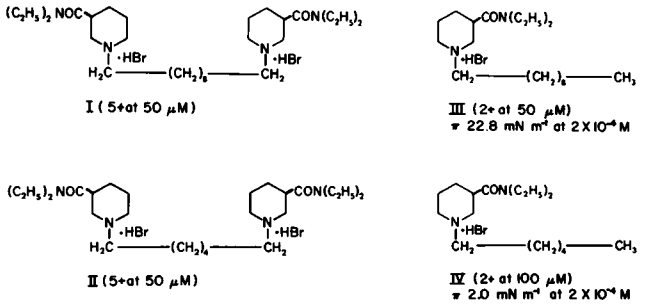

FIGURE 1 Influence of surface activity

RESULTS AND DISCUSSION

Among the highlights reported here, compounds I and II (Fig. 1) are equally active. From this and findings discussed later in this paper we conclude that the distance between the ring nitrogens separated by a hexamethylene link (C_6) matches closely that of the target sites on the platelet. The ring nitrogens on the decamethylene chain (C_{10}), through appropriate buckling of that flexible link, can also connect readily with the same target sites. However, because the ring nitrogens of the bis-dialkylcarbamoylpiperidino-substituted ethane separated by merely two methylene groups (C_2) could not accommodate a comparable reach, this analogue is 10 times weaker (1+ at 100 μM). In contrast, the mono-substituted decane (III) is twice as potent as the hexane (IV) (Fig. 1); the hydrophobic bonding of the former (C_{10}) on the platelet surface adjacent to the target site is so strong that it substantially augments the interaction of its single piperidino nitrogen with one target site; conversely, in the mono-substituted hexane, the shorter alkyl chain (C_6) is unable to exert hydrophobic bonding of comparable magnitude. Not surprisingly, such hydrophobic forces are not discernible at all in the mono-substituted ethane (C_2) which does not register any activity at these concentrations (0 at 100 μM).

While the contribution of lipophilicity to enhanced aggregation-inhibitory potency is evident, our results also indicate that our bis-substituted derivatives interact with two target sites on the platelet whenever the polymethylene chain is long enough to accommodate such intercourse. This is materially substantiated by the relative potencies of the bis(dialkylcarbamoylpiperidino)xylene analogues (Fig. 2). Interestingly, the para-congener (VI) can be envisioned as a close but substantially more potent (2+ at 5 μM) analogue of the bis-substituted hexane (II, Fig. 1), in which four of the six methylene units have been replaced by a phenylene moiety; while this should not perceptibly affect the hydrophobic character (15) of the molecule, the planarity of the ring structure renders VI much more rigid (16*a*) than II. Conformingly, the intramolecular distances between the ring nitrogens, computed with Godfrey space-filling models, register for the former a range of 7.8–8.3 Å with a flexibility variance of 0.5 Å, and for the latter a range of 4.4–9.1 Å with a flexibility variance of 4.7 Å. We suppose that II, in which the range of ring nitrogen distances encompasses those of VI, is less active due to the almost 10-fold greater flexibility variance. Remarkably, the mono-substituted analogue of VI is only half as active (1+) at 20 times higher concentrations (100 μM). The significance of the interatomic distance between the ring nitrogens in the bis-substituted compounds is further emphasized by the decreasing activities of the correspondingly substituted meta-xylene (VII, 5.3–7.8 Å, 3+ at 50 μM) and ortho-xylene (VIII, 4.8–5.9

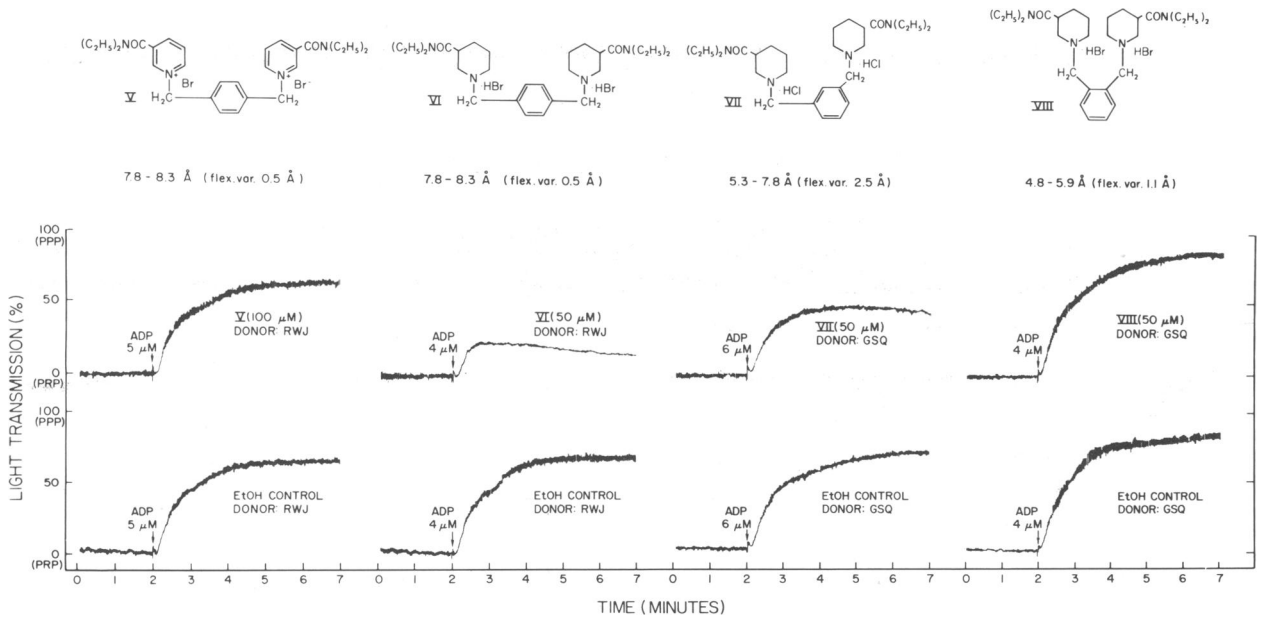

FIGURE 2 Effect of interatomic distance between aggregation-inhibitory specific functions and of N-charge/aromaticity levels.

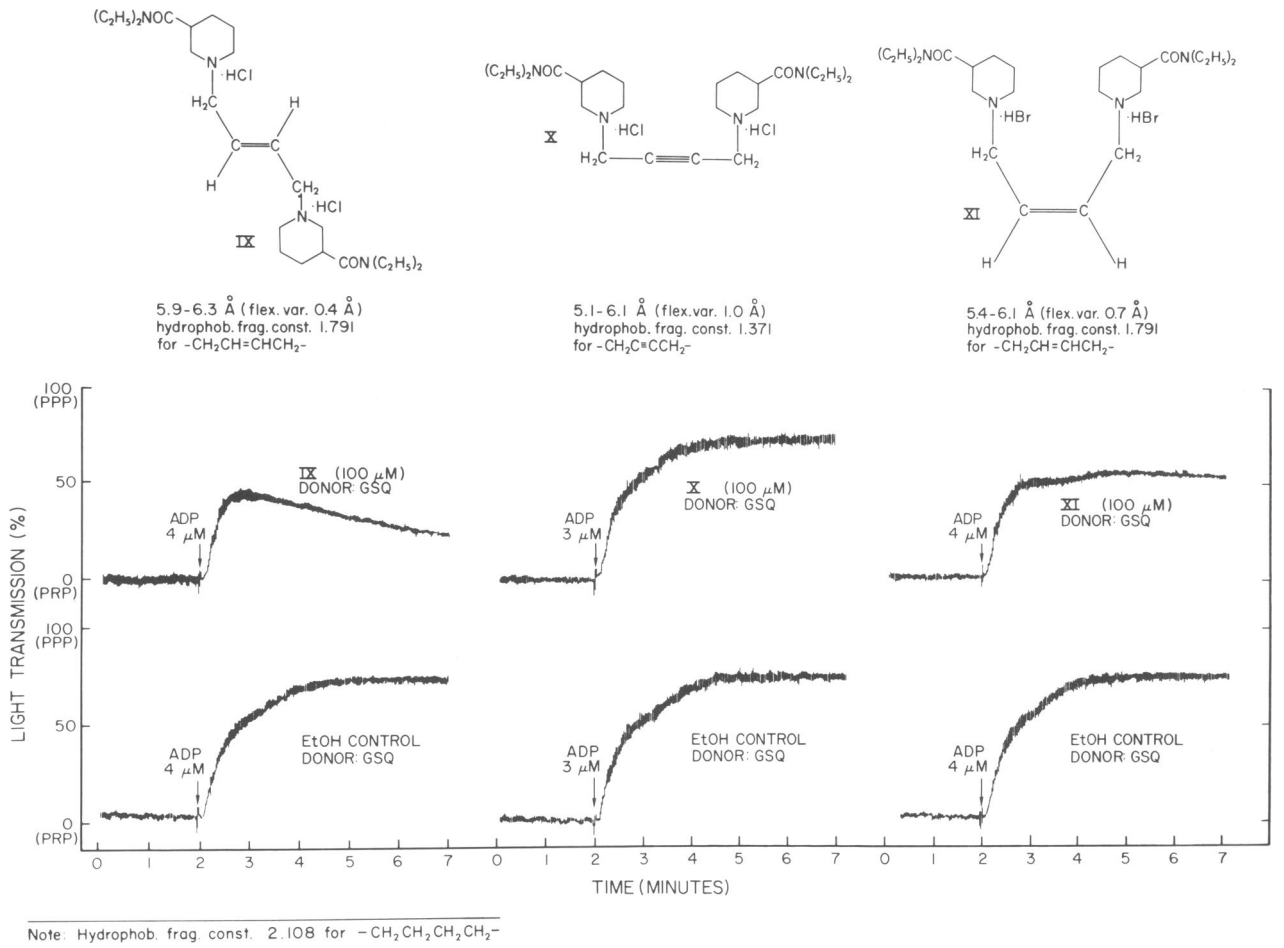

FIGURE 3 Effect of stereoisomerism and hydrophobic influence.

Å, 0 at 50 μM) analogues (Fig. 2). These data distinctly imply platelet membrane target sites spaced at 8 Å.

Despite the striking potency of VI, its bis(dialkylcarbamoylpyridinium) congener (V, Fig. 2) is completely inactive even at 20 times higher concentrations (0 at 100 μM); this and parallel findings with other comparable analogues could be attributed either to the aromatic character of the ring structure and its conjugation with the amide function (16*b*), or to the charge level of the ring nitrogen, or to both. Each factor individually, or both in combination, could lessen hydrophobicity and reduce aggregation-inhibitory potency. As quaternary amines, the heterocyclic nitrogens in V retain the same charge regardless of their environment and consequently, in a biosystem, their hydrophobicity does not even approximate that of corresponding tertiary amines (17, 18), which are generally subject to broad variances in protonation, contingent upon the pH of the medium and the specific compounds' pK_a values (6). This very trait of tertiary amines provides appropriate hydrophobic character for their penetration of the platelet membrane's lipid bilayer without interfering with the subsequent generation of adequate cationic species (19–21). The influence of this parameter is being examined in greater detail.

The impact of stereoisomerism is delineated in Fig. 3. The greater potency of the *trans*-isomer (IX) can also be attributed to the fact that the intramolecular distance of its ring nitrogens (5.9–6.3 Å) is closer to the postulated optimum of 8 Å than the lesser one computed for the rings' hetero-atoms in the *cis*-compound (XI, 5.4–6.1 Å). The increased polarity effected by unsaturation in the alkyl chain contributes considerably to the lower activity of both isomers. Accordingly, it should not be surprising that X registers no activity at 100 μM concentration; the hydrophobic fragmental constant (15) for its alkyl chain of four carbons with one triple bond is 1.371, compared with that of 1.791 for the corresponding linkage in IX and XI with one double bond segment, and with that of 2.108 for a corresponding alkyl chain without any unsaturation. It is obvious that X is the most polar compound among those depicted.

For a more extensive discourse on the subject matter the reader is referred to our most recent work (22).

Supported by National Institutes of Health grants HL-22236 and HL-25884, and by the Gustavus and Louise Pfeiffer Research Foundation.

Received for publication 18 March 1981.

REFERENCES

1. Lasslo, A., and P. D. Waller. 1957. 3-(*N,N*-Dialkylcarboxamido)piperidinoalkanes. *J. Org. Chem.* 22:837–838.
2. Lasslo, A., W. M. Marine, and P. D. Waller. 1956. Substituted piperidinecarboxamides. *J. Org. Chem.* 21:958–960.
3. Quintana, R. P., and W. A. Shrader. 1963. Synthesis of selected amides of mono- and bis(carboxypiperidino)alkanes. *J. Pharm. Sci.* 52: 1186–1188.
4. Beasley, J. G., R. P. Quintana, and G. G. Nelms. 1964. The effect of piperidinecarboxamide derivatives on isolated human plasma cholinesterase. II. Variations in the amide function. *J. Med. Chem.* 7:698–701.
5. Quintana, R. P., T. D. Smith, and L. F. Lorenzen. 1965. Synthesis of carbamoylpiperidine-type cholinesterase inhibitors. *J. Pharm. Sci.* 54:785–787.
6. Quintana, R. P., and W. R. Smithfield. 1967. Substituted l-benzyl-3-(N,N-diethylcarbamoyl)piperidine cholinesterase inhibitors. Relationships between molecular constitution, pKa′ values and partition coefficients. *J. Med. Chem.* 10:1178–1180.
7. Dachary-Prigent, J., J. Dufourcq, C. Lussan, and M. Boisseau. 1979. Propranolol, chlorpromazine and platelet membrane: a fluorescence study of the drug - membrane interaction. *Thromb. Res.* 14:15–22.
8. Vanderhoek, J., and M. B. Feinstein. 1979. Local anesthetics, chlorpromazine and propranolol inhibit stimulus-activation of phospholipase-A_2 in human platelets. *Mol. Pharmacol.* 16:171–180.
9. Chap, H. J., R. F. A. Zwaal, and L. L. M. Van Deenen. 1977. Action of highly purified phospholipases on blood platelets. Evidence for an asymmetric distribution of phospholipids in the surface membrane. *Biochim. Biophys. Acta.* 467:146–164.
10. Marcus, A. J., H. L. Ullman, and L. B. Safier. 1969. Lipid composition of subcellular particles of human blood platelets. *J. Lipid Res.* 10:108-114.
11. Quintana, R. P., A. Lasslo, M. Dugdale, L. L. Goodin, and E. F. Burkhardt. 1980. Effects of ethanol and of other factors on ADP-induced aggregation of human blood platelets in vitro. *Thromb. Res.* 20:405–415.
12. Quintana, R. P., A. Lasslo, G. S. Queen, and C. M. Baldwin. 1981. Aspirin analogs with enhanced interfacial activity. *J. Colloid Interface Sci.* In press.
13. Lasslo, A., and R. P. Quintana, Editors. 1973. Surface Chemistry and Dental Integuments. Charles C. Thomas, Springfield, IL.
14. Fisher, R. G., and R. P. Quintana. 1975. Surface-chemical studies on chlorhexidine and related compounds. II. Interactions with monomolecular-film systems. *J. Dent. Res.* 54:25–31.
15. Nys, G. G., and R. F. Rekker. 1974. The concept of hydrophobic fragmental constants (f-values). II. Extension of its applicability to the calculation of lipophilicities of aromatic and heteroaromatic structures. *Eur. J. Med. Chem.* 9:361–375.
16. Korolkovas, A. 1970. Essentials of Molecular Pharmacology; Background for Drug Design. Wiley-Interscience, New York; (*a*) 90, (*b*) 65–66.
17. Quintana, R. P. 1964. Relationships between the surface activity and cholinesterase inhibition of mono- and bis[3-(*N,N*-diethylcarbamoyl)piperidino]alkanes. *J. Pharm. Sci.* 53:1221–1223.
18. Quintana, R. P. 1965. Relationships between partition coefficients and cholinesterase inhibition of carbamoylpiperidinoalkanes. *J. Pharm. Sci.* 54:462–463.
19. Narahashi, T., and D. T. Frazier. 1971. Site of action and active form of local anesthetics. *Neurosci. Res.* 4:65–99.
20. Strichartz, G. R. 1973. The inhibition of sodium currents in myelinated nerve by quaternary derivatives of lidocaine. *J. Gen. Physiol.* 62:37–57.
21. Hille, B. 1977. Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. *J. Gen. Physiol.* 69:497–515.
22. Quintana, R. P., A. Lasslo, M. Dugdale, and L. L. Goodin. 1981. Relationships between the chemical constitution of carbamoylpiperidines and related compounds, and their inhibition of ADP-induced human blood platelet aggregation. *Thromb. Res.* 22:665–680. Cf. Quintana, R. P., A. Lasslo and L. T. Greer. Interaction of human blood platelet aggregation inhibitors with phospholipid films. Submitted for publication.

ENVIRONMENTALLY INDUCED REVERSIBLE PROTEIN TRANSLOCATION ACROSS INNER MITOCHONDRIAL MEMBRANE

A. WAKSMAN

Centre de Neurochimie du Centre National de la Recherche Scientifique, 67084 Strasbourg Cedex, France

Four molecular mechanisms account for protein translocation across biological membranes: translocation via vectorial translation, via vectorial processing, via subunit dissociation, and via environmental induction. Whereas the first three mechanisms are vectorial, the last one is reversible (1). Fig. 1 describes the general methodology and observations that led us to the discovery of this last mechanism.

RESULTS AND DISCUSSION

Externalization of a protein such as aspartate aminotransferase (AAT) from the inner face of inner mitochondrial membrane towards intermembranal fluid is shown in Fig. 1, step 1. This step is induced by movement effectors, e.g., succinate, fumarate, malate, pyruvate, citrate, phenylsuccinate, α-methylglutamate, etc., at concentrations ranging from 0 to 50 mM (2). Step 2 shows internalization of previously externalized AAT upon removal of the movement effector. This was demonstrated by testing the accessibility of the enzyme protein first on the outer face of the mitoplast (mitochondria from which the outer membrane was removed), then on the outer face of the corresponding inverted vesicle, which is equivalent to the inner face of the mitoplast (step 3). No permeant substrate, controlled