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Facile Platelet Adhesion to Collagen Requires Metabolic Energy and Actin Polymerization and Evokes Intracellular Free Calcium Mobilization

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Abstract The attachment of platelets to collagen-coated microtiter plates at 20°C was inhibited strongly by depletion of metabolic energy or by addition of cytochalasins and was slightly inhibited by the intracellular Ca^{2+} chelator BAPTA. In keeping with their respective potencies as inhibitors of actin polymerization, cytochalasins D and H were the most potent inhibitors of adhesion, while cytochalasin B was the least potent. Energy depletion, cytochalasin D or, to a much lesser extent, BAPTA also inhibited platelet adhesion to collagen in a suspension assay system at 37°C. Collagen-induced platelet cytosolic Ca^{2+} mobilization was inhibited up to 70% by cytochalasin D and abolished by energy depletion or BAPTA. Elevation of intracellular platelet calcium by treatment with ionomycin had little effect on platelet adhesion to collagen. We propose that rapid platelet spreading along collagen fibers is both energy- and actin-dependent and necessary to produce maximal adhesion needed to elicit Ca^{2+} mobilization required for subsequent responses.

Key words: cytochalasins, energy-depletion, hemostasis, kinetics, receptors, stimulus-response coupling

Following damage to a vessel wall, circulating blood platelets initially adhere to newly exposed collagen and then release ADP and thromboxane A_2 (TxA_2). Collagen thus acts both as a solid phase matrix for platelet adhesion and as a primary agonist to cause the release of ADP and TxA_2. The latter soluble agents act as secondary agonists to recruit more platelets to consolidate the hemostatic plug.

In the past, experiments studying the interaction of collagen with platelets have suffered from one or more of three major problems: 1) they have failed to measure platelet adhesion to collagen at the same time as studying stimulus-response coupling; 2) they have failed to distinguish the primary response to the agonist collagen from the secondary response induced by released ADP and TxA_2; and 3) they have been conducted at low collagen concentrations, where very few platelets interact directly with collagen.

In this light, while both Shadle and Barondes (1982) and Santoro (1986) developed assays in which the adhesion of platelets to collagen could be quantitated and showed that the process is Mg^{2+}-dependent, they did not study intracellular events or antagonize the effects of secondary agonists. Similarly, while Kinlough-Rathbone et al. (1980) showed that platelets are able to both adhere to collagen fibres and form pseudopods in the presence of an ADP removing system and the cyclooxygenase inhibitor indomethacin, they too did not study stimulus-response coupling. Watson et al. (1985) presented data which indicate that collagen stimulation of platelets proceeds along a path identical to that induced by other agonists. However, their experiments were performed with relatively low collagen concentrations and they did not attempt to remove released ADP. Low concentrations of collagen also were used by Pollock et al. (1986) who questioned whether or not collagen can directly activate phospholipase C or elevate $[Ca^{2+}]_i$ in platelets, as observed with other agonists. They suggested that the primary effect of collagen is to liberate arachidonic acid and that all the stimulatory effects of collagen on platelets are due to conversion of the arachidonic acid to thromboxanes. It is noteworthy that Pollock et al. (1986) did not detect a change in $[Ca^{2+}]_i$ in response to 10 μg/ml collagen if the platelets had been treated with aspirin.

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Recently, we (Smith and Dangelmaier, 1990) confirmed that adhesion of platelets to collagen is Mg^{2+}-dependent and further observed that under conditions where positive feedback by released agonists is blocked there is little change in $[Ca^{2+}]_i$ (an increase of 50 nM above basal) at 10 μg/ml of collagen where only 10% of the platelets adhere. However, at 100 μg/ml of collagen, where 60% of the platelets adhere, the $[Ca^{2+}]_i$ increased to 220 nM above the basal level.

Shadle and Barondes (1982) observed that cytochalasin D not only inhibited the adhesion of platelets to collagen by a maximum of about 50%, but also inhibited platelet spreading and partially inhibited the secretion of serotonin by adherent platelets. On the other hand, it was subsequently reported that platelets pretreated with cytochalasins are entirely unresponsive to collagen (Nakano et al., 1989). In view of the divergence of these reports and the fact that the former did not investigate intracellular events, while the latter used low concentrations of collagen and did not measure adhesion, we decided to investigate cytochalasins further, as well as studying the effects of various other interventions. In the present studies we show that the adhesion of platelets to high concentrations of collagen can occur slowly, without any energy requirement. However, facile adhesion depends on energy and actin polymerization, and leads to cytosolic Ca^{2+} mobilization which, while not important for adhesion, may be important for subsequent platelet responses, such as secretion and arachidonic acid liberation.

METHODS

Radiolabeled oleic acid and adenine were from New England Nuclear and collagen from Horm-Chemie, Munich, Federal Republic of Germany. Arg-Gly-Asp-Ser (RGDS) was from Calbiochem Corporation, La Jolla, CA, and the cytochalasins, creatine phosphokinase, creatine phosphate and bovine serum albumin from Sigma Chemical Company. The cytochalasins were dissolved in dimethylsulfoxide as a stock solution at 5 mg/ml and routinely added to platelet suspensions 10 min before exposure to collagen. The antagonist SQ 29,548 was a gift from D. Harris of Bristol-Myers Squibb. The stable PGI_2 analog Iloprost was from Berlex Laboratories Inc., Cedar Knolls, NJ. Fura-2 acetoxymethyl ester and BAPTA acetoxymethyl ester were from Molecular Probes, Inc., Junction City, OR and the nylon mesh from Small Parts Inc., Miami, FL.

Platelet Preparation

Human blood was obtained from healthy volunteers who denied taking any drug during the previous week. The blood was anticoagulated with citric acid-citrate-dextrose (Aster and Jandl, 1964) and centrifuged at 180*g* for 15 min at room temperature to obtain platelet-rich plasma. The platelet-rich plasma was centrifuged at 800*g* for 20 min at room temperature to obtain a platelet pellet.

Collagen Adhesion Assays

The platelet pellet was resuspended in 10 ml of wash buffer (5.5 mM dextrose, 128 mm NaCl, 4.26 mM Na_2HPO_4, 7.46 mM NaH_2PO_4, 4.77 mM trisodium citrate, 2.35 mM citric acid, pH 6.5) containing 0.35% bovine serum albumin and 28 nM Iloprost. After recentrifugation (800*g*, 15 min, room temperature) and resuspension in 3 ml of this same buffer, the platelets were incubated for 1 hr with 5 μCi/ml [9,10-^{3}H(N)]-oleic acid at 37°C. In some experiments, [U-^{14}C]-adenine (0.4 μM, 220 Ci/mmol) also was included. After gel filtration into calcium-free Tyrode's buffer containing 0.5% fatty acid free bovine serum albumin and 5 mM glucose (Lages et al., 1975), the platelet suspensions were adjusted to 2.5×10^8 cells/ml containing 28 nM Iloprost and 100 μg/ml RGDS.

The microtiter plate assay was based on a modification of the method of Tandon et al. (1989) and was performed in triplicate. Microtiter plates were coated with 1–5 μg collagen in a volume of 50 μl for 2 hr at room temperature. Following aspiration of the collagen, the wells were treated with 250 μl calcium-free Tyrode's solution containing 0.5% albumin for 1 hr at room temperature. Following aspiration of this solution, 50 μl of the suspension of [^{3}H]-oleic acid-labeled platelets (either containing the cytochalasins or other drugs or after energy depletion) was added and incubation was continued for up to 1 hr at room temperature without agitation. Subsequently, the platelets remaining in suspension were removed by aspiration and the wells were washed three times with 250 μl Ca^{2+}-free Tyrode's buffer. The platelets adhering to the wells were solubilized by two 30-min treatments at room temperature with 100 μl of 2% sodium dodecyl sulfate and radioactivity determined by liquid scintillation counting. Blanks,

in which the adhesion of platelets to wells not coated with collagen was measured, were typically less than 0.1% of the radioactivity.

For the suspension assays, the platelet pellet was resuspended in 5 ml of autologous plasma and incubated for 1 hr with 5 μCi/ml [9,10-^{3}H(N)]-oleic acid (8.9 Ci/mmol) at 37°C. In some experiments, either fura-2 acetoxymethyl ester (3 μM) or [U-^{14}C]-adenine (0.4 μM, 220 Ci/mmol) also was included. Following incubation, the platelets were separated from unincorporated radiolabel by gel filtration on a Sepharose 2B column using a calcium-free Tyrode's buffer as described above. The platelets were adjusted to 2.5×10^8 cells/ml and Iloprost (28 nM) was added. Samples of 1 ml were stirred at 800 rpm at 37°C for 1 min before the addition of feedback inhibitors and collagen, as described elsewhere (Smith and Dangelmaier, 1990). Adhesion was determined after vacuum filtration through a 10 μm nylon mesh. To control for nonspecific trapping of the platelets on the mesh, blanks were included in which collagen was added first, followed by the platelet suspension. These blanks ranged between 1 and 3% of the radioactivity passed through the filter.

Energy Depletion

Platelets simultaneously labeled with [^{3}H]-oleic acid and [^{14}C]-adenine (and in some cases fura-2) were gel-filtered into a calcium-free Tyrode's buffer modified to contain 0.5 mM glucose, but otherwise as described above. The platelet suspensions were divided into portions, one of which was treated with the combination of 50 mM 2-deoxyglucose, 10 μg/ml antimycin (or 0.5 μg/ml rotenone in the experiments measuring Ca^{2+}-mobilization) and 10 mM gluconolactone for 5 min at 37°C. The suspensions were then incubated with collagen either in suspension or attached to microtiter plates as described above. Analysis of metabolic adenine nucleotides as described (Dangelmaier et al., 1986) confirmed that this treatment reduced the ATP content by approximately 90%.

Measurement of Intracellular Free Ca^{2+}-Mobilization

Platelets were preincubated with 3 μM fura-2 acetoxymethyl ester for 45 min at 37°C prior to gel-filtration. When BAPTA acetoxymethyl ester was used, the chelator was added at a final concentration of 100 μM at the same time as the ^{3}H-oleic acid and fura-2. Fura-2 fluorescence was monitored continuously using settings of 340 nm (excitation) and 510 nm (emission). Fura-2 signals were calibrated as described elsewhere (Pollock et al., 1988). F_{max} was determined by lysing the cells with 40 μM digitonin in the presence of saturating $CaCl_2$. F_{min} was determined by the addition of 2 mM EGTA and 20 mM Tris base.

RESULTS

Microtiter Plate Studies

Using a microtiter plate assay, we examined the effects of collagen concentration, of an antagonist of the fibrinogen/fibronectin receptor (RGDS), and of time on platelet binding to collagen at room temperature. Figure 1A shows that the adhesion of platelets to collagen during a 60-min incubation increases as a function of the amount of collagen initially added to the well. Furthermore, adhesion was reduced by the antagonist RGDS (100 μg/ml), suggesting that some attachment was mediated via the glycoprotein IIb/IIIa fibrinogen/fibronectin receptor complex. To exclude the involvement of this receptor in all subsequent assays, RGDS was routinely included. Figure 1B illustrates the effect of incubation times on the adhesion of platelets to microtiter plate wells coated with different collagen concentrations. Based on these results 60-min incubations of platelets with wells coated with 5 μg/ml collagen were utilized in subsequent experiments with inhibitors.

Several sugars (glucose, mannose; 100 mM), sugar phosphates (fructose-6-phosphate, mannose-6-phosphate; 10 mM) and complex carbohydrates (mannan, fucoidan; 1 mg/ml), as well as colchicine (50 μg/ml), had no effect on the adhesion of platelets to collagen in the microtiter plate assay (data not shown). On the other hand, depletion of platelet metabolic energy with the combination of antimycin, deoxyglucose, and gluconolactone had a dramatic effect on platelet adhesion to microtiter plates (Fig. 2A). Furthermore, adhesion also was inhibited by cytochalasin D (20 μM), an inhibitor of actin polymerization, but not by BAPTA, a chelator of intracellular Ca^{2+} (Fig. 2A). Figure 2B shows the concentration-response characteristics for inhibition of platelet adhesion to collagen by five different cytochalasins. Cytochalasins H and D were the most effective inhibitors, while cytochalasin B was the least effective, in keeping with the known potencies of these agents as

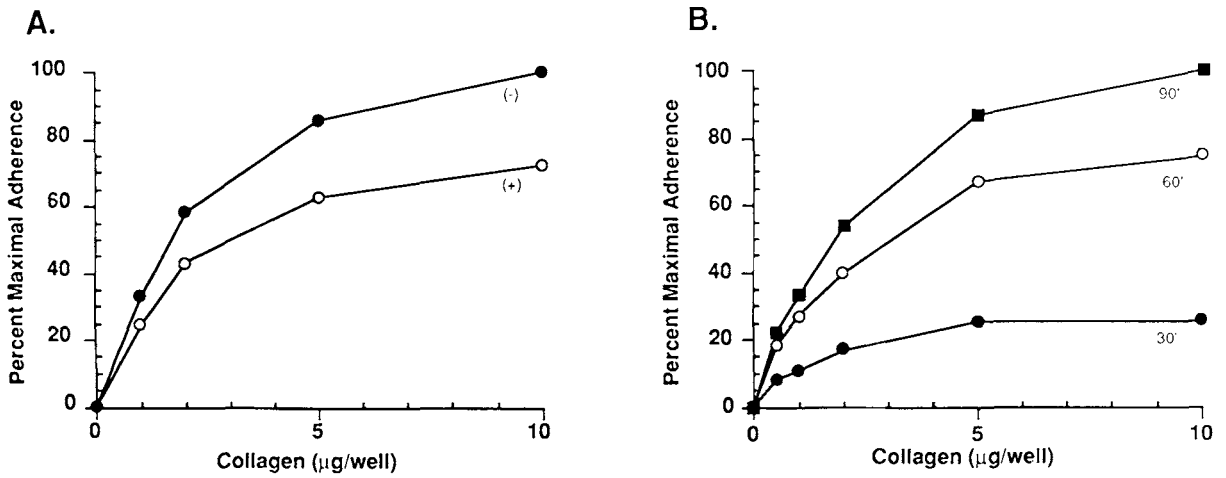

Fig. 1. **A:** Effect of different concentrations of collagen on platelet adhesion to collagen-coated microtiter plates at 20°C. The microtiter plates were coated for 2 hr with different concentrations of collagen as described in Methods. Platelets prelabeled with ^{3}H-oleic acid were incubated in the wells in the presence (+) or absence (−) of RGDS (100 μg/ml) for 60 min. The results shown are the mean of triplicate determinations from one experiment representative of two; 100% adhesion represents 17.2% of the number of platelets added. **B:** Effect of different concentrations of collagen and of incubation times on platelet adhesion to collagen-coated microtiter plates at 20°C. Incubations were for different times with ^{3}H-oleic acid labeled platelets in the presence of 100 μg/ml RGDS. The results shown are the mean of triplicate determinations from one experiment representative of two; 100% adhesion represents 15.7% of the number of platelets added.

inhibitors of actin polymerization (Fox and Phillips, 1981).

Suspension Assay

To study the kinetics of the effects of cytochalasin D, energy depletion, and BAPTA on platelet adhesion, a suspension assay was used in which platelet adhesion to collagen is essentially complete by about 2 min at 37°C (Smith and Dangelmaier, 1990). Figure 3A shows that cytochalasin D markedly inhibited the rate of platelet attachment to collagen during the first ten minutes of exposure but had almost no effect at longer incubation times. Similar but even more pronounced kinetic effects were observed when the adhesion of energy-depleted-treated platelets to collagen was studied (Fig. 3A). On the other hand, pretreatment of platelets with BAPTA had little effect on the rate or extent of platelet adhesion to collagen (Fig. 3B).

Signal Transduction

A major advantage of the suspension assay in studying the adhesion of platelets to collagen is that changes in cytosolic Ca^{2+} can be measured simultaneously. Figure 4 shows that collagen is able to elicit cytosolic Ca^{2+} mobilization in cells despite the presence of antagonists which block the Ca^{2+} mobilization induced by ADP and TxA_2. It can be seen that the extent of Ca^{2+} mobilization increases markedly as the collagen concentration is increased from 5 μg/ml to 50 μg/ml. Figure 5 shows that, in a concentration-related fashion, cytochalasin D reduces cytosolic Ca^{2+} mobilization induced by 50 μg/ml collagen from 140 nM to approximately 40 nM. No Ca^{2+} mobilization was observed in two experiments when energy-depleted platelets were treated with 50 μg/ml collagen while BAPTA treatment inhibited the increase in $[Ca^{2+}]_i$ by 80–100% (2 experiments). Treatment of platelets with ionomycin (1 μM) after the addition of 50 μg/ml collagen had little effect on the adhesion of platelets to collagen (Fig. 6). This was also true when ionomycin was added before the collagen (results not shown).

DISCUSSION

In 1970, it was observed that the antimotility agent cytochalasin B inhibited clot retraction in platelet-rich plasma (Shepro et al., 1970). Subsequently, evidence was provided that cytochalasins D and E, and to a lesser extent cytochalasin B, inhibit the rapid polymerization of actin in human platelets observed after thrombin stimulation (Fox and Phillips, 1981; Casella et al., 1981). It is generally agreed that cytochalasins

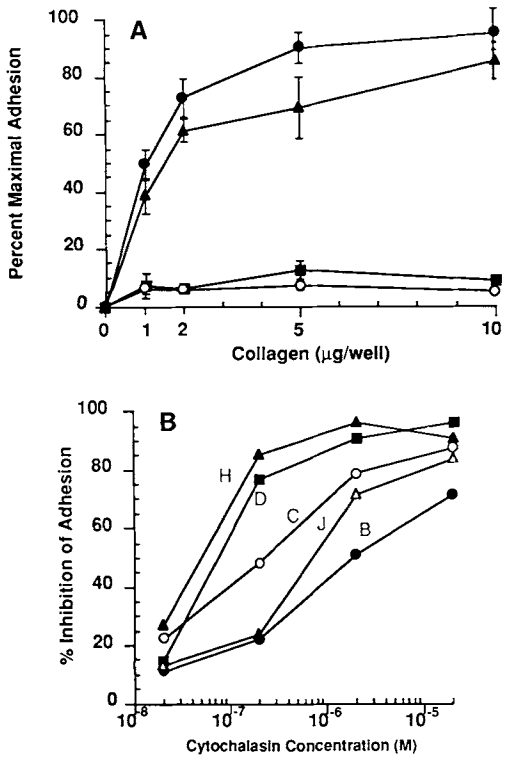

Fig. 2. **A:** Effect of energy depletion, cytochalasin D, or BAPTA on platelet adhesion to collagen-coated microtiter plates at 20°C. Platelets were incubated for 60 min in microtiter plates precoated with different concentrations of collagen in the presence of 100 μg/ml RGDS. The platelets were either untreated (closed circles), depleted of metabolic energy (open circles), preincubated with 100 μM BAPTA (closed triangles), or treated with 20 μM cytochalasin D (closed squares). The results shown are the mean of triplicate determinations from one experiment representative of three. 100% adhesion represents 12.2% of the number of platelets added. **B:** Comparison of different cytochalasins (marked on the figure) for their ability to inhibit the adhesion of platelets to collagen. The results shown or the mean of triplicate determinations from one experiment representative of two.

prevent assembly of actin and actin-binding protein bundles in platelets (Carroll et al., 1982; Hartwig and Stossel, 1979; Wheeler et al., 1984). Cytochalasins, however, do not inhibit thrombin-induced secretion or protein phosphorylation (Carroll et al., 1982).

We report here that cytochalasins and depletion of platelet metabolic energy inhibit platelet adherence to collagen. At 20°C in the microtiter plate assay, the effect was almost absolute during the 60-min incubation time. However, at 37°C in the suspension assay, the treated platelets were able to adhere to collagen almost as well as control platelets, given sufficient time. The enhanced sensitivity of the microtiter plate assay as compared with the suspension assay to measure the effects of cytochalasin D or energy depletion is presumably due to the lower agonist concentration, the lower temperature and the lack of stirring. In keeping with this, adhesion of control platelets was still occuring at 60 min and accounted for less than 20% of the number of platelets added in the microtiter plate assay (Fig. 1B), while it was essentially complete at 2 min and accounted for 56% of the number of platelets added in the suspension assay (Fig. 3A). The inhibitory treatments therefore do not appear to act by reducing the number of receptors available for adhesion to collagen, but rather by decreasing the rate at which the platelets bind to collagen. We postulate that this is because, after initial reversible association of an individual receptor on a platelet with collagen, rapid actin-dependent spreading is necessary to produce sufficient interaction at multiple receptors to prevent dissociation. A model for the adhesion of rat hepatocytes to collagen, where the formation of stable attachment bonds requires the binding of several low-affinity receptors clustered at the site of adhesion to collagen molecules in the substrate has been proposed previously (Rubin et al., 1981). A similar model also has been proposed for the attachment of platelets to collagen (Santoro and Cunningham, 1984).

The fact that sugars and sugar phosphates did not inhibit adhesion in our experiments indicate that lectins are not involved in the binding process. The weight of evidence at the present time indicates that the receptors involved in the Mg^{2+}-dependent binding of platelets to collagen consist of the integrin dimer, GPIa/IIa (Staatz et al., 1989). Based on their findings with cytochalasins B, D, and E, Nakano et al. (1989) proposed that the linkage of GPIa with actin filaments may play an important role in collagen-receptor mediated signal transduction. However, while Nakano et al. (1989), using low concentrations of collagen, observed that cytochalasins completely inhibited collagen-induced Ca^{2+} mobilization, the present studies show that cytochalasin D inhibits Ca^{2+} mobilization by a maximum of 70% (Fig. 5) and delays rather than abolishes platelet adhesion to collagen at 37°C (Fig. 3A). Thus, it appears that cytochalasin D does not prevent the initial Ca^{2+} signal associated with the first association of a platelet with collagen, but does prevent the subsequent Ca^{2+}-

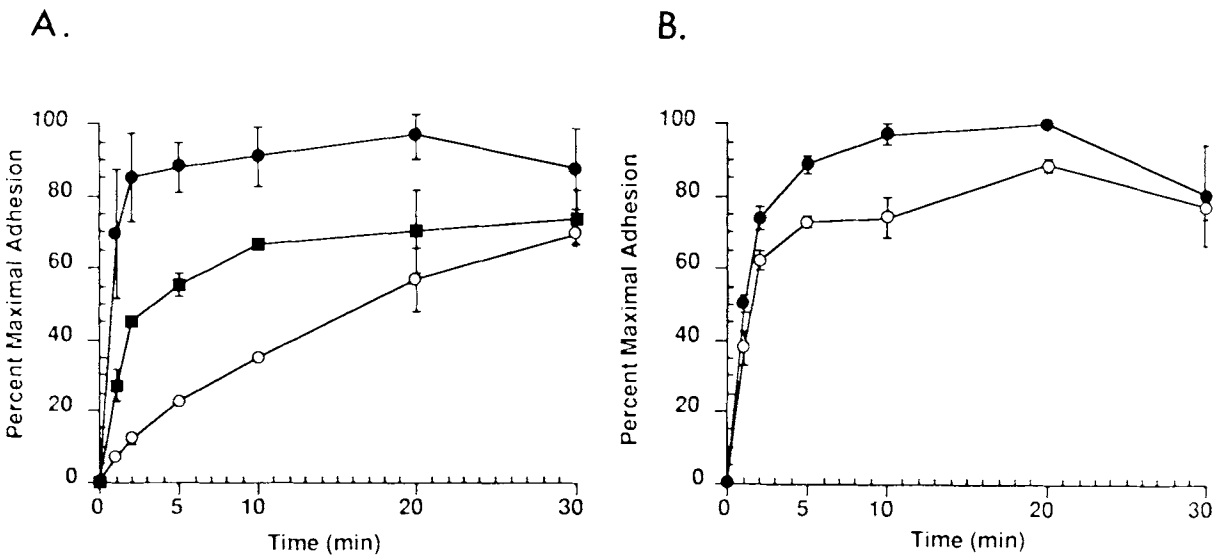

Fig. 3. **A:** Effect of energy depletion or cytochalasin D on platelet adhesion to 50 μg/ml collagen in suspension at 37°C. The platelets were untreated (closed circles), depleted of metabolic energy (open circles), or treated with 20 μM cytochalasin D (closed squares). The experiments were performed in the presence of SQ 29548, creatine phosphate/creatine phosphokinase, and RGDS to prevent feedback by released agonists. Individual values and mean of two experiments are shown; 100% adhesion represents 56% of the number of platelets added. **B:** Effect of BAPTA on platelet adhesion to 50 μg/ml collagen in suspension at 37°C. The platelets were either untreated (closed circles) or treated with 100 μM BAPTA (open circles). Individual values and mean of two experiments are shown; 100% adhesion represents 39% of the number of platelets added. Other details were as in Fig. 3A.

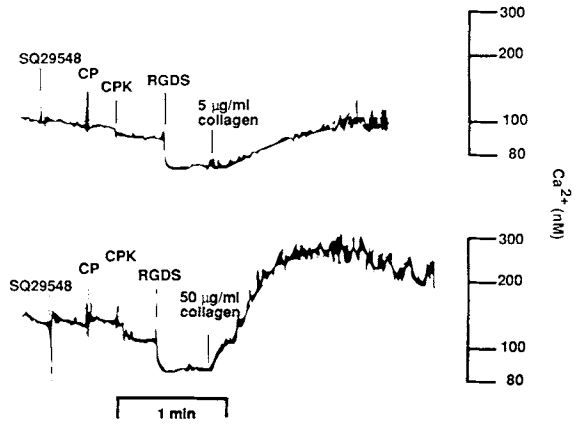

Fig. 4. Platelet cytosolic Ca^{2+} mobilization induced by collagen in suspension at 37°C in the presence of SQ 29548, creatine phosphate (CP)/creatine phosphokinase (CPK), and RGDS. In separate experiments it was established that the presence of the inhibitors blocked Ca^{2+} mobilization induced by 5 μM U46619 and 10 μM ADP. Typical of ten experiments.

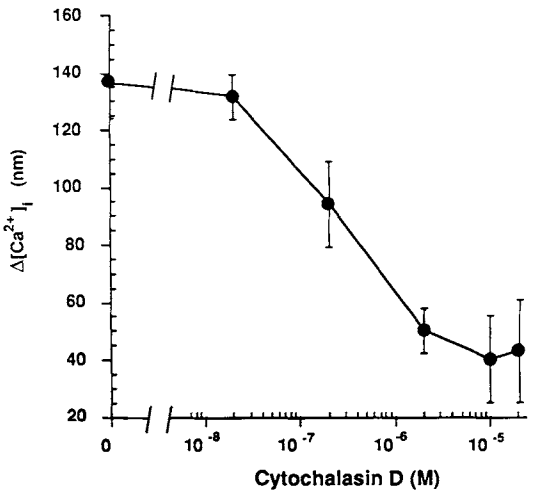

Fig. 5. Inhibition of cytosolic Ca^{2+} mobilization by cytochalasin D. Platelets prelabeled with fura-2 were incubated with 50 μg/ml collagen in suspension at 37°C in the presence of inhibitors of feedback pathways. The maximal increase in cytosolic $[Ca^{2+}]_i$ above basal was determined at each cytochalasin D concentration. Mean values from two experiments are shown.

mobilization associated with platelets spreading along collagen fibers. We previously reported that collagen itself can elicit phosphatidic acid formation (Smith and Dangelmaier, 1990). Phosphatidic acid is thought to be formed as a result of stimulation of the phosphatidylinositol cycle, an event which also results in the transient increase in inositol 1,4,5-trisphosphate. Thus, at least some of the increase in $[Ca^{2+}]_i$ that occurs after stimulation of platelets with collagen presumably is due to the release of Ca^{2+} from internal stores.

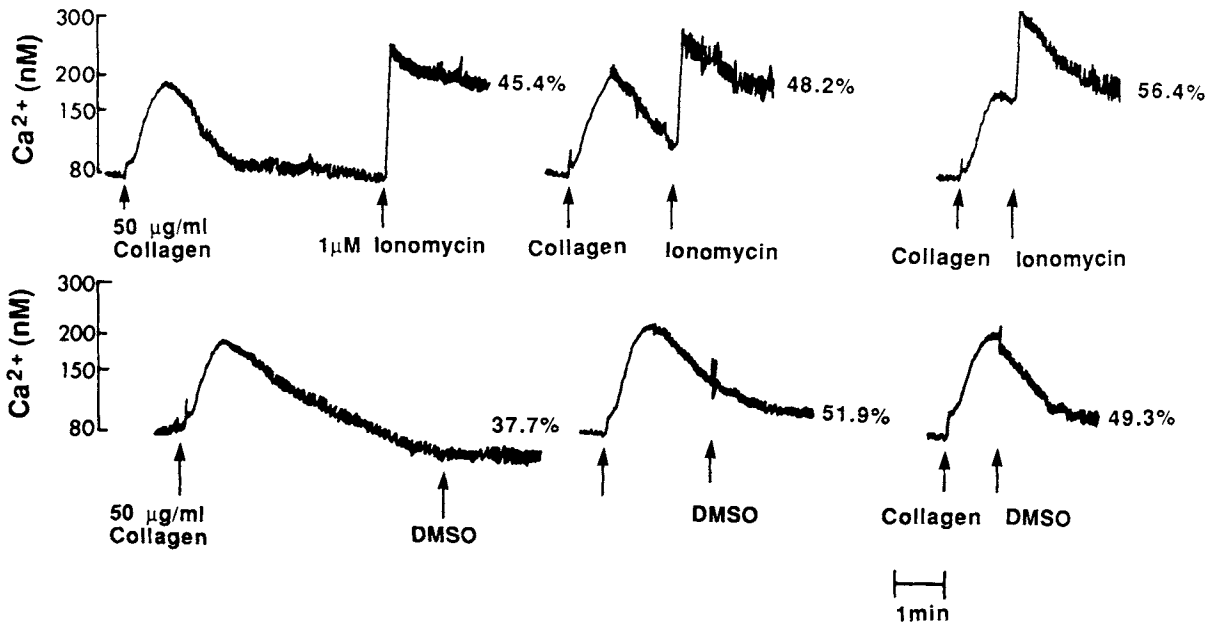

Fig. 6. Lack of effect of the calcium ionophore ionomycin on platelet adhesion to collagen. The scales on the ordinate indicate the $[Ca^{2+}]_i$ in nM as in Figure 4. The percentages refer to the number of platelets adhering to collagen at the end of the tracing.

The present study shows that collagen is able to elicit Ca^{2+} mobilization even in the presence of inhibitors of feedback pathways and that this increase in $[Ca^{2+}]_i$ depends to a major extent on processes which require metabolic energy and can be inhibited by cytochalasin D. On the other hand, the intracellular calcium chelator BAPTA had little effect on the rate of platelet adhesion to collagen (Figs. 2A and 3B), indicating that the increase in $[Ca^{2+}]_i$ is a consequence of the adhesive process and not essential for it to occur. This conclusion is supported by the observation that elevation of $[Ca^{2+}]_i$ with ionomycin, after the addition of collagen, had little effect on adhesion (Fig. 6). Moreover, in contrast to other soluble platelet agonists which elicit a very rapid Ca^{2+} mobilization, collagen evokes a slow but progressive elevation of the cytosolic free Ca^{2+} concentration (Fig. 4). The slower kinetics are consistent with those of a particulate agonist in which receptor occupancy is governed not only by the period of time in which a given platelet needs to associate with the available collagen, but also by the active movement of receptors (spreading) within the platelet so that it remains adhered. We hypothesize that the increase in cytosolic free Ca^{2+} observed is required for subsequent responses, such as secretion and arachidonate liberation to occur.

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REFERENCES

Aster RH, Jandl JH (1964): Platelet sequestration in man I. Methods. J Clin Invest 43:843–855.

Carroll RC, Butler RG, Morris PA, Gerrard JM (1982): Separable assembly of platelet pseudopodal and contractile cytoskeletons. Cell 30:385–393.

Casella JF, Flanagan MD, Lin S (1981): Cytochalasin D inhibits actin polymerization and induces depolymerization of actin filaments formed during platelet shape change. Nature 293:302–305.

Dangelmaier C, Daniel JL, Smith JB (1986): Determination of basal and stimulated levels of inositol trisphosphate in [^{32}P]-orthophosphate-labeled platelets. Anal Biochem 154: 414–419.

Fox JEB, Phillips DR (1981): Inhibition of actin polymerization in blood platelets by cytochalasins. Nature 292:650–652.

Hartwig JH, Stossel TP (1979): Cytochalasin B and the structure of actin gels. J Mol Biol 134:539–553.

Kinlough-Rathbone RL, Packham MA, Reimers HJ, Cazenave JP, Mustard JF (1977): Mechanisms of platelet shape change, aggregation, and release induced by collagen, thrombin or A23,187. J Lab Clin Med 90:707–712.

Lages B, Scrutton MC, Holmsen H (1975): Studies on gel filtered platelets: Isolation and characterization in me-

dium containing no Ca, Mg, or K. J Lab Clin Med 85:811–825.

Nakano T, Hanasaki K, Arita H (1989): Possible involvement of cytoskeleton in collagen-stimulated activation of phospholipases in human platelets. J Biol Chem 264:5400–5406.

Pollock WK, Rink TJ, Irvine RF (1986): Liberation of [^{3}H]arachidonic acid and changes in cytosolic free calcium in fura-2 loaded platelets stimulate by ionomycin and collagen. Biochem J 235:869–877.

Rubin K, Hook M, Obrink B, Timpi R (1981): Substrate attachment of rat hepatocytes: Mechanism of attachment to collagen substrates. Cell 24:463–470.

Santoro SA (1986): Identification of a 160,000 dalton platelet membrane protein that mediates the initial divalent cation-dependent adhesion of platelets to collagen. Cell 46:913–920.

Santoro SA, Cunningham LW (1977): Collagen-mediated platelet aggregation. Evidence for multivalent interactions of intermediate specificity between collagen and platelets. J Clin Invest 60:1054–1060.

Shadle PA, Barondes SH (1982): Adhesion of human platelets to immobilized trimeric collagen. J Cell Biol 95:361–365.

Shepro D, Belamarich FA, Robblee L, Chao FC (1970): Antimotility effect of cytochalasin B observed in mammalian clot retraction. J Cell Biol 47:544–547.

Smith JB, Dangelmaier C (1990): Determination of platelet adhesion to collagen and the associated formation of phosphatidic acid and calcium mobilization. Anal Biochem. 187:173–178.

Staatz WD, Rajpara SM, Wayner EA, Carter WG, Santoro SA (1989): The membrane glycoprotein Ia-IIa (VLA-2) complex mediates the Mg^{2+}-dependent adhesion of platelets to collagen. J Cell Biol 108:1917–1924.

Tandon NN, Kralisz V, Jamieson GA (1989): Identification of glycoprotein IV (CD36) as a primary receptor for platelet-collagen adhesion. J Biol Chem 264:7576–7583.

Watson SP, Reep B, McConnell RT, Lapetina EG (1985): Collagen stimulates [^{3}H] inositol trisphosphate formation in indomethacin-treated human platelets. Biochem J 226: 831–837.

Wheeler ME, Cox AC, Carroll RC (1984): Retention of the glycoprotein IIb-IIIa complex in the isolated platelet cytoskeleton. J Clin Invest 74:1080–1089.