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Initiation of RVD Response in Human Platelets: Mechanical-Biochemical Transduction Involves Pertussis-Toxin-Sensitive G Protein and Phospholipase A_2

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Abstract. Platelets revert hypotonic-induced swelling by the process of regulatory volume decrease (RVD). We have recently shown that this process is under the control of endogenous hepoxilin A_3. In this work, we investigated the mechanical-biochemical transduction that leads to hepoxilin A_3 formation. We demonstrate that this process is mediated by pertussis-toxin-sensitive G protein, which activates Ca^{2+}-insensitive phospholipase A_2, and the sequential release of arachidonic acid. This conclusion is supported by the following observations: (i) RVD response is blocked selectively by the phospholipase A_2 inhibitors manoalide and bromophenacyl-bromide (0.2 and 5 μM, respectively) but not by phospholipase C inhibitors. The addition of arachidonic acid overcame this inhibition; (ii) extracellular Ca^{2+} depletion by EGTA (up to 10 mM) does not affect RVD; (iii) intracellular Ca^{2+} depletion by BAPTA-AM (100 μM) inhibits RVD but not hepoxilin A_3 formation, as tested by the RVD reconstitution assay; (iv) RVD is inhibited by the G-protein inhibitors, $GDP_\beta S$ (1 μM) and pertussis toxin (1 ng/ml). This inhibition is overcome by addition of arachidonic acid or hypotonic cell-free eluate that contains hepoxilin A_3; (v) NaF, 1 mM, induces hepoxilin A_3 formation, tested by the RVD reconstitution assay; and (vii) $GDP_\beta S$ inhibits hepoxilin A_3 formation associated with flow. Therefore, it seems that G proteins are involved in the initial step of the mechanical-biochemical transduction leading to hepoxilin A_3 formation in human platelets.

Key words: Human platelets — Regulatory volume decrease — Hepoxilin A_3 — Phospholipase A_2 — G proteins — Mechanically induced activation

Introduction

When human blood platelets are exposed to hypotonic medium, they swell first, but, shortly thereafter, revert toward their original volume, expressing the process of regulatory volume decrease (RVD). Platelet RVD is mediated by enhanced independent K^+ and Cl^- effluxes and associated water (Livne, Grinstein & Rothstein, 1987). The RVD response of human platelets is controlled by a distinct lipoxygenase-derived product, which elevates exclusively K^+ current (Margalit & Livne, 1991). This product has been identified as hepoxilin A_3 (Margalit et al., 1993). Hepoxilin A_3 was also detected after submission of platelets to centrifugation and laminar flow (Margalit & Livne, 1992), which indicates that hepoxilin A_3 metabolism in human platelets is initiated by a common mechanical-biochemical transduction mechanism.

Although the RVD response has been investigated in many cell types, the mechanisms by which the cells sense volume change and initiate RVD response are not well understood (for review, *see:* Hoffmann & Simonsen, 1989; Sarkadi & Parker, 1991). Stretch-activated K^+ channels (Sackin, 1989) or stretch-activated Ca^{2+} channels (Christensen, 1987; Lansman, Hallam & Rink, 1987) have been suggested as mechanoreceptors for RVD, but since these experiments were done by patch clamping of membranes, it is not clear if such mechanisms exist in vivo. At least in human platelets, stretch-activated K^+ channels are not involved, since K^+ cur-

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rents associated with RVD are inhibited by lipoxygenase inhibitors (Margalit & Livne, 1991).

Hepoxilin A_3 is an arachidonic acid (AA) metabolite of the 12-lipoxygenase pathway (Pace-Asciak & Asorta, 1989). Its biosynthesis in RVD would be initiated by the release of AA from the cell membrane by phospholipases. Several mechanisms of the activation of phospholipases have been suggested, including $[Ca^{2+}]_i$ elevation (Baron & Limbird, 1988), phosphorylation (Wijkander & Sundler, 1992, Nemenoff et al., 1993) and involvement of GTP-binding proteins (Axelrod, 1990; Fain, 1990).

In this work, we study the mechanism by which human platelets initiate RVD response. It is demonstrated that hepoxilin A_3 metabolism in intact platelets is initiated by Ca^{2+}-insensitive phospholipase A_2 (PLA_2). This phospholipase is activated by pertussis-toxin-sensitive G protein. On the basis of our findings, we suggest that this G protein is activated by mechanical stresses, probably by G-protein-associated mechanoreceptors.

Materials and Methods

Reagents and Solutions

Nordihydroguaiaretic acid (NDGA), guanosine 5′-*O*-(2-thiodiphosphate) ($GDP_\beta S$), pertussis toxin (PTX), neomycin sulfate, staurosporine and arachidonic acid (AA) were obtained from Sigma (St. Louis, MO). Nicotinamide adenine [adenylate-^{32}P] dinucleotide (^{32}P NAD) was obtained from Amersham (Buckinghamshire, UK). [bis-(*o*-aminophenoxy)-ethane-N,N,N′,N′-tetraacetic acid, tetra(acetoxymethyl)-ester] (BAPTA-AM) was obtained from Molecular Probes (Eugene, OR). Wortmannin was purchased from Sandoz (Basel, Switzerland). Manoalide was kindly provided by Dr. U. Zor, The Weizmann Institute of Science, Rehovot, Israel. The acid-citrate-dextrose (ACD) solution was composed of 65 mM citric acid, 11 mM glucose and 85 mM trisodium citrate. The standard isotonic medium contained (mM) 137 NaCl, 1 KCl, 0.42 NaH_2PO_4, 0.5 $MgCl_2$, 5.5 glucose, and 20 HEPES, pH 7.4, adjusted to 285 mOsm. Hypotonic solutions were prepared by 1 : 1 dilution of the standard isotonic medium with distilled water. Media were filtered through a 1.2 μ membrane filter (Schleicher & Schuell, AE 9S) to remove particles that interfere with the cell sizing measurement. Stock solution of NDGA (20 mM), AA (10 mM) and manoalide (1 mM) were made in ethanol, stock solutions of $GDP_\beta S$ (1 mM) and PTX (0.1 μg/ml) were made in water. Stock solutions of wortmannin (100 mM), neomycin (100 mM), and BAPTA-AM (50 mM) were made in dimethyl sulfoxide. The organic solvent concentrations in the experiments did not exceed 0.2%. Incubation of platelets with the organic solvents (up to 60 min) did not, by itself, affect RVD.

Preparation of Platelet-rich Plasma (PRP)

Venous blood was obtained from healthy volunteers, aged 20–40 years, who had not taken any medication in the preceding 14 days. Blood was collected in plastic tubes with ACD solution at a volume ratio of blood/anti-coagulant of 6/1. PRP was obtained by centrifugation at 150 × *g* for 10 min and had a pH of 6.5 ± 0.1. Platelets were used within 3 hr of the collection time.

Volume Measurements and RVD Assessments

Cell volume distribution curves were obtained using a Coulter Counter model ZM with Coulter Channelizer 256, with an orifice diameter of 70 μm. The mean cell volume was calculated as the median of the cell volume distribution curves. Volume measurements commenced within 20 sec after the addition of platelets and lasted for 3 min. The relative volume relates to the cell volume in isotonic medium. The RVD rate was determined by the logarithmic change in relative volume during the time interval of 40–150 sec after the exposure of the platelets to hypotonic medium.

RVD Reconstitution Assays

The RVD reconstitution assays were based on the ability of endogenous hepoxilin A_3, present in cell-free eluates, to reform RVD of platelets in the presence of lipoxygenase inhibitors (Margalit et al., 1993). Hypotonic cell-free eluates were prepared as follows: aliquots of 200 μl PRP, containing 4–5 × 10^7 platelets, were mixed with an equal volume of distilled water and 600 μl of hypotonic solution. After 15 sec (or as indicated), NDGA was added and the suspension was centrifuged in a Juan microfuge (Hawksley, UK) for 30 sec. A sample of 200 μl of the cell-free eluate was added prior to PRP, to a Coulter vial containing 10 ml of hypotonic solution in the presence of various RVD inhibitors. The volume measurements were preformed as described above. Flow-derived cell-free eluates were prepared as follows: aliquots of 200 μl PRP, containing 4–5 × 10^7 platelets, were mixed with 800 μl of isotonic solution. The suspensions were drawn into a 1 ml syringe and exposed to flow stress by utilizing a "controlled-flow device" as described previously (Margalit & Livne, 1992). Thereafter, the cell-free eluates were prepared as described for hypotonic eluate and tested for their ability to reconstitute RVD.

ADP-Ribosylation

PRP (20 ml, 4 × 10^8 cells/ml) were centrifuged once (750 × *g*, 10 min) and the platelet pellet was suspended in 4 ml of isotonic solution in the presence of 0.3% bovine serum albumin (Sigma). For each experiment, 475 μl of the platelet suspension was mixed with an equal volume of isotonic solution (control) or distilled water (for hypotonic shock). The incubation was terminated, at the indicated times, by the addition of 50 μl of 20 × ADP-ribosylation cocktail, as described by Lapetina, Reep and Chang (1986), 1 ×: 25 μg/ml saponin, 5 μg/ml pertussis toxin (preactivated with 1 mM dithiothreitol), 20 μM NAD, 10 μCi/ml ^{32}P-NAD, 1 mM ATP, 1 mM EDTA and 10 mM thymidine. The suspension was incubated for 30 min, 37°C. After incubation, saponized platelets were fractionated by centrifugation for 160 sec in a Juan microfuge (Hawksley, UK). The supernatant was discarded and the pellet resuspended in 100 μl of sample buffer, consisting of 3% SDS, 0.0015% bromophenol blue, 5% 2-mercaptoethanol, 11% glycerol, 70 mM Tris HCl, pH 6.8. The samples were heated to 100°C

for 5 min and subjected to 11% SDS-PAGE. ADP-ribosylation of proteins was determined by autoradiography.

Results

Phospholipase A_2 Initiates Hepoxilin A_3 Metabolism

To study the role of different phospholipases in platelet RVD, we incubated 10 μl of PRP for 30 min in 5 ml of isotonic solution in the presence of different phospholipases inhibitors. At the end of the incubation, the platelets were submitted to hypotonic shock by the addition of 5 ml of distilled water, and tested for RVD. The phospholipase A_2 (PLA_2) inhibitor manoalide (Lister et al., 1989), inhibited RVD in a dose-dependent manner. Complete inhibition of RVD was obtained at 0.2 μM (Fig. 1*A*). Platelet-RVD was also inhibited by the PLA_2 inhibitor, bromophenacyl bromide (Blackwell & Flower, 1983). By contrast, the phospholipase C inhibitor, neomycin (Burch, Luini & Axelrod, 1986) and the phospholipase C/D inhibitor, wortmannin (Bonser et al., 1991), were ineffective (Table). As seen in Fig. 1*B*, addition of AA (1 μM) to platelets pretreated with manoalide (0.2 μM) restored the RVD response. By contrast, AA was ineffective when RVD was inhibited with the lipoxygenase inhibitor NDGA (20 μM), which acts downstream to AA liberation (Fig. 7). We conclude, therefore, that phospholipase A_2 modulates arachidonic acid liberation and hepoxilin A_3 metabolism in response to hypotonic shock.

Ca^{2+} Mobilizing and Protein Kinase Inhibitors Do Not Affect RVD Response

To evaluate a possible role of Ca^{2+} influxes on the initiation of RVD response, we exposed platelets to standard hypotonic solution in the presence of the Ca^{2+} chelator EGTA at concentrations of 1 and 10 mM. As seen in Fig. 2*A*, EGTA (10 mM) did not affect platelet RVD. These results are in agreement with the EGTA effect on RVD response of lymphocytes (Grinstein, Dupre & Rothstein, 1982) and Ehrlich ascites tumor cells (Hoffmann, Simonsen & Lambert, 1984). To study the role of intracellular Ca^{2+} on RVD, we incubated human platelets for 60 min with 100 μM of the intracellular Ca^{2+} chelator BAPTA-AM (Smith et al., 1992). At the end of the incubation, the platelets were subjected to hypotonic shock and tested for RVD response. As seen in Fig. 2*B*, BAPTA-AM did indeed inhibit platelet RVD. To study if the Ca^{2+} effect on platelet RVD is downstream or upstream to hepoxilin A_3 formation (*cf.* Fig. 7.), we submitted platelets pretreated with BAPTA-AM to the RVD reconstitution assay. As seen in Fig. 2*C*, addition of hypotonic cell-free eluate from platelets pretreated with BAPTA-AM, an indication for endogenous hepoxilin A_3 production, resumes RVD of platelets in the presence of NDGA. By contrast, addition of hypotonic-derived eluate to platelets pretreated with BAPTA-AM, an indication for external hepoxilin A_3 effect, did not restore RVD (*data not shown*). These observations indicate that Ca^{2+} depletion by BAPTA-AM does not block hepoxilin A_3 synthesis, but rather inhibits the effect of hepoxilin A_3 on platelet RVD.

Lin, Lin and Knopf (1992) reported that the serine/threonine kinase inhibitor, staurosporine, inhibits AA release by cytosolic PLA_2. To test the

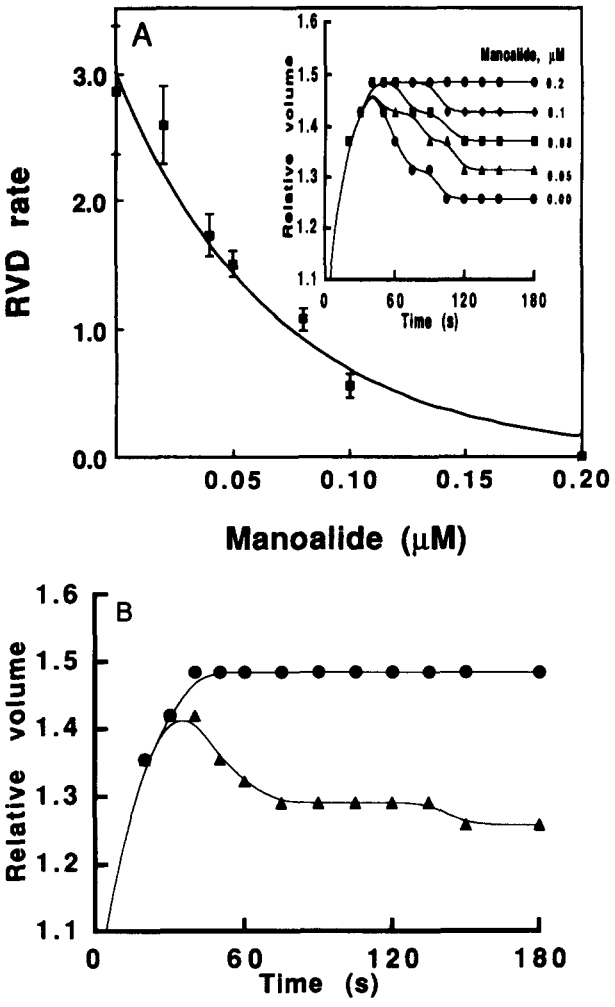

Fig. 1. Effect of manoalide on platelet RVD. (*A*) Inhibition of RVD rate by manoalide (mean ± SE, $n = 3$). RVD rate is determined as the log (relative vol/sec) × 10^3. Representative experiment is shown in the inset. (*B*) RVD reconstitution by 1 μM arachidonic acid of platelets pretreated with manoalide 0.2 μM (▲); or NDGA, 20 μM (●). (representative data, $n = 3$).

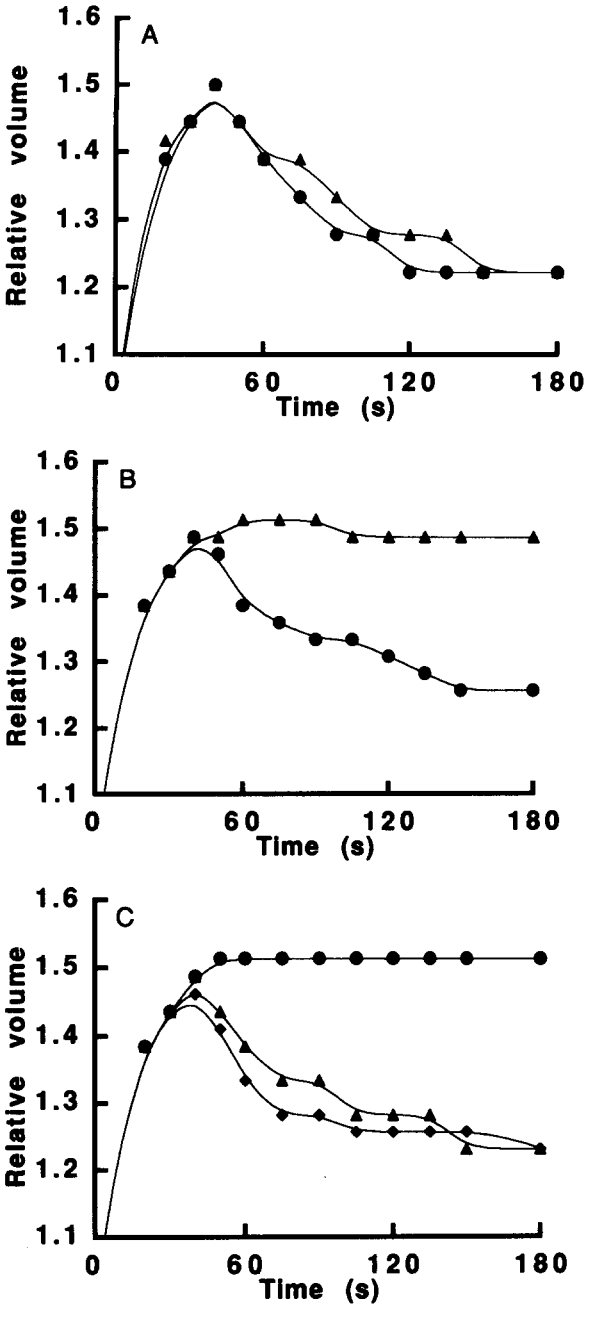

Fig. 2. Effect of Ca^{2+} on platelet RVD. (*A*) External Ca^{2+} depletion by EGTA. Control, (●); EGTA 10 mM, (▲) (representative data, $n = 3$). (*B*) Internal Ca^{2+} depletion by BAPTA-AM. Control, (●); platelets pretreated with BAPTA-AM (100 μM, 60 min), (▲) (representative data, $n = 3$). (*C*) RVD reconstitution assay in the presence of NDGA (20 μM). Isotonic cell-free eluate, (●); hypotonic cell-free eluate (exogenous hepoxilin A_3), (◆); hypotonic cell-free eluate, platelets pretreated with BAPTA-AM for 60 min, (▲) (representative data, $n = 3$).

effect of this inhibitor on hepoxilin A_3 metabolism, we incubated platelets with increasing concentrations of staurosporine before submission to hypotonic shock. As shown in the Table, staurosporine,

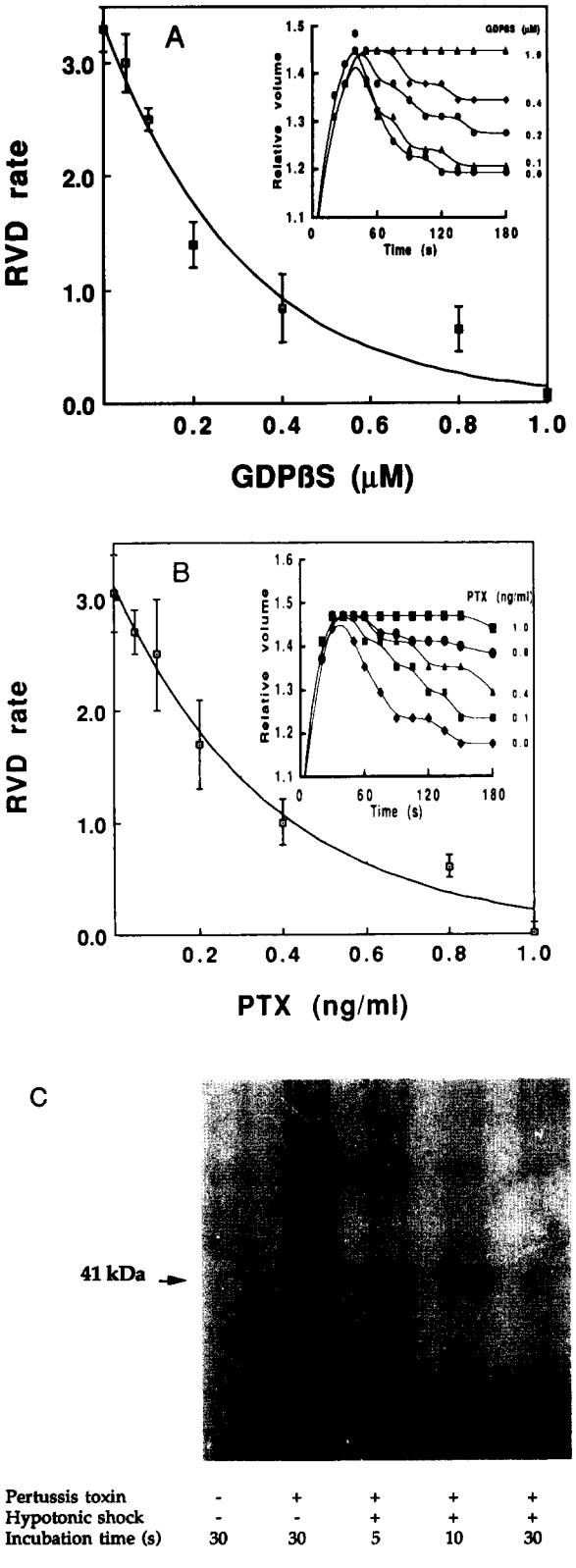

Fig. 3. Inhibition of RVD rate by (*A*) $GDP_\beta S$ (mean ± SE, $n = 3$). (*B*) PTX (mean ± SE, $n = 4$). RVD rate is determined as in Fig. 1; representative experiments are shown in the insets. (*C*) Inhibition of PTX-induced ADP-ribosylation by hypotonic shock as a function of incubation time.

Table. The effect of different inhibitors on RVD of human platelets

Compound	Site	Effect on RVD
Manoalide	Phospholipase A_2	Inhibition, IC_{50} = 0.076 ± 0.004 μM*
Bromophenacyl bromide	Phospholipase A_2	Inhibition, IC_{50} = 1.2 ± 0.3 μM*
Neomycin	Phospholipase C	No inhibition at 200 μM
Wortmannin	Phospholipase C/D	No inhibition at 20 μM
Staurosporine	Serin/threonine kinases	No inhibition at 10 μM

* Mean ± SE.

up to 10 μM, did not affect RVD. At this concentration, staurosporine inhibits both serine/threonine as well as tyrosine kinase activities (Fujita-Yamaguchi & Kathuria, 1988).

Pertussis Toxin and $GDP_\beta S$ Inhibit Platelet RVD

Human PRP (10 μl, 3–4 × 10^6 cells), were incubated for 30 min in 5 ml of isotonic solution in the presence of various concentrations of $GDP_\beta S$ or PTX. At the end of the incubation, we added 5 ml of distilled water and performed volume measurements. As seen in Fig. 3*A* and *B*, both compounds inhibited the RVD response in a dose-dependent manner. Complete inhibition of RVD was obtained with 1 μM $GDP_\beta S$ and 1 ng/ml PTX, indicating the high sensitivity of this response to G-protein inhibitors. Moreover, a hypotonic shock of 10 sec, inhibited ADP-ribosylation induced by PTX (Fig. 3*C*). This inhibition is associated with G-protein activation and the dissociation of α and βγ subunits (Lapetina et al., 1986; Gennity & Siess, 1991). The conclusion that G proteins are involved in RVD response is in agreement with the finding of le Maout et al. (1990) in rabbit kidney cells. Since hepoxilin A_3 is released into the medium in response to hypotonic shock (Margalit & Livne, 1991; Margalit et al., 1993) and may act externally on the cell surface, G proteins may be involved in either the hypotonic shock-induced formation of hepoxilin A_3, or the increased K^+ permeability induced by hepoxilin A_3, or both. To distinguish between these possibilities, we tested hepoxilin A_3 formation of platelets pretreated with $GDP_\beta S$ (1 μM, 30 min) by the RVD reconstitution assay. As demonstrated in Fig. 4*A*, addition of external hepoxilin A_3 (hypotonic-derived eluate) restored RVD of platelets pretreated with $GDP_\beta S$. By contrast, when the cell-free eluates were prepared from platelets pretreated with $GDP_\beta S$, no hepoxilin A_3 production could be detected by the RVD reconstitution assay. We conclude, therefore, that G proteins are involved in the formation of hepoxilin A_3 rather than in its effect. To determine if G proteins are involved prior to AA release, we added 1 μM of

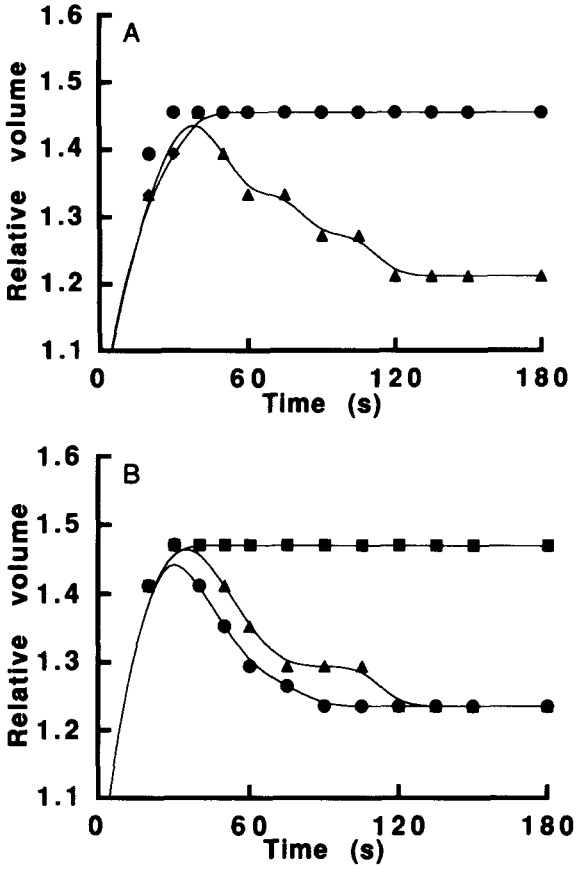

Fig. 4. (*A*) RVD reconstitution of platelets pretreated with 1 μM $GDP_\beta S$, by platelet-derived hypotonic eluate (▲), or hypotonic-derived eluate from platelets pretreated with $GDP_\beta S$ (●) (representative data, n = 3). (*B*) The effect of AA (1 μM) on RVD response of platelets pretreated with $GDP_\beta S$, 1 μM (●); PTX, 1 ng/ml (▲); or NDGA, 20 μM (■) (representative data, n = 3).

AA to platelets pretreated with $GDP_\beta S$, PTX or the lipoxygenase inhibitor NDGA. As seen in Fig. 4*B*, addition of AA restored RVD of platelets pretreated with $GDP_\beta S$, or PTX but not with NDGA (*cf.* Fig. 7).

Effect of NaF on Hepoxilin A_3 Production

NaF is a known activator of G proteins (Bigay et al., 1987; Brom et al., 1989). To test if G-protein-

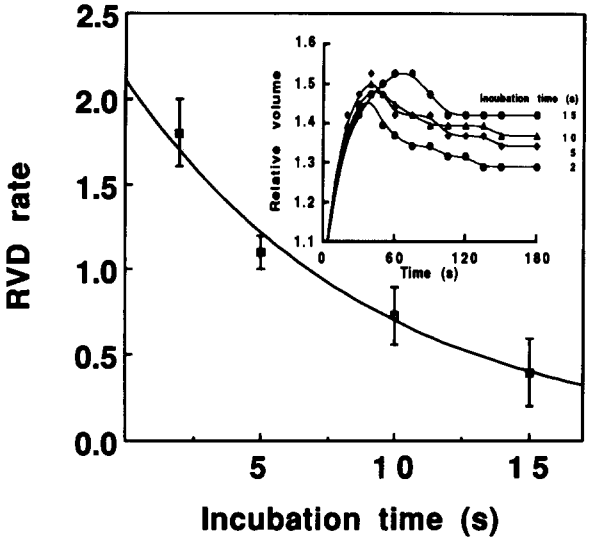

Fig. 5. NaF (1 mM) effect on hepoxilin A_3 production as a function of incubation time. Hepoxilin A_3 production is measured by the RVD reconstitution assay and expressed as RVD rate (mean ± SE, n = 3). Representative experiment is shown in the inset.

activation by NaF results in hepoxilin A_3 formation, we assayed cell-free eluates from platelets exposed to 1 mM NaF during various incubation times for RVD reconstitution. As seen in Fig. 5, cell-free isotonic eluates from platelets preincubated with NaF reconstitute RVD of platelets exposed to hypotonic solution in the presence of NDGA. This activity was transient with $t_{1/2}$ of 8 sec, which is identical to the $t_{1/2}$ of hepoxilin A_3 catabolism in platelet suspensions (Margalit et al., 1993). We conclude that at this relatively low concentration of NaF, the hepoxilin A_3 pathway is only transiently activated.

Effect of $GDP_\beta S$ on Hepoxilin A_3 Production in Response to Laminar Flow

We have previously shown that platelets produce the same lipoxygenase-derived product in response to hypotonic shock, laminar flow and centrifugation (Margalit & Livne, 1992) and that this product could be identified as hepoxilin A_3 (Margalit et al., 1993). To verify if this type of activation is also under G-protein control, we subjected platelets pretreated with $GDP_\beta S$ (1 μM, 30 min) to a flow velocity of 250 cm/sec in 5 cm vinyl tubing, 1 mm ID. As seen in Fig. 6, $GDP_\beta S$ completely blocked RVD reconstitutive activity induced by laminar flow. These data indicate that hypotonic shock and flow-associated stress share the same activation mechanism involving G proteins.

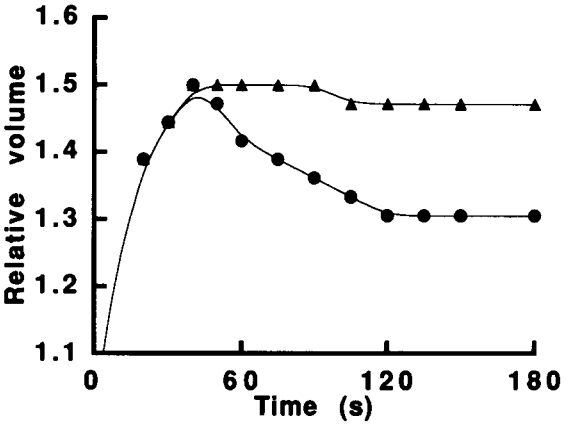

Fig. 6. RVD reconstitution assay of platelets in the presence of NDGA (20 μM), by platelet-derived isotonic eluate, (200 μl), from platelets exposed to a flow of 250 cm/sec, in vinyl tubing, 5 cm long, 1 mm, ID. Control, (●); platelets pretreated with $GDP_\beta S$, (1 μM, 30 min), (▲) (representative data, n = 3).

Discussion

In this work, we studied the activation mechanism of the RVD response in human platelets. We have demonstrated that the AA metabolism which leads to hepoxilin A_3 formation is initiated by Ca^{2+}-insensitive PLA_2 and that this phospholipase is activated by PTX-sensitive G proteins. This conclusion is based on the following findings: (i) RVD response is inhibited selectively by PLA_2 inhibitors and the site of inhibition is upstream to AA liberation (Fig. 1 and Table). (ii) Hepoxilin A_3 formation is not sensitive to external Ca^{2+} depletion by EGTA (Fig 2*A*) or internal Ca^{2+} depletion by BAPTA-AM (Fig. 2*C*). (iii) RVD response is inhibited by $GDP_\beta S$ and PTX (Fig. 3). The site of inhibition is upstream to AA release (Fig. 4). (iv) NaF 1 mM induces hepoxilin A_3 formation as tested by the RVD reconstitution assay (Fig. 5). (v) Laminar flow-induced formation of hepoxilin A_3 is inhibited by $GDP_\beta S$. We conclude, therefore, that both hypotonic and flow-induced hepoxilin A_3 formation share the same type of mechanical-biochemical-induced activation, which involves both PLA_2 and PTX-sensitive G proteins. This conclusion is mainly based on pharmacological studies. It is possible that some of the agents we used, such as boromophenacyl bromide (Blackwell & Flower, 1983) and wortmannin (Bonser et al., 1991) may have nonspecific effects. However, this conclusion is strongly supported by: (i) the RVD reconstitution approach which locates the site of inhibition and (ii) the use of more than one inhibitor for each target enzyme.

Ca^{2+}-sensitive PLA_2 from human platelets has been purified and characterized (Kim, Kodo & In-

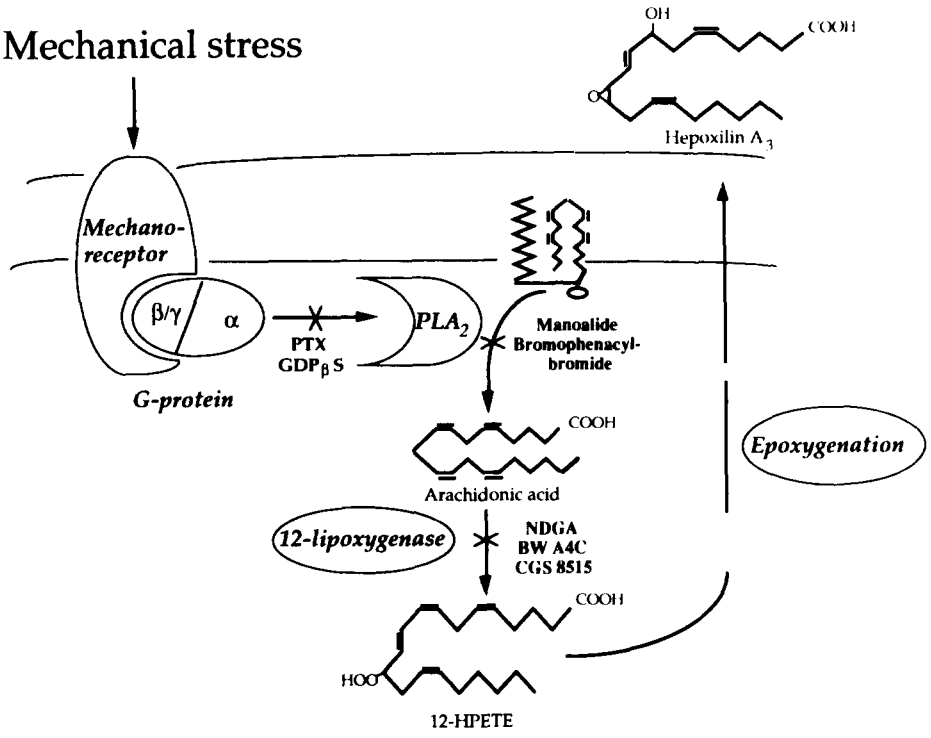

Fig. 7. Model of hypotonic and flow-induced formation of hepoxilin A_3. Application of mechanical stress to the cell surface induces G-protein activation, which is mediated by a surface receptor. The dissociated G protein activates phospholipase A_2 and the sequential release of AA. The AA is further metabolized into hepoxilin A_3 by the 12-lipoxygenase pathway. RVD inhibitors are in boldface. The 12-lipoxygenase inhibitors of RVD were described previously (Margalit & Livne, 1991).

oue, 1988) and the dependence of thromboxane metabolism on Ca^{2+} has been demonstrated (for review, *see:* Arita, Nakano & Hanasaki, 1989). The existence of Ca^{2+}-sensitive and Ca^{2+}-insensitive PLA_2 activities has been demonstrated in vascular smooth muscle cells (Miyake & Gross, 1992), pancreatic islet cells (Gross et al., 1993), and platelets (Ballou, DeWitt & Cheung, 1986). Our results indicate that hepoxilin A_3 metabolism is mediated by Ca^{2+}-insensitive PLA_2.

The role of G proteins in RVD response was studied using $GDP_\beta S$, PTX and NaF. Kucera and Rittenhouse (1988) had argued against the possibility that $GDP_\beta S$ penetrates human platelet membranes. However, in their system the platelets were incubated with $GDP_\beta S$ for 1 min, while in our system RVD inhibition is obtained after 30 min of incubation. Therefore, it seems that this G-protein antagonist diffuses the membranes and affects the cells internally. Although it was argued (Ui, 1984) that platelets do not have PTX receptors, we have shown that platelet RVD is inhibited by PTX (Figs. 3*B*, 4*B*). One possible explanation for these results is that different signal transduction pathways are placed in different compartments of the same cellular system and therefore may be affected differently by the same agent.

G proteins are regarded as mediators which couple the receptor to an appropriate "signal generator." It is generally accepted that this type of activation is the result of receptor-ligand interaction (Neer & Clapham, 1988; Hepler & Gilman, 1992). The possibility that a biochemical ligand is generated and released from the platelets prior to G-protein activation was not tested in this work. However, since the minimal period tested, between application of flow-associated stress and the termination of hepoxilin A_3 metabolism by NDGA, was only 5 msec (Margalit & Livne, 1992), it is not apparent that such a mechanism exists. It is suggested, therefore, that mechanical activation causes conformational changes in G-protein coupled receptors in analogy to the conformational changes induced by agonists such as epinephrine (Jackson, 1991). This receptor acts as a mechanoreceptor and transforms the mechanical stimulus to the biochemical response. In Fig. 7 we summarize our findings and illustrate the model for the mechanical-biochemical transduction which leads to hepoxilin A_3 formation in human platelets.

Eicosanoids are known to be formed by mechanical activation of endothelial cells (Frangos et al., 1985; Nollert et al., 1989). Recently, pertussis toxin was found to inhibit Rb^+ currents in rabbit kidney cells (le Maout et al., 1990). Therefore, it seems that the involvement of G proteins in the hypotonic and flow-associated formation of hepoxilin A_3 may represent a general pattern for inducing mechanical activation in different cell types.

This work is dedicated to the memory of Prof. A.A. Livne. It was carried out at the Amelia (Mimi) Rose Laboratory for Cellular Signal Transduction at the Department of Life Sciences, Ben-Gurion University of the Negev. We thank A. Dannon for helpful discussion.

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