Thrombin Receptors Define Responsiveness of Cholesterol-modified Platelets*

Narendra Tandon†, Joan T. Harmon‡, David Rodbard§, and G. A. Jamieson†‡

From the †American Red Cross, Blood Services Laboratories, Bethesda, Maryland 20814 and the §Laboratory of Theoretical and Physical Biology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892

The microviscosity of human platelet membranes was changed by incubating platelets with liposomes containing various ratios of cholesterol and lecithin. Binding of 125I-thrombin to the modified platelets was measured together with platelet aggregation and secretion. In cholesterol-normal platelets (mole ratio of cholesterol to phospholipid (C:PL) = 0.553; η = 2.40 poise), weighted nonlinear least squares curve fitting indicated that a model involving two classes of sites was adequate to describe the binding isotherm (K1 = 8.3 x 10^5 M^-1; R1 = 150 sites/platelet; K2 = 6.4 x 10^6 M^-1; R2 = 16,000 sites/platelet). In cholesterol-enriched platelets (C:PL = 0.857; η = 3.05 poise), the apparent affinities for the two classes of sites decreased to 55 and 53%, respectively, while the binding capacities increased to 170 and 180%, respectively. In contrast, in the cholesterol-depleted platelets (C:PL = 0.435; η = 2.03 poise), the affinities increased to 220 and 180%, respectively, while the binding capacities decreased to 53 and 46%, respectively. In cholesterol-enriched, cholesterol-normal, and cholesterol-depleted platelets, the thrombin concentrations required for half-maximal aggregation were 0.17, 0.35, and 0.52 nM, respectively, while the values for half-maximal secretion of ['4C]serotonin were 0.17, 0.40, and 0.55 nM, respectively. Plots of receptor occupancy versus biological response showed that maximum response in cholesterol-enriched, cholesterol-normal, and cholesterol-depleted platelets occurred with occupancy of 30, 50, and 70% of the high affinity sites, respectively. In all three treatment groups, occupancy of 40–50 high affinity sites results in 50% aggregation. These results show that (i) modification of platelet membrane microviscosity results in changes in the number and affinity of both high and low affinity thrombin receptors, (ii) the change in receptor number rather than affinity is the determinant for platelet responsiveness, and (iii) the changes in membrane microviscosity do not appear to alter the coupling between occupied receptor and subsequent bioresponse.

Elevated serum cholesterol is one of the most consistent risk factors for atherosclerosis and related vaso-occlusive disorders. Platelets from patients with hypercholesterolemia have an increased sensitivity to aggregating agents, especially epinephrine (1–3). A series of in vitro studies has sought to establish the basis for this hypersensitivity; when the cholesterol:phospholipid ratio of platelets was elevated by exposure to cholesterol-rich liposomes, the platelets were found to have increased membrane microviscosity (4), increased sensitivity to epinephrine and ADP (5), and increased basal levels of adenylate cyclase (6). Although thrombin-induced platelet aggregation was originally reported to be unaffected by changes in membrane cholesterol (5), more recent studies, reported since the completion of our own work, have shown that increases in the cholesterol:phospholipid ratio result in increases in platelet aggregation with thrombin and increases in the liberation of arachidonate (7, 8) and the levels of thromboxane B2 secretion (9).

We have now studied the effect of changes in platelet membrane microviscosity on the number and affinity of receptors for thrombin with both increased and decreased ratios of cholesterol:phospholipid in comparison with cholesterol-normal platelets and have studied the bioresponse (both aggregation and secretion) in the three treatment groups. Our results show that the number of thrombin receptors increases with increasing membrane microviscosity while the affinity of binding decreases and that receptor number, rather than affinity, is the principal determinant of platelet responsiveness but that coupling between occupied receptor and bioresponse mechanism is unaffected by change in membrane microviscosity. Preliminary experiments leading to these studies have been published elsewhere (10).

EXPERIMENTAL PROCEDURES

**Materials—L-α-Dipalmitoyllecithin, bovine serum albumin (essentially free of fatty acids), and 1,6-diphenyl-1,3,5-hexatriene were obtained from Sigma. Unesterified cholesterol (99%) was from Miles Laboratories. Garamycin (gentamycin sulfate) was from Schering-Plough Corp., Kenilworth, N.J. Human serum albumin USP (25% aqueous solution) was from Armour. Lecithin, 1,6-diphenyl-1,3,5-hexatriene, and cholesterol were used without further purification. All other chemicals used were of reagent grade. Iodogen was obtained from Pierce Chemical Co. Human α-thrombin was purified by the procedure of Fenton et al. (11) and had a specific activity in the clotting assay of 3400 NIH units/mg.

**Platelet Preparation—**Fresh human blood anticoagulated with citrate/phosphate/dextrose/adenine was obtained from the Washington Region, American Red Cross Blood Services from volunteer donors. Platelet-rich plasma was obtained from 1 unit of whole blood by centrifugation at 3200 x g for 3.5 min at 22 °C. Platelet-rich plasma was transferred to the satellite bag and spun at 3200 x g for 10 min to sediment the platelets and to obtain platelet concentrate, while the supernatant plasma was removed and used as platelet-poor plasma.

For the preparation of washed platelets, the pH of the platelet-rich plasma was adjusted to 6.5 with citric acid (150 mM), platelets were sedimented by centrifugation at 1800 x g for 10–12 min, and the pellet was resuspended in platelet wash buffer (110 mM dextrose, 129...
Effect of Platelet Membrane Fluidity on Thrombin Receptors

mm NaCl, 4.26 mM NaHPO₄, 7.46 mM trisodium citrate, and 2.35 mM citric acid, pH 6.5) supplemented with 0.35% bovine serum albumin. Contaminating erythrocytes were removed by centrifugation at 800 x g for 15 min. The platelet suspension was then centrifuged at 3,000 x g for 5 min, and the pellet was suspended in modified Tyrode's buffer (136 mM NaCl, 5.5 mM dextrose, 2.7 mM KCl, 0.07 mM Na₂HPO₄, 0.01 mM NaHCO₃). Platelets were counted electronically in a Coulter counter (Coulter Electronics Inc., Hialeah, FL).

Preparation of Liposomes—Cholesterol- and lecithin-containing liposomes were prepared as described by Shatil and Cooper (4) in modified Tyrode's buffer. Weight ratios (milligram/mg) of cholesterol-lecithin used to prepare lipid dispersions (liposomes) were 80:40 for cholesterol-rich, 23:40 for cholesterol-normal, and 0:40 for cholesterol-poor. After sonication for 1 h (Branson sonifier, model 350, setting 5), the dispersions were centrifuged at 50,000 x g for 30 min to remove undispersed lipid. The supernatant suspension was stored at 4 °C and was used within 1 week. Immediately before use, the dispersions were made 2.5 g/100 ml with respect to human serum albumin, incubated for 20–30 min at 37 °C, and centrifuged at 21,000 x g for 30 min to sediment any aggregated material.

Aggregation of Platelet Membrane Lipid—The following procedures were performed under sterile conditions. One unit of platelet concentrate was adjusted with platelet-poor plasma to a platelet concentration of 1.5 x 10⁹/ml. Ten-milliliter portions of this diluted platelet concentrate were transferred to three separate 150-ml blood transfer packs and incubated with equal volumes (10 ml) of each lipid dispersion in modified Tyrode's buffer containing 2.5 g/100 ml human serum albumin, pH 7.2. Gartsmycin (30 µg/ml final concentration) was added, and the mixtures were incubated for 18-22 h at 22 °C in an incubator equipped with a horizontal shaking device (Forma Scientific platelet incubator) of the type used for storage of platelets for clinical use (15). This incubation resulted in an insignificant increase in the pH (0.05-0.1 pH unit) of the platelet suspension. The platelet suspension was adjusted to 6.5 with citric acid, and the platelets were pelleted by centrifugation at 1,600 x g for 12 min at 22 °C, resuspended in platelet wash buffer, washed twice with platelet wash buffer, and finally suspended in Tyrode's buffer or thrombin binding buffer, as required.

A crossover experiment was carried out to establish that changes in platelet responsiveness and in binding data were not due to differing protective effects of the three liposome populations. In this experiment, platelets originally rendered cholesterol-enriched or cholesterol-depleted by incubation with liposomes for 5 h at 37 °C (4) were used for 18 h at 37 °C with cholesterol-depleted and cholesterol-enriched liposomes, respectively.

Platelet Liquid Analysis—Thrice-washed platelets in platelet wash buffer were extracted by shaking with 15-20 volumes of chloroform:methanol (2:1) for 3–4 h. The organic layer was washed twice with one-tenth volume of 0.15 M KCl in methanol. The aqueous phase was evaporated under a stream of dry nitrogen at 40 °C. The contents of cholesterol and lipid phosphorus were measured in the dried residue by the methods of Zlatkis et al. (13) and Chen et al. (14), respectively.

Platelet Liquid Analysis—Thrice-washed platelets in platelet wash buffer were extracted by shaking with 15-20 volumes of chloroform:methanol (2:1) for 3–4 h. The organic layer was washed twice with one-tenth volume of 0.15 M KCl in methanol. The aqueous phase was evaporated under a stream of dry nitrogen at 40 °C. The contents of cholesterol and lipid phosphorus were measured in the dried residue by the methods of Zlatkis et al. (13) and Chen et al. (14), respectively.

Platelet Liquid Analysis—Thrice-washed platelets in platelet wash buffer were extracted by shaking with 15-20 volumes of chloroform:methanol (2:1) for 3–4 h. The organic layer was washed twice with one-tenth volume of 0.15 M KCl in methanol. The aqueous phase was evaporated under a stream of dry nitrogen at 40 °C. The contents of cholesterol and lipid phosphorus were measured in the dried residue by the methods of Zlatkis et al. (13) and Chen et al. (14), respectively.

Platelet Liquid Analysis—Thrice-washed platelets in platelet wash buffer were extracted by shaking with 15-20 volumes of chloroform:methanol (2:1) for 3–4 h. The organic layer was washed twice with one-tenth volume of 0.15 M KCl in methanol. The aqueous phase was evaporated under a stream of dry nitrogen at 40 °C. The contents of cholesterol and lipid phosphorus were measured in the dried residue by the methods of Zlatkis et al. (13) and Chen et al. (14), respectively.
from those previously used by Shattil and Cooper (4) and others (7-9) which involved incubation at 37 °C for 5 h with frequent inversion. For this reason, the extent of incorporation was determined by cholesterol and phospholipid analysis and changes in fluorescence polarization. The results obtained (Table I) show values for C:PL ratios and for microviscosity similar to those previously reported for cholesterol-enriched, cholesterol-normal, and cholesterol-depleted platelets (4). The values obtained for platelets incubated with cholesterol-normal liposomes were identical with those obtained with unmodified platelets incubated with Tyrode’s buffer under similar conditions showing that incubation, as such, did not affect the C:PL ratio.

Platelet Aggregation and Secretion—In order to determine platelet responsiveness by aggregation and secretion, the three classes of cholesterol-enriched, cholesterol-normal, and cholesterol-depleted platelets were subjected to aggregation by titrating them with increasing levels of thrombin from 0-1 nM (0-100 milliunits/ml). As shown in Fig. 1A, a clear-cut pattern of responses was observed. Cholesterol-enriched platelets gave a 35% aggregation response after 3 min at a thrombin concentration of 0.15 nM (15 milliunits/ml), while the cholesterol-normal and cholesterol-depleted platelets showed essentially no response to thrombin at this concentration. At the next higher thrombin concentration (about 0.25 nM), aggregation of cholesterol-enriched platelets reached 80% of their final value by 3 min, while aggregation with cholesterol-normal platelets was barely detectable. At a thrombin concentration of 0.4 nM (40 milliunits/ml), aggregation of cholesterol-enriched platelets had reached 90% of its final value; with cholesterol-normal platelets it had reached 70%, while only a minimal excursion of the aggregometer recorder was detected with cholesterol-depleted platelets.

From these data it appears that essentially maximum aggregation was reached with cholesterol-enriched platelets at thrombin concentrations of 0.4 nM (40 milliunits/ml), with cholesterol-normal platelets at 0.60 nM (60 milliunits/ml), and with cholesterol-depleted platelets at 0.75 nM (75 milliunits/ml). Values for half-maximal aggregation from the dose response curves of Fig. 1 were 0.17, 0.35, and 0.53 nM. While there were minor shifts in values between the 8 and 10 individual platelet preparations examined in this way, this general pattern was observed in all cases.

Similar results were obtained when the release of serotonin was examined at different thrombin concentrations in the three platelet populations. The maximum release for cholesterol-enriched, cholesterol-normal, and cholesterol-depleted platelets was achieved at thrombin concentrations of 0.50, 0.70, and 1.0 nM, respectively, and the same type of differential curves were obtained as were seen in the case of the aggregation response (Fig. 1B).

Thrombin Binding Studies—The thrombin binding data could be fit successfully using a model involving two independent classes of binding sites for all experiments and for each of the three platelet treatment groups, but they could not be fit satisfactorily using a one-site model. All of the data from five separate binding studies for each of the three separate treatment groups of cholesterol-enriched, cholesterol-normal, and cholesterol-depleted platelets were submitted to computer curve fitting (Fig. 2 and Table II). The shapes of the Scatchard plots for each of the three platelet treatment groups differ significantly. For cholesterol-normal platelets, the association constant for high affinity sites (8.3 ± 2.3 × 10^6 M^-1) and the number of high affinity receptors (150 ± 36) were similar to that found in previous studies (20-22) as were the association constant for low affinity sites (6.4 ± 1.6 × 10^6 M^-1) and the number of receptors (16,000 ± 3,200). With cholesterol-enriched platelets, the association constant for high affinity binding decreased to 4.6 ± 1.1 × 10^6 M^-1, while the number of high affinity sites increased to 260 ± 64. A similar relationship was found for the low affinity binding, where the association constant decreased to 3.4 ± 0.97 × 10^6 M^-1, while the number of low affinity receptors increased to 26,000 ± 7,900. Conversely, with cholesterol-depleted platelets, the high affinity association constant (18 ± 4.7 × 10^6 M^-1) was greater than that in cholesterol-normal platelets, while the number of receptors (79 ± 20) was smaller. A similar pattern was obtained with regard to the low affinity association constant (12 ± 2.2 × 10^6 M^-1) and the corresponding number of receptors (7,300 ± 1,200). In all cases, nonspecific binding remained constant at about 3% of the total counts added. The changes in affinities and receptor numbers in the three platelet treatment groups did not cause changes in the per cent binding of trace concentrations of labeled thrombin (0.1 nM); that is, KxR1 + KzR2 were approximately equal for each platelet treatment group.

Since the results of the binding studies indicated that both the affinity of the receptors and the number of receptors change upon modification of membrane cholesterol, we examined the relationship between thrombin receptor occupancy and biological response, that is, thrombin-induced aggregation. Results of this analysis are shown in Fig. 3 for the high affinity sites. Cholesterol-enriched platelets were more responsive to the thrombin, as indicated by the leftward shift in the curve, than were cholesterol-normal platelets which were, in turn, more responsive than cholesterol-depleted platelets. The maximum response in cholesterol-enriched platelets occurred with 30% occupancy of the high affinity receptors; the maximum response in cholesterol-normal platelets occurred with 50% occupancy of the high affinity receptors; while the maximum response in cholesterol-depleted platelets occurred with 70% occupancy of the high affinity

![Table I](image)

**Fluidity data for cholesterol-modified platelets**

| Present data | Literature values (4) |
|--------------|-----------------------|
| **C:PL**    | **η**                 | **C:PL** | **η** |
| Cholesterol-enriched | 0.85 ± 0.044 | 3.65 ± 0.20 | 1.07 | 3.20 |
| Cholesterol-normal | 0.553 ± 0.018 | 2.40 ± 0.14 | 0.57 | 2.84 |
| Cholesterol-depleted | 0.438 ± 0.074 | 2.03 ± 0.11 | 0.376 | 2.47 |
| Tyrode’s buffer  | 0.5728 ± 0.020 | 2.48 ± 3.086 | 0.55 | NR* |

* η, microviscosity (poise) measured at 37 °C.
* NR, not reported.

![Fig. 1](image)

**Fig. 1.** Thrombin responsiveness of cholesterol-modified platelets. ○, cholesterol-enriched; Δ, cholesterol-normal; ●, cholesterol-depleted. A, aggregation response: B, secretion of [14C]serotonin. 5HT, 5-hydroxytryptamine.
Effect of Platelet Membrane Fluidity on Thrombin Receptors

FIG. 2. Scatchard plots of thrombin binding data for cholesterol-modified platelets. Five independent experiments were carried out and are combined in this presentation. A-C are the expanded graphs for the high affinity regions, while the A'-C' are the complete Scatchard plots. A and A', cholesterol-depleted platelets; B and B', cholesterol-normal platelets; C and C', cholesterol-enriched platelets.

**TABLE II**

| Cholesterol Enriched (a) | Cholesterol Normal (b) | Cholesterol Depleted (c) |
|--------------------------|------------------------|--------------------------|
| Receptor Numbers (sites/platelet) | Receptor Numbers (sites/platelet) | Receptor Numbers (sites/platelet) |
| 10^-4 Kalpha | 10^-6 Kalpha | 10^-4 Kalpha | 10^-6 Kalpha | 10^-4 Kalpha | 10^-6 Kalpha |
| 4.6 ± 1.1 | 260 ± 64 | 3.4 ± 2.00 | 26,000 ± 7,900 | 0.027 ± 0.005 |
| 8.3 ± 2.3 | 150 ± 36 | 6.4 ± 1.6 | 16,000 ± 3,200 | 0.032 ± 0.003 |
| 18.0 ± 4.7 | 79 ± 20 | 12.0 ± 2.2 | 7,300 ± 1,200 | 0.054 ± 0.002 |

Ratios

| Ratio |  |
|-------|---|
| a/b   | 0.55 |
| c/b   | 2.2 |
| a/c   | 0.26 |

receptors. Thus, the change in receptor number appears to be the determinant for platelet responsiveness.

Since biological response is related to the number of occupied receptors (23), we calculated the number of occupied high affinity receptors necessary to elicit 50% aggregation. In the three treatment groups, occupancy of 40–50 high affinity receptors resulted in 50% aggregation; specific values for cholesterol-enriched, cholesterol-normal, and cholesterol-depleted platelets were 36, 50 and 48, respectively. Therefore, modification of platelet membrane microviscosity by changing the C:PL ratio appears to alter the number of thrombin receptor sites which in turn alters the responsiveness of the platelet, but this does not appear to alter the coupling between the occupied high affinity receptor and the bioresponse mechanism.

When similar calculations were made for all receptors, that is, both high and low affinity, the number of occupied receptors necessary to elicit 50% aggregation for the cholesterol-enriched, cholesterol-normal, and cholesterol-depleted were 156, 112, and 95, respectively. This relationship is contrary to the observed dependence of platelet responsiveness on membrane microviscosity and suggests that the low affinity sites are not involved in this response.

**Crossover Experiment**—The results of the crossover experi-
significant difference between the three treatment groups. This was confirmed by simultaneously fitting multiple experiments within a treatment group giving rise to more precise estimates of parameters because of the increased number of observations relative to the number of fitted parameters. Although graphical analysis of the Scatchard plots (Fig. 2) might suggest gross similarities of shape, this hypothesis could be unequivocally rejected \((P < 0.001)\), fitting all curves from the three treatment groups simultaneously with the same affinity constants \((K_1, K_2)\) and the same ratio of receptor number \((R_1/R_0)\) resulted in a drastic increase of the sum of square and appearance of severe nonrandomness of residuals.

These changes in surface expression of thrombin receptors were accompanied by differences in thrombin-induced aggregation and release between the three platelet treatment groups. Thus, the thrombin concentrations required for half-maximal aggregation in cholesterol-enriched, cholesterol-normal, and cholesterol-depleted platelets were 0.17, 0.35, and 0.52 nM, respectively. These results confirm and extend the observations of Kramer et al. \((7)\) who showed that cholesterol-enriched platelets exhibit greater aggregation than cholesterol-normal platelets at thrombin concentrations in the range 2–10 nM. The differences we are observing are at approximately 1 order of magnitude lower concentrations of thrombin and are observed for both cholesterol-enriched and cholesterol-depleted platelets. The differences observed are in the range of the high affinity association constants for the thrombin receptor, and no differences were observed between the three platelet treatment groups at higher concentrations of thrombin. We have previously observed that differences in reactivity between normal and Bernard-Soulier platelets are most readily detected at low thrombin concentrations \((\sim 0.3 \text{ nM})\) \((23)\). Thus, titration of the aggregation response of platelets with low concentrations of thrombin may have value in detecting a variety of clinical platelet defects.

The crossover experiment designed to determine differential effects of the three liposome populations was not entirely satisfactory because of the additional periods of incubation and increased handling of all samples. However, the results with the platelets which were first depleted and then enriched are clear with respect to C:PL ratio, high and low affinity binding, and thrombin sensitivity. These experiments show that the observed differences are not due to differential effects of the liposome preparations.

Similar results to those observed for thrombin-induced aggregation were obtained for the thrombin concentrations required for half-maximal release of serotonin in the three platelet treatment groups at values of 0.17, 0.40, and 0.55 nM, respectively. These results show an exact parallel between platelets with low concentrations of thrombin may have value in detecting a variety of clinical platelet defects.

The present study clearly indicates the advantages of an objective computer-assisted statistical analysis of complex ligand binding systems. Casual inspection of the Scatchard plots (Fig. 2) fails to convey the necessary information because it is impossible to view the entire binding isotherm \((\sim 5 \text{ orders of magnitude})\) on one graph. Examination of the computer-generated curves indicates a consistent effect of either cholesterol enrichment or cholesterol depletion relative to cholesterol-normal platelets. Analysis using LIGAND permitted us to obtain estimates for the affinity constants and receptor number for both the high and low affinity sites, for nonspecific binding in each experiment, as well as the best scaling factor to correct for variations in platelet number and in density of receptors/platelet. The logarithmic mean of the affinity constants and receptor numbers showed a statistically

**DISCUSSION**

The present study clearly indicates the advantages of an objective computer-assisted statistical analysis of complex ligand binding systems. Casual inspection of the Scatchard plots (Fig. 2) fails to convey the necessary information because it is impossible to view the entire binding isotherm (over 5 orders of magnitude) on one graph. Examination of the computer-generated curves indicates a consistent effect of either cholesterol enrichment or cholesterol depletion relative to cholesterol-normal platelets. Analysis using LIGAND permitted us to obtain estimates for the affinity constants and receptor number for both the high and low affinity sites, for nonspecific binding in each experiment, as well as the best scaling factor to correct for variations in platelet number and in density of receptors/platelet. The logarithmic mean of the affinity constants and receptor numbers showed a statistically

**TABLE III**

| C:PL | Thrombin concentration for half-maximal aggregation | High affinity sites | Low affinity sites |
|------|--------------------------------------------------|---------------------|-------------------|
|      | Before crossover | After crossover | Before crossover | After crossover | \(10^{-4} K_a\) | No. | \(10^{-4} K_a\) | No. |
| Cholesterol-depleted to enriched | 0.50 | 0.86 | 0.37 | 0.22 | 5.63 | 167 | 1.96 | 37,000 |
| Cholesterol-normal to normal | 0.66 | 0.64 | 0.26 | 0.38 | 7.85 | 78 | 14.60 | 3,400 |
| Cholesterol-enriched to depleted | 0.88 | 0.76 | 0.12 | 0.30 | 2.94 | 276 | 2.10 | 28,000 |

**Fig. 3. Relationship of high affinity receptor occupancy and thrombin-induced aggregation response in cholesterol-modified platelets.**
lipid-lipid interactions and affecting the packing density within the membrane (16). These changes in membrane microviscosity can influence expression of receptors by two different mechanisms. First, changes in membrane fluidity could affect the association of receptors to form oligomeric groups; second, changes in fluidity may cause passive modulation resulting from vertical deflection of receptors in relation to the plane of the membrane. At present, we cannot differentiate between these two possible mechanisms with regard to platelets although both receptor number and affinity are affected in an inverse relationship.

Interestingly, similar trends with regard to fluidity and binding have been observed in the interaction of [3H] serotonin with mouse brain membranes where increases in receptor number at higher membrane microviscosities were associated with decreases in receptor affinity (24). In this case, the changes were interpreted as possibly being due to passive modulation by vertical displacement of the high and low affinity sites along the vertical axis of the receptor molecule. Whether similar relationships exist for high and low affinity receptors for thrombin in platelet membranes or whether the observed changes are due to combined effects on receptor association and vertical displacement remain to be determined.

The mechanism of the interaction of platelets with thrombin remains unresolved. We have proposed that thrombin binds first to a platelet receptor which, in a second step, interacts with a platelet effector leading to platelet aggregation and release (25). The alternative hypothesis suggests that platelet activation is entirely due to the proteolytic activity of thrombin and that thrombin binding, as such, is not a determinant of platelet responsiveness (26). Although it is possible that changes in membrane fluidity could affect the susceptibility of the proteolytic substrate to thrombin proteolysis, the present results show that platelet response is directly proportional to the amount of thrombin bound for each of the three platelet treatment groups examined.

The present work on dose response to thrombin suggests that half-maximal aggregation and secretion occur when the same number (40–50) of high affinity receptors are occupied in each of the three treatment groups, but that progressively higher concentrations of thrombin would be required to occupy this number in the cholesterol-enriched, cholesterol-normal, and cholesterol-depleted populations. The extensive metabolic changes observed in cholesterol-modified platelets in previous work (5–9) have been carried out at thrombin concentrations far above the threshold thrombin concentra-

Effect of Platelet Membrane Fluidity on Thrombin Receptors

REFERENCES

1. Carvalho, A. C. A., and Colman, R. W. (1974) N. Engl. J. Med. 290, 434–438
2. Nordoy, A., and Rodset, J. M. (1974) Acta Med. Scand. 188, 133–137
3. Mustard, J. F., Packham, M. A., and Kinlough-Rathbone, R. L. (1978) Adv. Exp. Med. Biol. 104, 127–144
4. Shattil, S. J., and Cooper, R. A. (1976) Biochemistry 15, 4832–4837
5. Shattil, S. J., Aneya-Galuda, R., Bennett, J., Colman, R. W., and Cooper, R. A. (1975) J. Clin. Invest. 55, 636–642
6. Sinha, A. K., Shattil, S. J., and Colman, R. W. (1977) J. Biol. Chem. 252, 3310–3314
7. Kramer, R. M., Jakubowski, J. A., Vaillancourt, R., and Deykin, D. (1982) J. Biol. Chem. 257, 6844–6849
8. Worner, P., and Patscheke, H. (1980) Thromb. Res. 18, 439–451
9. Stuart, M. J., and White, J. C. (1980) N. Engl. J. Med. 302, 6–10
10. Jamieson, G. A., Jung, S. M., and Ordinas, A. (1981) Ann. N. Y. Acad. Sci. 370, 96–100
11. Fenton, J. W., Il, Fasce, M. J., Stackrow, A. B., Aronson, E. L., Young, A. M., and Finlayson, J. S. (1977) J. Biol. Chem. 252, 3587–3598
12. Holme, S., Vaidja, K., and Murphy, S. (1978) Blood 52, 425–435
13. Zlatkis, A., Zak, B., and Boyle, A. J. (1953) J. Lab. Clin. Med. 41, 466–492
14. Chen, P., Toribara, T. Y., and Warrer, H. (1956) Anal. Chem. 28, 1756–1758
15. Shinitzky, M., and Lubar, M. (1976) Biochim. Biophys. Acta 433, 133–149
16. Shinitzky, M., and Barenholz, Y. (1978) Biochim. Biophys. Acta 515, 367–384
17. Kiemmeli, U. K. (1970) Nature (Lond.) 227, 680–685
18. Rodbard, D., Munson, P. J., and Thakur, A. Y. (1980) Cancer (Phil.) 46, 2907–2918
19. Scatchard, G. (1949) Ann. N. Y. Acad. Sci. 51, 666–672
20. Tollefsen, D. M., Feagler, J. R., and Majerus, P. W. (1974) J. Biol. Chem. 249, 2646–2651
21. Martin, B. M., Feinman, R. D., and Detwiler, T. C. (1975) Biochemistry 14, 1308–1314
22. Ganguly, P. (1974) Nature (Lond.) 247, 306–307
23. Jamieson, G. A., and Okumura, T. (1978) J. Clin. Invesst. 61, 861–864
24. Haron, D., Shinitzky, M., Hershkowitz, M., and Samuel D. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 7463–7467
25. Okumura, T., Haisz, M., and Jamieson, G. A. (1978) J. Biol. Chem. 253, 3435–3445
26. Berndt, M. C., and Phillips, D. R. (1981) J. Biol. Chem. 256, 59–65
Thrombin receptors define responsiveness of cholesterol-modified platelets.
N Tandon, J T Harmon, D Rodbard and G A Jamieson

J. Biol. Chem. 1983, 258:11840-11845.

Access the most updated version of this article at http://www.jbc.org/content/258/19/11840

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/258/19/11840.full.html#ref-list-1