Activated platelets release a potent inhibitor of factor XIa previously identified as a Kunitz proteinase inhibitor domain-containing form of the β-amyloid precursor proteins (βAPP). Two carboxy-terminal truncated forms of the βAPP, βAPP-751 and βAPP-770, are shown to be the predominant isoforms secreted by platelets. The release of βAPP from platelets is responsible for the higher concentration of βAPP in serum compared with plasma, and thrombin dose-response data show that release of βAPP is most consistent in serum compared with plasma, and thrombin dose-response data show that release of βAPP is most consistent.

Materials and Methods

Reagents. All reagents were obtained from Sigma Chemical Co (St Louis, MO) except: chromogenic substrate S-2366 from Kabi (Stockholm, Sweden); 125I from Amersham (Arlington Heights, IL); Iodogen from Pierce (Rockford, IL); Polybrene from Aldrich Chemical Co, Inc (Milwaukee, WI), and 14C-serotonin from Dupont NEN Products (Boston, MA).

Preparation of clotting factors and factor inhibitors. Factor XI was purified from outdated frozen human plasma using a modification of the method described by Bouma and Griffin.24 Dialyzed plasma, 3.5 L, was loaded at 300 cm²/h into a 5-L column of DEAE Sephadex A-50 equilibrated in 0.04 mol/L Tris, 0.01 mol/L.
Platelet βAPP and the Effect of Thrombin

**Fig 1.** (A) Types of βAPP. The five identified alternatively spliced products of the βAPP gene on human chromosome 21 are shown. The arrow indicates the site of proteolysis for releasable forms of transmembrane βAPP. (B) Localization of antisera recognition sites. Antibodies were developed against the amino terminus, the Kunitz domain insert, the MRC OX-2 insert, the amino-terminus of exon 14, the βAP, and the cytosolic carboxy-terminus. The exon 14 antibody was developed against a polypeptide region of the βAPP immediately carboxy-terminal to the Alu repeat insertion site of βAPP.

Human blood using differential centrifugation. Platelet preparations contained no detectable leukocytes or red blood cells (RBCs) as determined by Coulter counter (Coulter Electronics, Hialeah, FL) analysis and Wright's stain. Platelets at 1 × 10^9/mL were activated using 0.5 U/mL human thrombin or 1 mmol/L calcium ionophore A23187 (Sigma) at room temperature. For ligand and Western blot experiments, platelet incubation with thrombin was performed for 15 to 30 minutes with intermittent stirring to induce aggregation. Incubation with the calcium ionophore A23187 was extended to 3 to 4 hours.

The thrombin dose/response data was obtained under different conditions. Before activation, platelets were loaded with 1 to 5 μL 14C-labeled serotonin (DuPont NEN Products) per milliliter of 1 × 10^9 platelets for 1 hour at 37°C. Labeled platelets were then centrifuged, resuspended in the same volume of Tris-saline and 1 mg/mL dextran, and divided into aliquots before incubation with varying concentrations of thrombin diluted in TBSA (0.05 mol/L Tris, 0.10 mol/L sodium chloride, 0.02% NaN₃, 0.1% bovine serum albumin [BSA], pH 7.5). Samples were vortexed for 1 to 3 seconds after the addition of thrombin and incubated at room temperature without stirring. The reaction was stopped after 15 minutes by the addition of hirudin, after which releasate samples were immediately separated from platelets by centrifugation over a mixture of Apezon oil and N-butyl phthalate. Samples were measured for release of dense body (14C-serotonin), α granule (platelet factor 4 [PF4]), and lysosomal β-hexosaminidase (β-hex) compartments; release of platelet βAPP was determined by factor Xla ligand blot (see below). 14C-serotonin in these samples was determined using a Beckman LS 1701 scintillation counter (Beckman Instruments, Fullerton, CA). Alpha granule release was determined by PF4 radioimmunossay (RIA) (Abbott Labs, Chicago, IL) using releasate samples diluted 1:30 to TBSA. β-Hex activity was determined by incubating 100 μL releasate samples with 200 μL substrate mixture (50 μL of 0.01 mol/L para-nitrophenol-labeled N-acetylβ-D-glucosaminide in water; 15 μL 1 mol/L citrate, pH 4.0; 0.05 μL 20% Triton X-100; water to 200 μL) for 60 to 90 minutes at 37°C. The reaction was stopped after the addition of 1 mL 0.2 mol/L Na₂CO₃ and the absorbance at 405 nm measured. The experiments were performed in duplicate for each of 3 to 4 platelet preparations.

The functional factor Xla inhibitory activity in platelet releasate was determined using the factor Xla chromogenic substrate S2366 (Kabi). Factor Xla at 100 ng/mL final concentration was incubated with varying volumes of platelet releasate brought to a final volume of 900 μL with TBSA. After 15 minutes incubation at room temperature, 100 μL of 3 mmol/L S2366 in water was added and the absorbance at 405 nm measured three times per minute for 60 to 90 minutes on a spectrometer (Response UV-VIS; Gilford Systems, Ciba Corning, Oberlin, OH). The degree of inhibition was determined by comparing the remaining steady-state factor Xla activity with that of factor Xla in the absence of releasate.

Releasate was selectively cleared of Kunitz domain-containing forms of βAPP by affinity chromatography using a MoAb directed against human factor Xa (see below). Releasate was loaded onto a 2 to 3 cm³ MoAb-Affigel 10 column at a rate of 5 to 10 mL/h. The flow-through was collected in 750-μL samples and the column washed with Tris-saline before elution with 2 mol/L NaSCN. After dialyzing (2,000 molecular weight cut-off) the eluent into Tris-saline, samples were subjected to factor Xla ligand blot and assayed for functional factor Xla activity as described. A similar experiment was performed in parallel using an unrelated MoAb-affinity column (antifactor X, see below) as a control for nonspecific binding.

Factor Xla ligand blotting was performed on samples following SDS-PAGE and subsequent transfer to nitrocellulose paper. Blots
were blocked with 5% defatted milk and incubated overnight with 125I-factor Xla at room temperature. Autoradiography for 3 to 12 hours at -70°C was performed following extensive washing of the blot in 5% defatted milk. Quantitation of released factor Xla inhibitor was determined by densitometry measurement of bound 125I-labeled factor Xla using an LKB 2222-020 Ultrascan XL laser densitometer (LKB, Bromma, Sweden).

WBCs were separated into a polymorphonuclear fraction and a mononuclear fraction consisting of monocytes and lymphocytes using Mono-Poly Resolving Medium Ficoll-Hypaque following the manufacturer’s recommendations (ICN Flow, Costa Mesa, CA). Final samples contained 10,000 to 20,000 mononuclear cells/mm³ (10% to 25% monocytes, 75% to 90% lymphocytes) and 30,000 to 60,000 polymorphonuclear cells/mm³. For ligand blots, approximately 20 μL of each preparation was loaded per lane. Less than 5% cross-contamination between leukocyte fractions was observed, and fewer than 5,000 platelets/mm³ were present in either leukocyte fraction as determined by Coulter counter.

Antibody preparation. New Zealand white rabbits were immunized with βAPP purified from HepG2 cells or peptides representing different domains of the βAPP coupled to keyhole lymphocyte hemocyanin (KLH). The peptides used were EVPTDGNAGLLAEQPQAMF of the amino-terminus (anti-NH2); RAMISRWYFDVTEGKC of the Kunitz protease inhibitory domain (anti-Kunitz); MSQSLKTTQEPAPKVL representing the 19 amino acid MRC OX-2 insert encoded by exon 8 (anti-MRC OX-2); DELLQKEQNYSDVLANMIS representing the amino-terminus of the region encoded by exon 14 (anti-exon 14); DAEFRHDSGYEVHHQKLVFFAEDV of the amino terminus of the β-amyloid protein (anti-βAPI); and GYENPTYKFEEQMQN of the cytoplasmic tail/carboxy-terminus (anti-COOH). The peptide representing the βAP was also used to immunize rabbits without prior coupling to KLH (anti-βAP2). An MoAb against the Kunitz domain of βAPP was obtained from Athena Neurosciences (San Francisco, CA).

Samples for Western blotting were subjected to SDS-PAGE and transferred to nitrocellulose paper. Antiseras were used at 1:100 dilutions in TBSA/Tween (0.15 mol/L Tris, 0.10 mol/L NaCl, 0.1% TBSA, 0.05% Tween 20, pH 7.5) and allowed to incubate with the samples for 2 to 4 hours. Following extensive washing with 5% defatted milk, the blots were incubated with the secondary antibody coupled to alkaline phosphatase (Sigma) for 30 to 90 minutes and developed per manufacturer’s protocol. To confirm specificity, antisera were preincubated with appropriate peptide at concentrations of 10 to 100 μg/mL for 6 to 12 hours before use. The polyclonal antibody developed against Hep G2 factor Xla inhibitor, the monoclonal preparation against the Kunitz domain, and a monoclonal developed against factor X (3618) were chromatographed over Protein Aagarose (Repligen Corp, Cambridge, MA) before linking to Affigel 10 (Bio Rad, Richmond, CA) at 3 to 5 mg immunoglobulin/mL Affigel 10 per manufacturer’s specifications.

Transfection of human kidney 293 cells. Cultured human kidney 293 (ATCC CRL 1573) cells were cotransfected with vector containing the BAPP-751 sequence (pSV-751-TM from Athena Neurosciences) and a vector conferring neomycin resistance (pSV2-neo) using a calcium phosphate protocol (Stratagene, La Jolla, CA). The antibiotic G418 was used for selection, and clones were screened for production of BAPP-751 by ligand blot as described above.

The transfected 293 cells were harvested with trypsin, washed in sterile Tris-saline, and resuspended in Tris-saline containing 1% Triton X-100. Following sonication and centrifugation the supernatant was subjected to SDS-PAGE with subsequent transfer to nitrocellulose paper and blotting with various anti-βAPP antisera.

RESULTS

Proteolysis of βAPP in platelet releasate. Factor Xla ligand blotting of SDS-lysed platelets detects a broad band of factor Xla binding at 112 Kd (Fig 2). Following platelet activation with 1 mmol/L calcium ionophore A23187 or 0.5 U/mL thrombin, this factor Xla binding activity is found in the platelet releasate and is no longer associated with the platelet pellet. Figure 2 shows also that affinity chromatography using an MoAb directed against the Kunitz domain of βAPP removes all factor Xla binding detectable by ligand blot from platelet releasate.

Following activation of platelets by thrombin, platelet βAPP undergoes additional proteolytic cleavage with a reduction in the intensity of the broad signal at 112 Kd and the appearance of a doublet with a relative molecular mass of 90 to 95 Kd (Figs 2, 3). With higher concentrations of thrombin (Fig 3A) or longer incubations (Fig 3B) the entire

Fig 2. Ligand blot of platelet and platelet releasate. Following SDS-PAGE, samples were transferred to nitrocellulose paper and probed with 116.5 Kd is shown on the left.
PLATELET βAPP AND THE EFFECT OF THROMBIN

Fig 3. Thrombin cleavage of βAPPs. (A) Dose-response of thrombin in platelet releasate. Radiolabeled [125I] factor Xa ligand blot of platelet samples activated with varying concentrations of thrombin for 10 minutes. Units per milliliter of NIH standardized thrombin are shown along the bottom of the figure. (B) Time course of thrombin cleavage of radiolabeled rβAPP 751. Radiolabeled [125I]-rβAPP-751 was incubated with 3 U/mL thrombin. Samples were taken at the times shown and immediately added to nonreducing SDS sample buffer for subsequent SDS-PAGE and autoradiography. No additional cleavage sites were detected when SDS-PAGE was performed under reducing conditions (5% β-mercaptoethanol) (not shown). Some degradation of [125I]-βAPP-751 occurs during purification and storage (time 0) but is not augmented by incubation in the absence of thrombin (control). (C) Western blot of thrombin-cleaved βAPP 751. A Western blot of samples obtained as detailed in (B) was performed using an antibody directed against the NH2-terminus of βAPP. Lane 1, rβAPP-751; lane 2, rβAPP-751 incubated with 3 U/mL thrombin for 180 minutes; lane 3, same as lane 2 with the addition of 5 U/mL hirudin.

112-Kd signal is converted to the lower molecular weight doublet. There does not seem to be additional sites of proteolysis and, more specifically, there is no indication that the lower molecular weight member of the doublet is a proteolytic fragment of the higher molecular weight member of the doublet (Figs 3A, 3B).

Thrombin seems responsible for this proteolysis because:
1. it is not seen to the same degree during the same time period when calcium ionophore A23187 is used as the platelet agonist (Fig 2);
2. there is a dose-response relationship between thrombin concentrations and the degree of βAPP cleavage in platelet releasate (Fig 3A);
3. thrombin cleaves rβAPP-751 in a purified system (Fig 3C); and
4. hirudin, a potent and specific inhibitor of thrombin, blocks the proteolysis of both purified rβAPP-751 and βAPP present in platelet releasate (Fig 3C). Furthermore, thrombin's cleavage of rβAPP-751 involves a similar carboxy-terminal truncation as seen in platelet releasate, because the amino-terminal–directed antibody recognizes the cleaved moiety (Fig 3C). This carboxy-terminal proteolysis does not significantly alter the factor Xa inhibitory properties of βAPP (data not shown).

Types of βAPP in platelet releasate. Western blots using polyclonal antibodies developed against specific regions of the βAPP indicate that carboxy-terminal truncated forms of βAPP containing the Kunitz domain predominate in platelet releasate. The factor Xa binding activity at 112 Kd comigrates with bands recognized by antibodies directed against the βAPP purified from Hep G2 media as well as antipeptide antibodies developed against the amino-terminus, the Kunitz domain, the MRC OX-2 insert, and the βAP of βAPP (Fig 4A). Antibodies developed against the carboxy-terminal peptide sequence do not recognize proteins in platelet releasate (Fig 4A) but do recognize full-length forms of βAPP in a transfected cell lysate (Fig 4B). This pattern is similar to that previously reported for secreted forms of βAPP with the 15 amino acid βAP remnant on the secreted form accounting for the retained signal observed with antisera directed against the βAP (Fig 1).18,19 In addition, when platelet releasate is selectively

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cleared of Kunitz domain-containing forms of βAPP by monoclonal affinity chromatography, none of the βAPP-specific antibodies recognize proteins in the flow-through releasate, suggesting that Kunitz domain-containing forms of the βAPPs account for the large majority of the signal (data not shown).

When thrombin is used to activate platelets the βAPP signal consists of a doublet at 90 to 95 Kd in addition to the band at 112 Kd (Figs 2 and 3). Figure 4C shows a Western blot of a releasate sample from thrombin-activated platelets in which this conversion is approximately half complete. The 112-Kd parent species and both members of the 90- to 95-Kd doublet are recognized by antibodies directed against the amino-terminus, Kunitz domain, and exon 14 region of the βAPP. The antisera to the βAP region recognizes the 112-Kd band but neither member of the 90- to 95-Kd doublet suggesting proteolytic loss of this carboxy-terminal epitope in the doublet. Only the higher molecular weight moiety in the 90- to 95-Kd doublet yields a signal with antisera against the MRC OX-2. This pattern is most consistent with the 95-Kd band being a carboxy-terminal-truncated form of βAPP-770 and the 90-Kd band being a carboxy-terminal–truncated form of βAPP-751.

Characteristics of βAPP release by platelets. Figure 5 compares the thrombin-mediated release of platelet βAPP with that of known α granule (PF4), dense body (serotonin), and lysosomal (βhex) constituents. Each of these separate platelet compartments has a different threshold for release in response to thrombin stimulation. Using four platelet preparations and thrombin concentrations run in duplicate, the thrombin concentration required for 50% maximal inducible release of PF4 was determined to be 0.045 ± 0.009 U/mL, whereas the thrombin concentration for 50% release of serotonin and βhex was 0.078 ± 0.01 U/mL and 0.3 ± 0.03 U/mL, respectively. The concentration of thrombin required for 50% release of platelet βAPP was 0.037 ± 0.015 U/mL, nearly identical to that of PF4. Two-tailed t-test showed a significant difference in thrombin concentrations inducing 50% release of βhex and serotonin (P < .001), PF4 and serotonin (P < .01), and release of βAPP compared with βhex and serotonin (P < .001 and P < .01, respectively). The thrombin concentrations required for 50% release of PF4 and βAPP were not statistically different.

Contribution of βAPP to Xla inhibitory activity in platelet releasate. To determine the contribution of platelet-releasable βAPP to the overall factor Xla inhibitory activity released from activated platelets, releasate was cleared of Kunitz domain-containing forms of βAPP by monoclonal affinity chromatography (see Materials and Methods). Greater than 95% of all factor Xla inhibitory activity contained in platelet releasate is removed with the βAPP (data not shown) as is all radiolabeled factor Xla binding on ligand blot (Fig 2). Furthermore, factor Xla inhibitory activity (data not shown) and factor Xla ligand-binding activity (Fig 2) is recovered in the eluate of the monoclonal column. When an unrelated monoclonal affinity column is used (antifactor X) 90 ± 2% of the factor Xla inhibitory activity is recovered in the effluent and 0 ± 1% is detected in the elution. No radiolabeled factor Xla binding activity is found on ligand blot of the elution (data not shown).

Further evidence of the significance of the contribution of platelets to the βAPP factor Xla inhibitory activity is suggested by evaluation of βAPP in plasma and serum. Plasma contains less than 5% of the βAPP factor Xla binding activity found in serum, suggesting that coagulation results in the release of significant amounts of Kunitz domain-containing forms of βAPP (Fig 6). This is probably caused by the release of βAPP from platelets, because

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**Figure 5.** Differential release of granular compartments of platelets as a function of thrombin concentration. PF4 (α granule), serotonin (dense body), and βhex (lysosome) activities were measured as described in Materials and Methods and compared with release of βAPP as determined by densitometry analysis of ligand blot. Data are expressed as a percentage of the value obtained in platelet lysate (freeze-thaw and Triton lysed). The dose response of βAPP release by thrombin most closely parallels that of PF4.
circulating mononuclear cells and polymorphonuclear cells do not contain significant amounts of Kunitz-containing forms of βAPP as determined by factor Xla ligand blot (Fig 6).

**DISCUSSION**

Previous studies have demonstrated that a factor Xla inhibitory activity is present in platelet releasate, and that a Kunitz domain-containing form of the βAPP found in platelets is a potent inhibitor of factor Xla. Other reports have demonstrated several forms of the βAPP in platelets and platelet releasate, leaving the precise identification of the isoforms open to speculation. The difficulty in identifying which of the various βAPP species is present in platelets is compounded by the presence of up to five different βAPP(α) isoforms, four of which can be present in both full-length (transmembrane) and secreted (truncated) forms, any and all of which may be susceptible to variable degrees of degradation. The panel of antibodies used in this study clarifies the moieties present in platelet releasate.

The 112-Kd factor Xla binding activity detected by ligand blot (Fig 2) comigrates with bands recognized by antipeptide antibodies directed against several regions of the βAPP (Fig 4A). The antibody pattern is consistent with that predicted of carboxy-terminal-truncated forms of the βAPP as the antisera directed against the common cytosolic tail fails to recognize this moiety. The presence of signal seen with antisera directed against the amino-terminus and the βAP suggest that the carboxy-terminal truncation in platelet βAPP occurs at or near the previously described processing site for the transmembrane form of the βAPP. The staining with antisera toward the Kunitz inhibitory domain verifies the presence of at least one of the three Kunitz domain-containing forms of the βAPP in this band, whereas the signal seen with the anti-MRC OX-2 antisera confirms the presence of at least one of the two βAPP isoforms that contain this insert. However, this antibody pattern does not allow a clear distinction between the presence of one or more of the possible forms of βAPP as the relatively broad band at 112-Kd could conceivably represent several forms of the βAPP migrating in close proximity to each other.

To more specifically identify which forms of the βAPP are present in platelet releasate, we took advantage of the observation that the broad band at 112 Kd is reduced to a doublet migrating at 90 to 95 Kd during thrombin activation of platelets. Both proteolytic fragments of the parent species continue to be recognized by antisera to the amino-terminus, the Kunitz domain, and the region encoded by exon 14; however, the anti-βAP signal has been lost, suggesting further carboxy-terminal proteolysis. Only the higher molecular weight member of the doublet is recognized by antisera to the MRC OX-2 region that, combined with the presence of the Kunitz domain signal, identifies this band as a proteolytic fragment of βAPP-770. The lower molecular weight member of the doublet has the same antisera recognition pattern except for the absence of the MRC OX-2 signal, which identifies it as a proteolytic fragment of βAPP-751. The persistence of a signal detected with the anti-exon 14 antisera excludes the possibility that the doublet represents sequential proteolysis, because the epitope for this antisera lies carboxy-terminal to the MRC OX-2 sequence (Fig 1B). The presence of detectable quantities of βAPP-770 is similarly excluded, because all bands either before or after thrombin-mediated proteolysis are recognized by the anti-exon 14 antisera, which is directed against a peptide sequence not present in this species. Comigration of βAPP-751 with one of the detected bands is unlikely, because the 112-Kd band is completely converted to the doublet and no band is seen in the 90- to 95-Kd range before thrombin’s effect. Because the effect of thrombin is localized to an amino acid sequence not present in βAPP-770, no change in apparent molecular weight of this species would be anticipated.

Non-Kunitz domain-containing forms of βAPP are not detected in platelet releasate as all signals obtained with other antipeptide antibodies are also recognized by antisera against the Kunitz domain. Furthermore, all specific antibody staining is lost after selective removal of Kunitz domain-containing forms of βAPP by monoclonal affinity chromatography (not shown). This suggests that carboxy-truncated forms of βAPP-770 and βAPP-751 are the predominant forms of the βAPP in platelet releasate and is
consistent with previous reports comparing the forms of βAPPs in nonneural tissue.13,31

Reports of the presence of full-length (transmembrane) forms of βAPP in platelets have been published.28,29 In studies purporting to show full-length forms of βAPP, high concentrations of platelets or concentrated platelet membrane fractions were required for their detection. Microvesicle localization of transmembrane forms of βAPP has also been suggested.30,31 The present studies suggest that full-length βAPP represents a small percentage of βAPP in platelet releasate. However, it is not conclusively demonstrated whether truncated forms of the βAPP are stored and released as such by platelets on activation, or whether platelet activation results in the proteolysis of the transmembrane forms and the release of carboxy-terminal–truncated species. Messenger RNA encoding for transmembrane forms of βAPP have been found in platelets,30 and megakaryocytes have been shown to contain abundant βAPP.29 Therefore, it is likely that platelet βAPP is an endogenous platelet product and the coexistence of both full-length and truncated forms is conceivable. However, the identical size of βAPP in SDS-lysed platelets and platelet releasate argues against large-scale proteolytic conversion of full-length to secreted forms as a consequence of platelet activation. Moreover, sucrose density gradient studies23 and the thrombin dose-response data of this report suggest that the released βAPP are stored in α granules.

Serum contains substantially more βAPP than plasma (Fig 6). Our data suggest that this increase is a direct result of the release of βAPP from platelets. Circulating WBC do not bind radiolabeled XIa on ligand blot (this report) and do not contain immunologically detectable concentrations of βAPP.32 The high avidity of Kunitz domain-containing forms of βAPP for factor XIa22 implies that platelets contribute significantly to the total factor XIa inhibitory activity found in serum.

Our results suggest that βAPPs account for the majority of the factor XIa inhibitory activity released from platelets. Another factor XIa inhibitor, unrelated to βAPP, has recently been identified in platelet releasate.33 This inhibitor, called PIXI, is of low molecular mass (~8 Kd), is found in a low molecular weight filtrate (YM 10; Amicon, Danvers, MA) of platelet releasate, and reportedly accounts for 18% to 54% of the total factor XIa inhibitory activity in platelet releasate.33 Why this moiety escaped detection in this report is presently unclear but may be related to differences in methodology, as our attempts to locate an ultrafilterable factor XIa inhibitory activity have not as yet been successful.

Thrombin cleaves the secreted forms of βAPP in the platelet releasate. This proteolysis is associated with the loss of reactivity to anti-βAPP antiserum, whereas that to the region encoded by exon 14 is retained. There is no loss of epitopes amino terminal to the exon 14 region and the 90- to 95-Kd doublet is stable even in the presence of high concentrations of thrombin or prolonged incubation (Fig 3). Thus, the thrombin-mediated degradation seems to be restricted to a site within 100 amino acids of the amino-terminus of the βAP. Conceivably the same proteolysis occurs in βAPP-751 and βAPP-770, because both isoforms have identical amino acid sequence in this region, including a single potential thrombin cleavage site (X-Pro-Arg) at amino acid 564-566 (βAPP-751). Cleavage at this site would result in loss of 102 amino acids from the carboxy-terminal end of the secreted forms of βAPP-751 and βAPP-770 consistent with the observed reduction in apparent molecular weight. The relatively high concentrations of thrombin used in these experiments may belie the physiologic relevance of this observation, because the local accumulation of thrombin at the site of a thrombus may be much higher than plasma or serum concentrations. Furthermore, the involvement of as yet unidentified cofactors may obviate the need for high thrombin concentrations. The loss of the carboxy-terminal fragment does not seem important for factor XIa inhibitory activity or heparin binding, because no difference in either of these functions was detected between βAPP-751 before or after thrombin-mediated proteolysis. Nevertheless, this observation may be more than trivial, because similar thrombin cleavage of the transmembrane forms of the βAPP would result in a membrane remnant that contains the intact βAP. Further processing of this remnant could yield the intact 42 amino acid βAP, which could then be deposited in tissues as amyloid under the appropriate pathologic conditions. Recent reports describing the presence of prothrombin message36 and thrombin receptor (S. Coughlin, personal communication, December 1991) in the brain suggest the possible interaction of βAPP and thrombin need not be limited to the circulation and coagulation.

REFERENCES

individuals near the interface of 21q21 and q22.1. Ann Genet 30:68, 1987


Characterization of platelet-releasable forms of beta-amyloid precursor proteins: the effect of thrombin

RP Smith and GJ Jr Broze