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Role of chondroitin 4-sulphate as a receptor for polycation induced human platelet aggregation

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1 Proteoglycans provide negatively charged sites on the surface of platelets, leukocytes and endothelial cells. Since chondroitin 4-sulphate is the main proteoglycan present on the platelet surface, the role of this molecule in mediating the activation of human platelets by polylysine was studied.

2 Platelets were desensitized with phorbol 12-myristate 13-acetate (PMA, 10 nM) 5 min before the addition of polylysine to platelet-rich plasma (PRP). Changes in the intracellular Ca^{2+} concentration were measured in fura2-am (2 μM) loaded platelets and protein phosphorylation was assessed by autoradiography of the electrophoretic profile obtained from [^{32}P]-phosphate labelled platelets. The release of dense granule contents was measured in [^{14}C]-5-hydroxytryptamine loaded platelets and the synthesis of thromboxane (TXA_2) was assessed by radioimmunoassay. Surface chondroitin 4-sulphate proteoglycan was degraded by incubating platelets with different concentrations of chondroitinase AC (3 min, 37°C). The amount of chondroitin 4-sulphate remaining in the platelets was then quantified after proteolysis and agarose gel electrophoresis.

3 The addition of PMA to PRP before polylysine inhibited the aggregation by $88 \pm 18\%$ ($n=3$). Staurosporine (1 μM, 5 min) prevented the PMA-induced inhibition. Chondroitinase AC (4 pu ml^{-1} to 400 μu ml^{-1}, 3 min) abolished the polylysine-induced aggregation in PRP but caused only a discrete inhibition of ADP-induced aggregation. The concentration of chrondroitin 4-sulphate in PRP (0.96 ± 0.2 μg/10^8 platelets, $n=3$) and in washed platelets (WP; 0.35 ± 0.1 μg/10^8 platelets, $n=3$) was significantly reduced following incubation with chondroitinase AC (PRP $= 0.63 \pm 0.1$ μg/10^8 platelets and WP $= 0.08 \pm 0.06$ μg/10^8 platelets).

4 Washed platelets had a significantly lower concentration of chondroitin 4-sulphate than platelets in PRP. The addition of polylysine to WP induced a rapid increase in light transmission which was not accompanied by TXA_2 synthesis or the release of dense granule contents. This effect was not inhibited by sodium nitroprusside (SNP), iloprost, EDTA or the peptide RGDS. This event was accompanied by the discrete phosphorylation of plekstrin and myosin light chain, which were inhibited by staurosporine (10 μM, 10 min). The hydrolysis of platelet surface chondroitin 4-sulphate strongly reduced the polylysine-induced phosphorylation.

5 Our results indicate that polylysine activates platelets through a specific receptor which could be the proteoglycan chondroitin 4-sulphate present on the platelet membrane.

Keywords: Chondroitin 4-sulphate; polylysine; protein phosphorylation; phorbol 12-myristate 13-acetate (PMA); calcium mobilization

Introduction

Basic proteins are released mainly by activated leukocytes and platelets (Peterson *et al.*, 1985; Tetta *et al.*, 1985; Camussi *et al.*, 1986). Such proteins binds to negative sites on the surface of platelets, neutrophils and endothelial cells (Skutelsky *et al.*, 1975; Simionescu *et al.*, 1981) leading to a wide range of biological responses such as an increase in vascular permeability (Needham *et al.*, 1988; Antunes *et al.*, 1990; Rosengren & Arfors, 1991), the release of storage granule contents from neutrophils (Efferink & Deierkanf, 1986) and mast cells (Suzuki-Nishimura *et al.*, 1989; Sekino *et al.*, 1990; Yoshino *et al.*, 1990), inflammation in rat lungs (Santana *et al.*, 1993) and platelet aggregation (Jenkins *et al.*, 1971; Massini *et al.*, 1974; Guccione *et al.*, 1976; Marcondes *et al.*, 1993).

Polycationic substances such as platelet factor-4 (PF4) are able to bind to the plasma membrane proteoglycans of endothelial and other cell types through heparan sulphate chains (Hook, 1984). However, there is no evidence that proteoglycans can participate in cell activation by exerting a receptor function. The platelet aggregation induced by various agonists is accompanied by the release of chondroitin 4-sulphate (Nader, 1991; Donato *et al.*, 1994) which is thought to be stored primarily in the α-granules (Barber *et al.*, 1972; Donato *et al.*, 1994). Heparan sulphate and chondroitin 4-sulphate are also present on the platelet surface and account for a proportion of the anionic sites in the platelet membrane (Nader, 1991).

In order to understand further polylysine-induced platelet aggregation, we have assessed the role of proteoglycans in this activation. The results suggest that chondroitin 4-sulphate proteoglycan could be the platelet receptor for polylysine. Therefore the study of cationic proteins on platelets is a good model to understand the interaction of physiological cationic proteins such as those released by platelets or eosinophils on the homeostatic process.

Methods

Platelet-rich plasma preparation

Blood from healthy donors who had not taken any medication for at least ten days was anticoagulated with 0.1 vol of 3.8% (w/v) sodium citrate. Platelet-rich plasma (PRP) was obtained

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by centrifugation of whole blood at 200 × g for 15 min at room temperature. The supernatant was collected and the precipitate centrifuged at 2,000 × g for 15 min to obtain the platelet-poor plasma (PPP) used to calibrate the aggregometer.

Washed platelet (WP) preparation

Blood was collected into plastic tubes containing citric acid/citrate/dextrose (ACD-C, 1:9 v/v), centrifuged at 200 × g for 15 min at room temperature and the resulting supernatant (PRP) collected. Iloprost (0.8 μM) was added to the PRP which was then centrifuged at 800 × g for 12 min. The supernatant was discarded and the pellet was carefully resuspended in calcium-free Krebs-Ringer solution (composition in mM: NaCl 118, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4.7H_2O$ 1.17 and glucose 5.6). Iloprost (0.8 μM) was again added and the suspension centrifuged at 800 × g for 10 min (Radomski & Moncada, 1983). The pellet was resuspended in Krebs and the number of platelets was adjusted to 1.5×10^8 ml^{-1}. Finally, $CaCl_2$ (1 mM) was added to the platelet suspension.

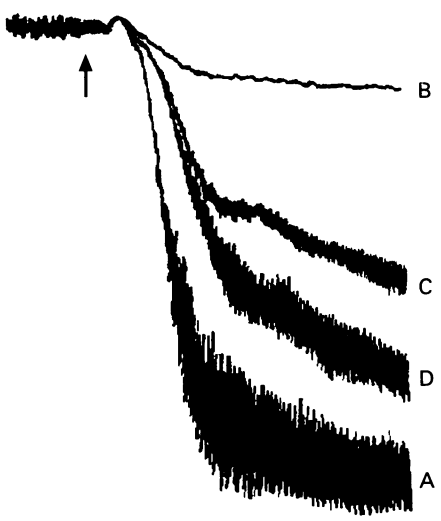

Figure 1 The effect of staurosporine on the desensitization caused by phorbol ester. PRP was stimulated with poly-L-lysine (132.3 kDa; 0.2 μM, at arrow, trace A) alone or after a 5 min preincubation with 10 nM PMA (B). In (C) and (D), the PRP was incubated for 5 min with 0.1 and 1.0 μM staurosporine, respectively, before the addition of PMA and polylysine. The results shown are representative of three such experiments.

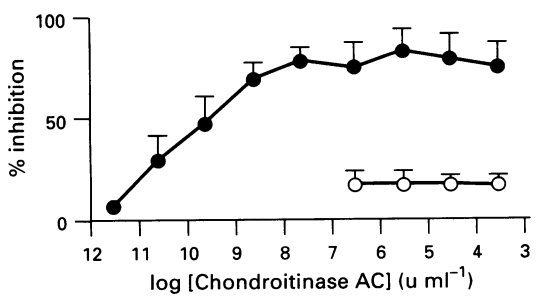

Figure 2 Chondroitinase AC inhibits the platelet aggregation in PRP induced by poly-L-lysine (87.4 kDa, 0.2–0.8 μM, ●), but not by ADP (5–10 μM, ○). Values are the mean of four experiments for each agonist; vertical lines indicate s.e.mean.

Platelet aggregation

Stirred (900 r.p.m.) platelet samples (0.5 ml) were prewarmed for 1 min at 37°C in a two channel Payton aggregometer (Payton Scientific Instruments, Inc., Buffalo, NY). Inhibitors were incubated at 37°C with the platelets for 1–5 min before addition of the agonist (except for indomethacin for which the incubation time was 20 min). The results are expressed as the percentage of maximum light transmission obtained when the aggregometer was calibrated for 100% transmission with PPP or Krebs solution.

Platelet desensitization

Platelets in PRP were desensitized by incubation with 10 nM phorbol 12-myristate 13-acetate (PMA) for 5 min with stirring at 37°C. After this incubation, poly-L-lysine (0.2 μM) was added and the aggregation followed for 5 min. To confirm the role of protein kinase C (PKC) in platelet desensitization, the PRP samples were pre-incubated with 0.1 or 1.0 μM staurosporine for 5 min.

Release reaction

Platelets in PRP were labelled by incubation with 0.5 mM [^{14}C]-5-hydroxytryptamine ([^{14}C]-5-HT, 50 Ci mmol^{-1}) for 30 min at 37°C. The platelets were washed as already described. Before stimulation, the PRP or WP were incubated with imipramine (1 μM) for at least 15 min in order to minimize the reuptake of released 5-HT. After addition of agonist, aggregation was allowed to proceed for 5 min and the reaction then stopped by the addition of 100 μl of cold EDTA (100 mM). The aggregated platelets were then centrifuged at 12,000 g for 2 min at room temperature and the extent of granule [^{14}C]-5-HT release was determined by quantifying the radioactivity in 100 μl of the resulting supernatant. The results are presented as a % of the total [^{14}C]-5-HT content in 100 μl of platelet suspension (Holmsen & Weiss, 1979).

TXA_2 formation

The synthesis of thromboxane A_2 (TXA_2) was assessed by the measurement of TXB_2 accumulation. Initially, platelet samples aggregated with either polylysine or thrombin were centrifuged for 3 min at full speed in a Beckman microfuge and the supernatants removed and stored at −20°C until assayed. The procedure for the determination of TXB_2 levels by radioimmunoassay with [^{3}H]-TXB_2 and the specificity of the antiserum employed have been described previously (Salmon, 1978).

Measurement of intracellular Ca^{2+}

The PRP obtained from ACD-C anticoagulated blood was centrifuged at 800 × g for 12 min as detailed above. The platelets were resuspended in Krebs-Ringer solution at 3×10^8 cells ml^{-1} and incubated with 2 μM of fura2-am for 45 min at room temperature (Pollock *et al.*, 1986). Iloprost (0.8 μM) was

Table 1 Changes in light transmission induced by positively charged polyaminoacids in washed platelets

Polyaminoacid	*MW (10^3)*	*ED_{50} (nM)*
Poly-L-lysine	26.5	140±30
Poly-L-lysine	289.0	1.5±0.4
Poly-D-lysine	106.0	35±3.0
Poly-L-arginine	139.0	3±0.5
Poly-L-ornithine	23.0	530±30

The change in light transmission was monitored for 5 min. The results represent the mean±s.e.mean of 5 experiments.

Table 2 Thromboxane B_2 and dense granule release induced by polylysine and thrombin in human washed platelets

Conditions	*Thromboxane B_2* *(ng ml^{-1})*	n	*Granule release* *(%)*	n
Control	1.7±0.3	6	0	5
Thrombin (100 mu ml^{-1})	87.8±24.5	6	74.4±8.7	5
Poly-L-lysine (0.8 μM)	12.1±3.0	3	0	4
Poly-D-lysine (0.2 μM)	3.0±0.7	3	ND	–
Thrombin + indomethacin (10 μM)	3.5±2.0	6	58.7±12.3	5
Poly-L-lysine + indomethacin	1.3±0.2	3	0	4
Poly-D-lysine + indomethacin	0.6±0.2	3	ND	–

Indomethacin was added 20 min before the stimulus. The results represent the mean±s.e.mean of the number of experiments (*n*) shown. ND-not determined.

added and the suspension centrifuged at 800 × *g* for 12 min. The pellet was resuspended in Krebs-Ringer solution and the number of platelets was adjusted to 2 × 10^8 ml^{-1}. Aliquots of platelets (1.5 ml) were dispensed into cuvettes thermostatted at 37°C (Hitachi F-3010, Japan) and equipped with a stirring device. The external Ca^{2+} concentration was adjusted to 1 mM with $CaCl_2$. Following equilibration for at least 3 min, agonists were added to the platelets as desired. To verify the Ca^{2+} mobilization from internal storage sites alone, 2 mM EGTA was added to chelate the extracellular Ca^{2+}. The fura2-am fluorescence was monitored continuously with monochromator settings of 339 nm (excitation) and 500 nm (emission). The $[Ca^{2+}]_i$ levels were calculated by use of a general formula as described by Pollock *et al.* (1986).

Detection of ^{32}P-labelled platelet proteins

Platelet-rich plasma was incubated with [^{32}P]-phosphate (0.5 mCi mol^{-1}, 0.05 mCi ml^{-1}) for 90 min at 37°C and the platelets were washed on a metrizamide gradient. The washed platelets were resuspended at a concentration of 3 to 4 × 10^8 ml^{-1} in Krebs-Ringer solution. Calcium was added immediately before the agonist, and the stimulation stopped by addition of 100 μl of a solution containing 12% sodium dodecyl sulphate (SDS) and 6 mM EDTA to the reaction cuvette. Platelet proteins were analysed by SDS-polyacrylamide gel electrophoresis (PAGE) under reducing conditions, with 12% (w/v) acrylamide in the resolving gel and 5% acrylamide in the stacking gel. After staining with Coomassie Blue, the gels were dried and exposed to Kodak XAR film for 5 to 10 days at −20°C.

Incubation of platelets with Flavobacterium heparinum *enzymes and the quantification of platelet glycosaminoglycans*

PRP was incubated with chondroitinase AC, heparitinases (I and II) and heparinase at 37°C under stirring for different time intervals, after which the platelets were then stimulated with polylysine (0.2 μM) or ADP (5 μM). Aggregation was allowed to proceed for 5 min and the reaction was stopped by adding 100 μl of 100 mM EDTA. The platelets were rapidly centrifuged at 12,000 *g* for 2 min and the chondroitin 4-sulphate present in both the soluble fraction and in the pellet was extracted after proteolysis with maxatase. Chondroitin 4-sulphate was identified by agarose electrophoresis and quantified by densitometry analysis (Nader, 1991).

Chemicals and enzymes

Sodium nitroprusside, indomethacin, bovine α-thrombin, poly-L-arginine (139 kDa), poly-L-ornithine (23 kDa), poly-L-aspartic acid (28.8 kDa), poly-L-glutamic acid (10.6, 46.2 and 74 kDa), poly-D-lysine 105.8 kDa), poly-L-lysine (26.5, 132.3 and 289 kDa) and (RGDS)-peptide, were obtained from Sigma (St. Louis, MO, U.S.A.). Iloprost was a gift from Schering (Germany). [^{14}C]-5-Hydroxytryptamine and [^{3}H]-thromboxane B_2 were purchased from Amersham (Amersham, U.K.). The TXB_2 anti-serum was a gift from Dr John Salmon (Wellcome Research Laboratories, U.K.). Ethylenediaminetetra-acetic acid (EDTA) was obtained from BDH Chemicals Ltd (Poole, England). The chemicals used for preparing the suspension media were obtained from Merck (Darmstadt, Germany). Low m_r agarose was bought from Biorad Laboratories (Richmond, CA, U.S.A.). 1,2 Diaminoethane and 1,3 diaminopropane were obtained from Aldrich Chemical Co (Milwaukee, WI, U.S.A.). Chondroitinase AC, heparitinases and heparinase were prepared from *Flavobacterium heparinum* as previously described (Nader *et al.*, 1990). Maxatase, a proteolytic enzyme from sporobacillus, was obtained from BIOCON (Rio de Janeiro, Brazil).

Table 3 The effect of poly-L-lysine and thrombin on platelet Ca^{2+} mobilization

	Ca^{2+} (1 mM)	*EGTA (2 mM)*
Basal	66±11	42±8
+Thrombin (100 mu ml^{-1})	1158±142	156±12
+Poly-L-lysine (0.4 μM)	227±21	72±1

Washed platelets were loaded with fura2-am (2 μM) and then incubated with either Ca^{2+} (1 mM) or calcium-free Krebs+EGTA (2 mM). The concentrations of $[Ca^{2+}]_i$ are expressed in nM and represent the mean±s.e.mean of 4 experiments.

Results

Poly-L-lysine effects on platelets in PRP

The addition of poly-L-lysine (132.3 kDa, 0.2 μM) to PRP induced a small shape change followed by a marked platelet aggregation (76±9%, $n=8$, Figure 1). Desensitization of platelets by incubation with the protein kinase C (PKC) activator PMA (10 nM, 5 min) inhibited the polylysine-induced aggregation by 88±19% ($n=3$). Figure 1 shows that in the presence of staurosporine (0.1 and 1 μM, 5 min), the inhibition of polylysine-induced platelet aggregation caused by platelet desensitization was significantly attenuated (57±13% and 23±9% of platelet aggregation inhibition, respectively; $n=3$). At higher concentrations (10 μM, 10 min), staurosporine *per se* abolished the aggregation (not shown).

Chondroitinase AC (4 pu ml^{-1} to 400 μu ml^{-1}, 3 min) dose-dependently inhibited polylysine-induced platelet aggregation. At the highest concentration (400 μu ml^{-1}, 3 min), chondroitinase AC induced only a discrete inhibition of ADP-induced platelet aggregation (Figure 2). The incubation of PRP for either 3 or 30 min with heparitinase I, heparitinase II and heparinase (same concentrations as used for chondroitinase AC) had no effect on either the polylysine- or ADP-induced platelet aggregation ($n=3$; not shown).

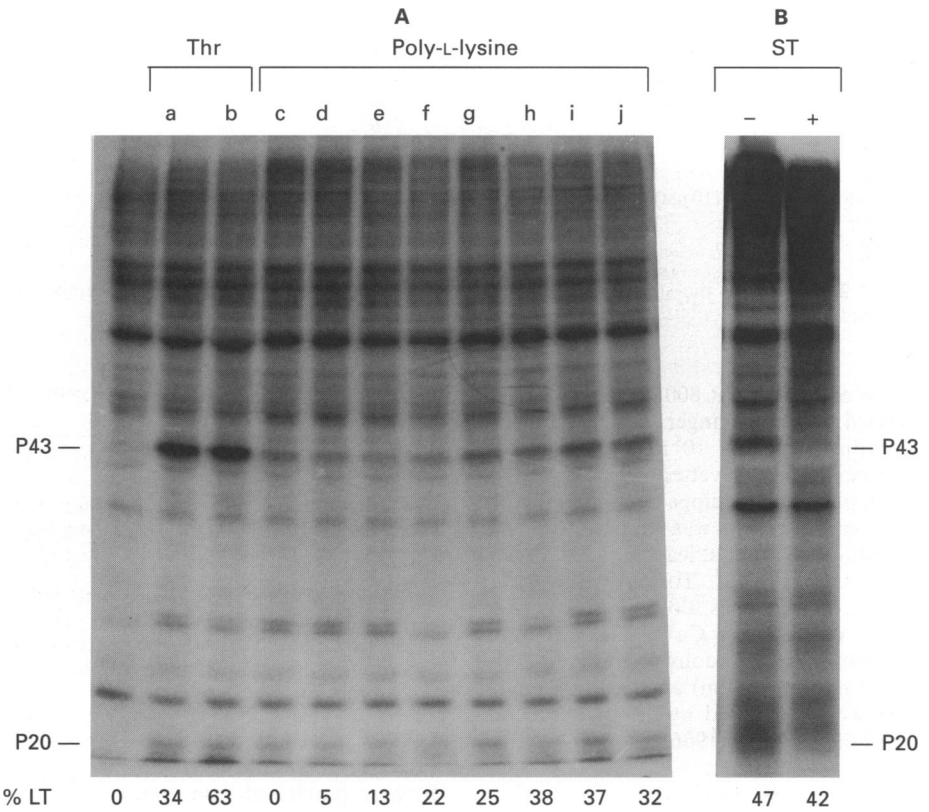

Figure 3 Autoradiograph of ^{32}P proteins in human platelets stimulated with polylysine or thrombin. (**A**) ^{32}P-labelled platelets were incubated with different concentrations of polylysine (c, 0.02; d, 0.04; e, 0.08; f, 0.2; g, 0.4; h, 0.8; i, 1.6 and j, 3.2 μM) or thrombin (Thr; a, 50 and b, 100 mu ml^{-1}) for 2 min at 37°C. (**B**). Platelets were incubated with staurosporine (ST, 10 μM) for 10 min at 37°C before stimulation with polylysine (0.4 μM) for 2 min at 37°C. The platelet proteins were then separated by electrophoresis in 12% polyacrylamide gel. The values at the base of the autoradiograph represent the increase in the light transmission (LT) after 2 min of stimulation. The locations of the P20 (myosin light chain) and P43 (pleckstrin) bands are indicated. The results are representative of four experiments.

Measurement of chondroitin 4-sulphate

The concentration of chrondroitin 4-sulphate in PRP (0.96 ± 0.2 μg/10^8 platelets, $n=3$) was significantly higher than that in WP (0.35 ± 0.1 μg/10^8 platelets, $n=3$). Pre-incubation of either PRP or WP with chrondroitinase AC (400 μu ml^{-1}, 3 min at 37°C) reduced the chondroitin 4-sulphate content to 0.63 ± 0.1 and 0.08 ± 0.06 μg/10^8 platelets, respectively.

Effect of polyaminoacids on washed platelets

The addition of polylysine to WP caused an immediate increase in light transmission which was not preceded by any shape change. This effect was dependent on the polylysine molecular weight (Table 1). Unlike polylysine or poly-L-arginine and poly-L-ornithine, negatively charged polyaminoacids such as poly-L-glutamic acid and poly-L-aspartic acid had no effect on the light transmission.

The response of WP to poly-D-lysine (105.8 kDa, 0.2 μM) and poly-L-lysine (26.5 kDa, 0.8 μM) was accompanied by the formation of a small amount of TXB_2 and no dense granule release. Under the same conditions, thrombin (100 mu ml^{-1}) induced a significant release of both TXB_2 and platelet dense granule contents. Although indomethacin (10 μM, 20 min) significantly reduced the TXB_2 release induced by either polylysine or thrombin (Table 2), it did not affect the change in light transmission mediated by these agents.

Prior incubation of WP with the prostacyclin analogue iloprost (0.53 μM, 3 min) abolished the thrombin (200 mu ml^{-1})-induced platelet aggregation. In contrast, at concentrations ten times higher (5.3 μM, $n=3$), iloprost had no effect on the increase in light transmission induced by poly-D-lysine (105.8 kDa, 0.2 μM). Addition of either the peptide RGDS (100 μM) or EDTA (3 mM) to WP had no effect on the increase in light transmission induced by poly-L-lysine (132.3 kDa, 0.4 μM, $n=3$). In thrombin-stimulated platelets, the aggregation was abolished by both compounds ($n=3$).

Previous incubation of WP with poly-L-glutamic acid (46.2 kDa, 0.07–17.3 μM, 1 min) abolished the increase in light transmission induced by poly-L-lysine ($n=3$). Poly-L-glutamic acid (same concentration range as above) also promptly reversed the increase in light transmission when added to WP during the maximal response to polylysine ($n=3$). Platelet activation induced by thrombin (100 mu ml^{-1}, $n=3$) was not affected by this polyanion (not shown).

$[Ca^{2+}]_i$ measurement

In the presence of external Ca^{2+}, the addition of either thrombin or poly-L-lysine to WP resulted in an immediate drop in the fura2 fluorescence indicating a rise in $[Ca^{2+}]_i$ to 1158.0 ± 142 and 227.3 ± 21 nM, respectively ($n=4$ each). When the external Ca^{2+} was removed by adding 2 mM EGTA, the rise in $[Ca^{2+}]_i$ in response to thrombin and poly-L-lysine was drastically reduced (Table 3).

Protein phosphorylation

Figure 3 shows that poly-L-lysine (0.02–3 μM) induced a dose-dependent phosphorylation of the plekstrin (P43) and myosin

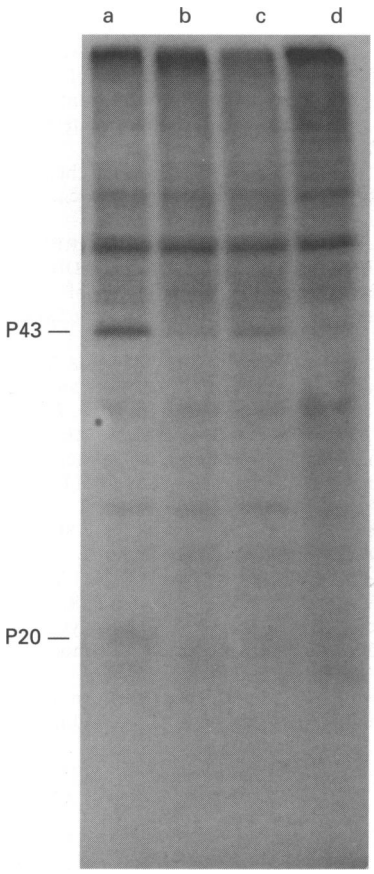

Figure 4 Effect of platelet membrane chondroitin 4-sulphate hydrolysis on platelet protein phosphorylation. The chondroitin 4-sulphate present in the membrane of [^{32}P]-phosphate labelled platelets was degraded by incubating the platelets with chondroitinase AC 400 mu ml^{-1} (b and c) for 3 min at 37°C. The platelets were stimulated with 0.2 μM polylysine for 2 min (a and c). Line d represents the basal phosphorylated protein profile in resting platelets. Autoradiography of the ^{32}P labelled proteins was performed as described in Methods. The locations of the P20 and P43 bands are indicated. Results are representative of four experiments.

light chain (P20). However, the intensity of this phosphorylation was less than that observed in thrombin-stimulated platelets. Pretreating the platelets with chondroitinase AC significantly reduced the P43 and P20 phosphorylation induced by poly-L-lysine (Figure 4).

Staurosporine did not significantly affect the increase in light transmission. However, it abolished the poly-L-lysine-induced phosphorylation of both P43 and P20. Both the platelet aggregation and protein phosphorylation induced by thrombin were inhibited by staurosporine (Figure 3).

Discussion

Activation of protein kinase C by phorbol esters such as PMA is able to inhibit, as well as promote, platelet activation. The inhibitory effects emerge when platelets are preincubated with PMA at a concentration not enough to induce platelet aggregation during a prolonged incubation period. In this case, the platelet responses usually seen with the agonist are attenuated or abolished (Zavoico *et al.*, 1985; Jakobs *et al.*, 1985). Normally, preincubation with PMA has been shown to suppress activation of phospholipase C and inhibition of adenosine 3′:5′-cyclic monophosphate formation by thrombin and adrenaline (Rittenhouse & Sasson, 1985; Crouch & Lapetina, 1988). Potentially, desensitization of platelets with PMA is a useful tool in studying receptor-mediated events (Carlson *et al.*, 1989; Brass, 1992). Thus, the finding that desensitization by PMA inhibits polylysine-induced platelet aggregation indicates the involvement of a receptor mechanism for this polycation. Interestingly, the PKC inhibitor staurosporine (Brass, 1992; Rajinder & Colman, 1993) almost completely prevented the desensitization induced by PMA preincubation, suggesting that PKC is responsible for this phenomenon. Indeed, PKC is able to phosphorylate and desensitize several G protein-coupled receptors, including those for thrombin (Rittenhouse & Sasson, 1985; Murayama *et al.*, 1990; Brass, 1992), platelet-activating factor (PAF; O'Flaherty *et al.*, 1989; Yamazaki *et al.*, 1989; Chao *et al.*, 1990), 5-hydroxytryptamine (5-HT), and adrenoceptors (Kelleher *et al.*, 1984; Leeb-Lundberg *et al.*, 1985).

To determine the existence of a specific receptor for polycations, we investigated the possible binding sites for polylysine on the platelet membrane surface. Reducing the platelet surface content of chondroitin 4-sulphate by treatment with chondrotinase AC abolished the platelet aggregation in PRP and markedly decreased the kinase activation in WP. Since ADP-induced aggregation was only mildly affected by chondroitinase AC treatment, these findings indicate that chondroitin 4-sulphate participates in the signal transduction events induced by polylysine and may function as a platelet surface receptor for this polycation.

As found for thrombin, collagen, PAF, 5-HT, arachidonic acid, and thromboxane analogues (Siess, 1989), polylysine also induced staurosporine-sensitive phosphorylation of P20 and P43 in WP. This phosphorylation was accompanied by a significant increase in the $[Ca^{2+}]_i$. Since both of these events were also observed in the absence of external calcium, they indicate that polylysine is able to activate a signal transduction pathway. As in PRP, the treatment of WP with chondroitinase AC reduced both the protein phosphorylation and the increase in $[Ca^{2+}]_i$, thus further confirming the pivotal role of chondroitin 4-sulphate in polylysine-induced platelet activation.

Polylysine was not able to induce WP aggregation. The increase in light transmission induced by this polycation was due to platelet agglutination rather than aggregation since it was not calcium-dependent, a similar phenomena to that observed for ristocetin (Weiss *et al.*, 1973; Allain *et al.*, 1975). Considering that chondroitin 4-sulphate may be the receptor for polylysine, the latter finding probably reflects the decrease in level of this proteoglycan observed in WP compared to PRP.

Platelets contain large amounts of proteoglycans in their membrane and chondroitin 4-sulphate accounts for more than 90% of the total platelet proteoglycan content (Barber *et al.*, 1972; Nader, 1991). Although chondroitin 4-sulphate is released during platelet activation (Donato *et al.*, 1994), its role outside the cell is unknown. Here we have provided some evidence that this proteoglycan may act as a receptor in polylysine-induced platelet activation.

Since most blood proteins are anionic under physiological conditions, any circulating cationic protein would most likely interact with plasma proteins, and would therefore probably not be available to modulate platelet reactivity *in vivo*. However, since eosinophils contain large amounts of cationic proteins (Gleich *et al.*, 1976), it is possible that chondroitin receptor activation may be relevant in allergic phenomena. Interestingly, the cellular perivascular cuff found in asceptic vasculitis in Henoch-Schönlein purpura is composed predominantly of eosinophils and platelet plugging (Gairdner, 1948; Vernier *et al.*, 1961). The finding that prophylactic therapy with heparin yields good clinical results in such individuals compared to an untreated population (Szelid *et al.*, 1986) may also be related to its ability to bind to cationic proteins and therefore to prevent platelet activation.

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