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Secretion of Alzheimer's Disease Aβ Amyloid Peptide by Activated Human Platelets

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SUMMARY: Alzheimer's disease (AD) is characterized by the deposition of Aβ (βA4) peptides of 39 to 43 amino acid residues, which are normal cellular metabolic products derived by proteolysis of the amyloid precursor protein (APP). The physiologic function of Aβ/APP in vivo is poorly understood. We analyzed human platelets for Aβ production by immunoprecipitation coupled to immunoblotting. A 4-kd Aβ fragment that comigrates with an Aβ40 synthetic peptide and reacts with several antibodies specific for the N- and C-termini of Aβ is detected. The majority of platelet Aβ appears to end at residue 40, as determined by immunoreactivity with an Aβ40-specific antibody. Furthermore, Aβ is secreted upon platelet stimulation with the physiologic agonists thrombin and collagen, together with secretion of soluble APP (sAPP). A comparison between serum and plasma shows a 1.6-fold increase in Aβ levels and a 2.4-fold increase in sAPP levels in serum. This is consistent with the view that platelets are the primary source of circulating Aβ and APP. The release of platelet Aβ by physiologic stimuli suggests that it may play a role in platelet aggregation and coagulation or in the repair mechanisms associated with injury. (*Lab Invest 1998, 78:461–469*).

Patients with Alzheimer's disease (AD) present pathologic brain lesions including amyloid deposition in the neuropil (as plaques), within neurons (associated with neurofibrillary tangles), and around blood vessels (as congophilic angiopathy). The principal components of AD amyloid are heterogeneous Aβ (βA4) peptides including full-length Aβ (1–39/43 residues) and p3(17–39/43) fragments, which are proteolytically derived from the amyloid precursor protein (APP) (Kang et al, 1987). The Aβ(1–39/40) species is the dominant form associated with blood vessel amyloid (Glenner and Wong, 1984; Masters et al, 1985a); whereas the longer species Aβ(1–42/43), which are less soluble and more cytotoxic (Hilbich et al, 1991; Jarrett et al, 1993; Roher et al, 1996), are the dominant forms found in plaques (Gravina et al, 1995; Masters et al, 1985b). Genetic and cell biologic evidence implicates Aβ/APP in the pathogenesis of AD. Mutations of the APP gene (on chromosome 21) within the Aβ region are causally linked to some familial forms of the disease (Ashall and Goate, 1994), causing either an increased secretion of Aβ or an increased relative production of Aβ(1–42) (Cai et al, 1993; Citron et al, 1992; Haass et al, 1994; Suzuki et al, 1994). Other pathogenic mutations that occur in the multitransmembrane proteins presenilin 1 and 2 (Levy-Lahad et al, 1995; Sherrington et al, 1995) also cause an increase in Aβ(1–42) production in plasma and fibroblasts of carriers (Scheuner et al, 1996), and in the brains of transgenic mice (Citron et al, 1997; Duff et al, 1996). However, the mechanisms by which Aβ deposition and accumulation occur in the more common sporadic forms of AD are not yet understood.

APP is a type I transmembrane protein that undergoes constitutive cleavage within the Aβ sequence by α-secretase to generate a large secreted N-terminal extracellular fragment of 100 to 130 kd (designated sAPPα) and a small 10-kd membrane-associated C-terminal fragment, which can be further processed by γ-secretase activity to produce a p3 fragment (Esch et al, 1990; Haass et al, 1992, 1993; Sisodia et al, 1990; Weidemann et al, 1989). This process precludes formation of a full-length Aβ peptide. In contrast, full-length Aβ generation may occur either at an early step of the endosomal-lysosomal pathway after endocytosis (Koo and Squazzo, 1994; Shoji et al, 1992) or in a late acidic compartment of the secretory pathway through the combined action of β- and γ-secretases (Busciglio et al, 1993; Haass et al, 1995; Xu et al, 1997). The secreted ectodomain (sAPPβ) generated by this pathway does not contain any Aβ sequence (Seubert et al, 1993). Low levels of Aβ/p3 are normally released by cultured cells (Cai et al, 1993; Fuller et al, 1995; Haass et al, 1992; 1993; Suzuki et al, 1994) and are present in plasma and cerebrospinal fluid (Seubert et al, 1992; Shoji et al, 1992). Recently, intracellular Aβ has been detected in platelets (Chen et

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al, 1995), neuronal (Fuller et al, 1995; Tienari et al, 1997; Turner et al, 1996), and non-neuronal cells (Iizuka et al, 1996; Wild-Bode et al, 1997).

The origin of the intracerebral amyloid deposits is obscure, but it is likely that it occurs locally, as neurons secrete substantial amounts of Aβ (Simons et al, 1996; Turner et al, 1996). It has been postulated that cerebral vascular amyloid deposits may be derived, in part, from the circulation and/or platelets (Chen et al, 1995). Numerous trophic or toxic actions have been suggested for sAPP and Aβ; however, their precise functions in vivo remain unclear. In an attempt to gain a better understanding of the contribution of APP/Aβ to the development of AD, we are studying the metabolism/function of APP in platelets. We have shown previously that platelets contain not only sAPP, but also full-length APP and Aβ-containing carboxyl terminal fragments (Li et al, 1994; 1995). Platelets may therefore contain the necessary machinery to generate Aβ. Platelet sAPP is primarily stored in the α-granules (Van Nostrand et al, 1990), contributing to > 90% of circulating APP, and is readily released into the medium upon physiologic agonist stimulation (Bush et al, 1990; Cole et al, 1990; Smith and Broze, 1992; Van Nostrand et al, 1991). The released APP potently inhibits coagulation factor IXa and XIa (Schmaier et al, 1993; Smith et al, 1990) and synthetic Aβ peptide itself is vasoactive (Thomas et al, 1996), suggesting a role for APP/Aβ in hemostasis.

In the present study, we were able to detect Aβ40 in unstimulated platelets by using the newly developed monoclonal antibodies (mAbs) W02 and G210 (Ida et al, 1996) and we demonstrate that Aβ is released by platelets during activation with physiologic stimuli, suggesting a physiologic role for platelet Aβ.

Results

The Major Aβ Species Present in Platelets Is Aβ(x-40)

Because we had demonstrated that human platelets contain the potentially amyloidogenic species of full-length APP and its C-terminal fragments (Li et al, 1994, 1995), the next step was to identify Aβ in platelets. Immunoprecipitation was coupled to a sensitive immunoblot analysis using the newly developed mAb W02 directed at residues 5 to 8 of Aβ (Ida et al, 1996). A 4-kd band was detected in unstimulated platelet lysates (Fig. 1A, Lane 2), which comigrated with the synthetic Aβ(1–40) peptide (Fig. 1A, Lane 1). This band was also immunoprecipitated by the monoclonal antibody 1E8, which recognizes residues 18 to 22 of Aβ (Allsop et al, 1997) (Fig. 1A, Lane 3).

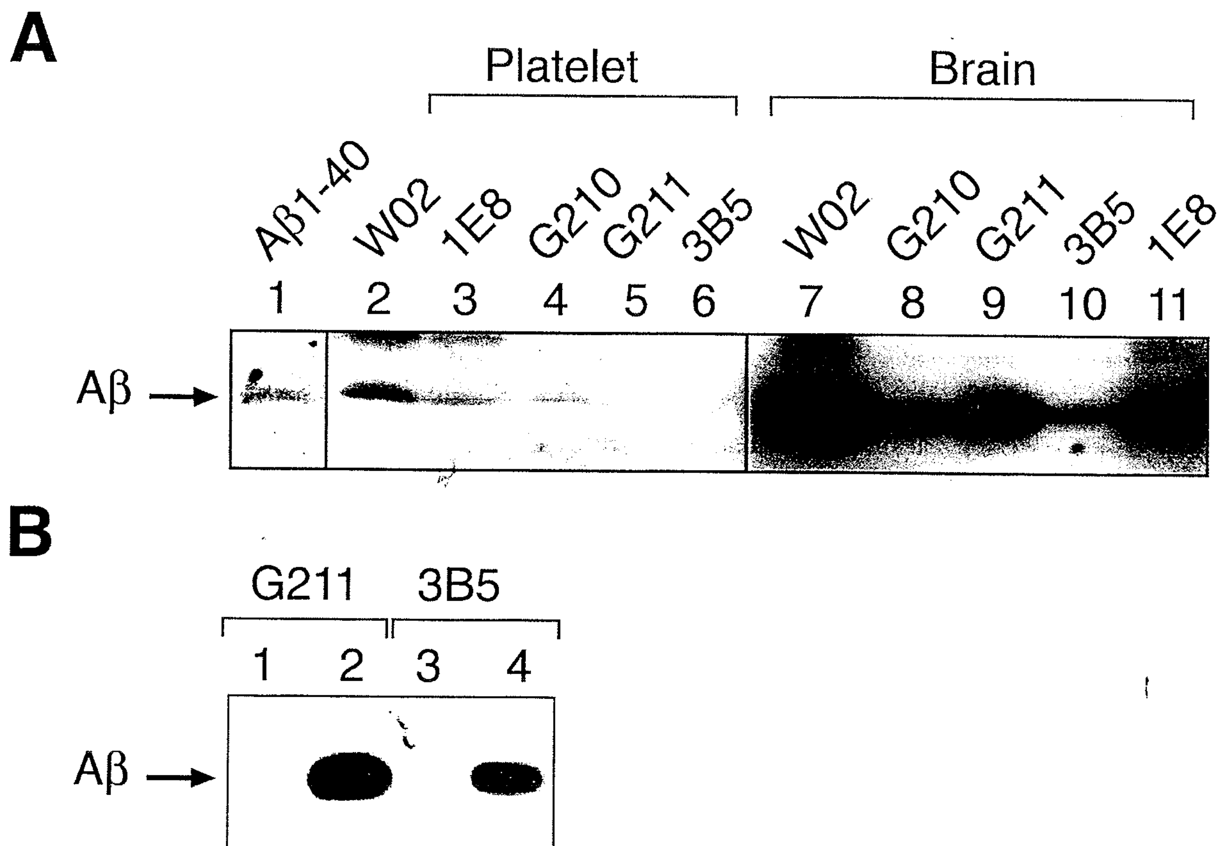

Figure 1.
A, Immunoprecipitation of platelet Aβ with monoclonal antibodies specific for the C-terminal residues of Aβ40 or Aβ42. Lysates of washed unstimulated platelets (from 1×10^9) or human brain homogenates (1 mg protein) were immunoprecipitated with the indicated antibodies. The immunoprecipitates were analyzed by immunoblotting with W02 as described in the "Materials and Methods." Synthetic Aβ1–40 (30 pg) was used as a standard (Lane 1). B, The specificity of Aβ42 antibodies. Synthetic peptides Aβ1–40 (Lanes 1 and 3) or Aβ1–42 (Lanes 2 and 4) were immunoprecipitated by mAb G211 (Lanes 1 and 2) or mAb 3B5 (Lanes 3 and 4) and detected by immunoblotting with W02.

To characterize the C-terminus of the platelet Aβ, we used the Aβ40-specific antibody mAb G210, and the Aβ42-specific monoclonal antibodies G211 (Ida et al, 1996) and 3B5 (R Jayasena et al, unpublished data). mAb G210 immunoprecipitated a 4-kd Aβ species, which comigrated with the immunoprecipitates of W02 and 1E8 (Fig. 1A, lane 4). The mAbs G211 and 3B5 specifically immunoprecipitated synthetic peptide Aβ(1–42) (Fig. 1B, Lanes 2 and 4) but not Aβ(1–40) (Fig. 1B, Lanes 1 and 3). However, neither of these Aβ42-specific antibodies immunoprecipitated detectable amounts of Aβ from platelet lysates (Fig. 1A, Lanes 5 and 6). To confirm the reactivity of the Aβ40 and Aβ42 antibodies against a native source of Aβ, the same set of antibodies was used to immunoprecipitate Aβ from an AD brain homogenate. Aβ species reacting with 40- and 42-specific antibodies were easily detected (Fig. 1A, Lanes 7 to 11). This result suggests that the main Aβ species in platelets is of x-40 residues. The Aβ(x-42) species, if present, are too low to be detected by our method.

Quantitative measurement of platelet Aβ using mAb W02 by ELISA was not possible, because W02 cross-reacts to sAPPα (see "*Platelets Release . . .*"), and all other available Aβ antibodies are not sensitive enough to detect Aβ in platelet lysates by ELISA.

Platelets Release Aβ Upon Activation

To investigate whether Aβ is released during platelet activation after agonist treatment, washed platelets were incubated with prostaglandin E_1 (PGE_1; unstimulated platelets) or with thrombin, collagen, phorbol 12-myristate 13-acetate (PMA), or calcium ionophore A23187 (stimulated platelets) as described in the "Materials and Methods." The media (releasates) derived from 2×10^9 unstimulated and stimulated platelets were immunoprecipitated by W02, and the products were detected by immunoblotting with W02 (Fig. 2A). We demonstrated that Aβ levels were significantly enhanced (Friedman ANOVA $p < 0.03$, t test $p < 0.05$ for each agonist) after stimulation by agonists such as thrombin (7.4 ± 2.8-fold), collagen (2.5 ± 0.3-fold), PMA (5.6 ± 0.6-fold), and A23187 (5.4 ± 0.7-fold), as compared with the medium from unstimulated (PGE_1-treated) platelets (Fig. 2, A and C). As a control for

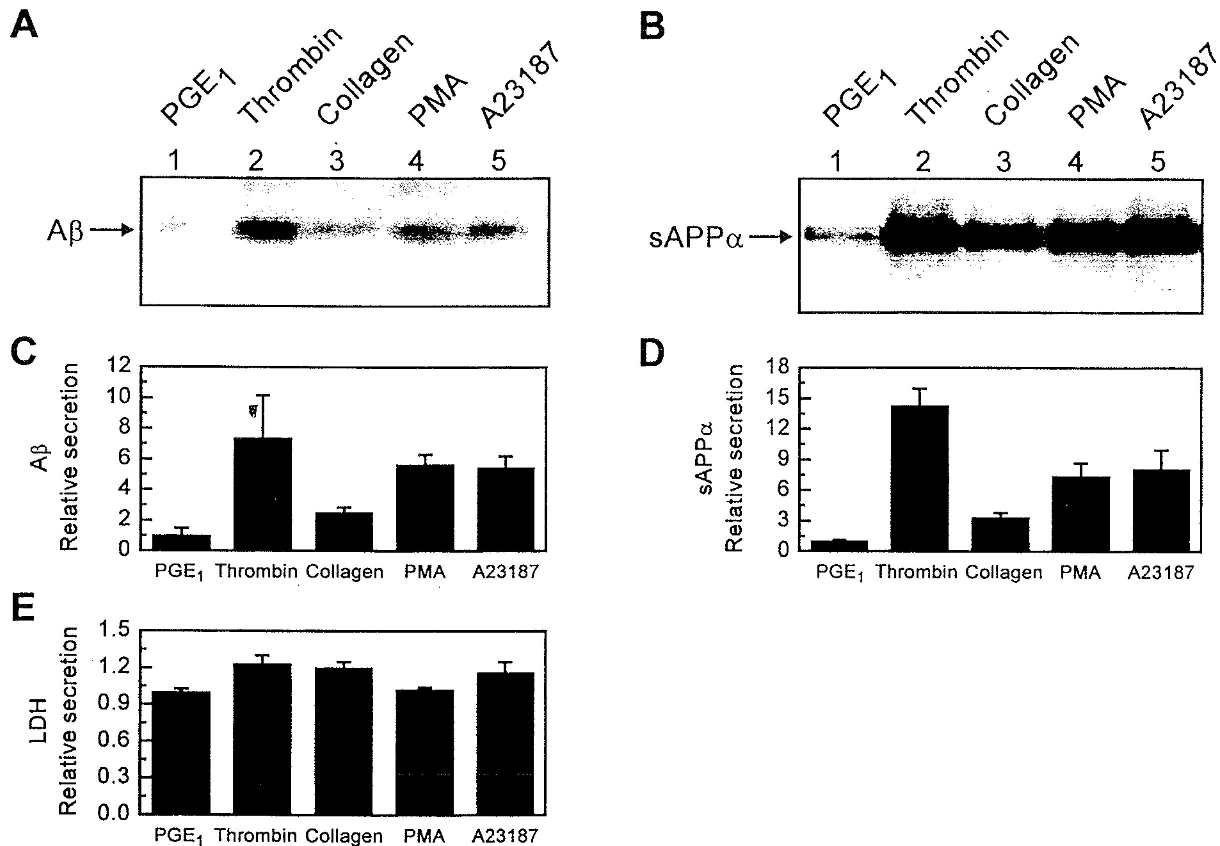

Figure 2.

Aβ is released during platelet activation. Washed platelets were activated with the indicated agonists in the presence of 2 mM $CaCl_2$, as described in "Materials and Methods." A, Platelet releasates obtained from 2×10^9 activated platelets were used for immunoprecipitation using W02 and immunoblotting with W02 to detect Aβ. B, Platelet releasates from 1×10^7 activated platelets were analyzed for soluble APP (sAPP) by direct immunoblotting with W02. C and D, Densitometry analysis of the bands corresponding to Aβ and sAPP, respectively. In each experiment, data were normalized to the control (prostaglandin E_1 [PGE_1]-platelets). Data represent means ± SEM from at least three independent experiments. For Aβ, Friedman ANOVA $p < 0.03$ and t test $p < 0.05$ for each agonist. For sAPP, Friedman ANOVA $p < 0.02$ and t test $p < 0.05$ for each agonist. E, Relative secretion of lactate dehydrogenase activity in releasates from unstimulated (PGE_1) or stimulated platelets (thrombin, collagen, phorbol 12-myristate 13-acetate [PMA], or A23187). Data represent means ± SEM from three independent experiments. Friedman ANOVA $p > 0.1$, not significant, comparing lactate dehydrogenase (LDH) from stimulated platelets and PGE_1 platelets.

secretion, we tested the above releasates for the presence of sAPP, a platelet-secreted protein (Bush et al, 1990; Cole et al, 1990; Li et al, 1994; Smith and Broze, 1992; Van Nostrand et al, 1990). In this experiment, releasates corresponding to 1×10^7 platelets were loaded into each gel track. Direct immunoblot analysis of sAPP with W02 demonstrated a significant increase of sAPP (Friedman ANOVA $p < 0.02$, t test $p < 0.05$ for each agonist) in the medium (releasate) obtained from treatment by thrombin (14.2 ± 1.7-fold), collagen (3.3 ± 0.4-fold), PMA (7.4 ± 1.3-fold), or A23187 (8.0 ± 1.9-fold), as compared with that in the PGE_1-treated medium (Fig. 2, B and D). As mAb W02 recognizes Aβ(5–8), the sAPP-released species is defined as sAPPα (secreted APP generated by α-secretase). To monitor platelet lysis accompanying activation, cytoplasmic lactate dehydrogenase (LDH) activity was measured in the releasates obtained from PGE_1, thrombin, collagen, PMA, or A23187 treatments. LDH activity was not elevated (Friedman ANOVA $p > 0.1$) in the stimulated releasates (thrombin, collagen, PMA, or A23187) (Fig. 2E), indicating that the Aβ release is a regulated secretion process.

Level of Aβ Is Higher in Serum than in Plasma

If platelets release Aβ during degranulation, serum (where complete platelet degranulation has occurred) should contain higher levels of Aβ than plasma. To detect Aβ in serum or plasma, samples (100 μl) were filtered through Centricon 50 and the filtrates analyzed by immunoblot using mAb W02. A representative blot is shown in Figure 3A, and the quantitative measure of 12 samples is shown in Figure 3C. A significant 1.6 ± 0.3-fold increase of serum Aβ, relative to plasma, was found ($p < 0.01$, Wilcoxon test). To measure sAPP, plasma or serum was passed through a Q-sepharose column, and sAPP was detected by immunoblot with mAb 22C11. Similar results were obtained when sAPP was detected with W02 (data not shown). Figure 3 (B and C) shows a significant 2.4 ± 0.4-fold increase of sAPP in serum as compared with plasma ($p < 0.002$, Wilcoxon test).

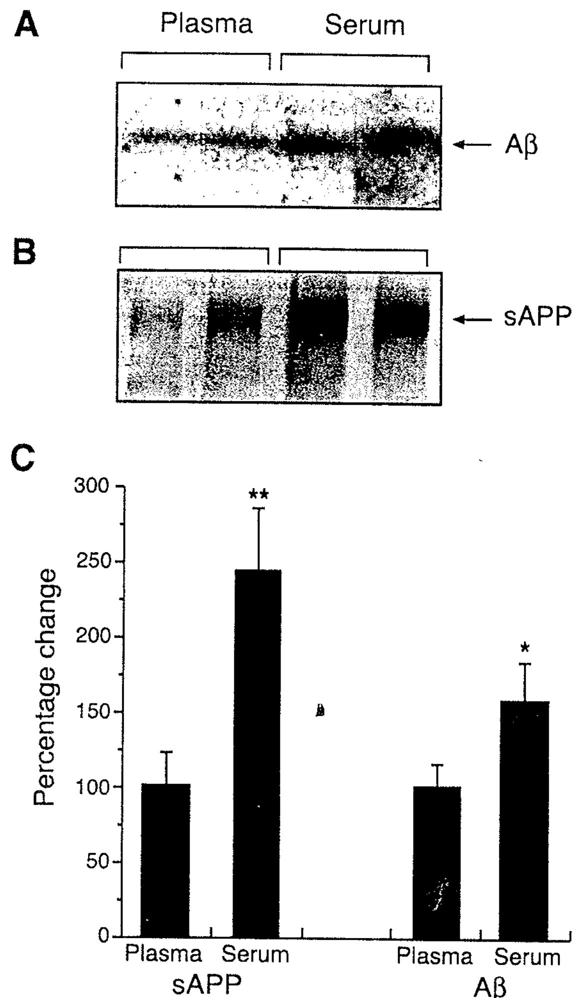

Figure 3.
Serum Aβ levels are elevated as compared with plasma. A, Immunoblot analysis of serum and plasma Aβ with mAb W02. A representative blot of Aβ (matched samples from two individuals) is shown. B, Immunoblot analysis of serum and plasma sAPP with mAb 22C11. A representative sAPP blot (matched samples from two individuals) is shown. C, Percentage change of Aβ and sAPP densities of serum compared with plasma. Each *bar* represents the average of 12 samples. The *error bars* refer to the standard error of the mean ($n = 12$). **, Difference from plasma sAPP ($p < 0.002$, Wilcoxon test); *, difference from plasma Aβ ($p < 0.01$, Wilcoxon test).

Discussion

The biogenesis and accumulation of cerebral Aβ from APP is a central issue in AD research. As a peripheral model system for studying neuronal processing, we have been investigating APP in platelets (Li et al, 1994, 1995). The present study identifies Aβ in unstimulated platelet lysates by immunoprecipitation with several Aβ-specific monoclonal antibodies coupled to a sensitive immunoblot detection assay. Evidence is also provided for the first time that Aβ is detected in the medium (releasate) derived from agonist-treated platelets, indicating that platelet Aβ is released upon degranulation. This finding suggests that Aβ may be localized to the secretory granules. The major species of Aβ in platelets ends at residue 40, as determined by immunoreactivity with mAb G210 (specific for Aβ40 C-terminus); this finding is in agreement with that of Chen et al (1995), who identified platelet Aβ by partial N-terminal sequencing. These data are consistent with other reports that Aβ(1–40) is the main species found in vascular amyloid (Glenner and Wong, 1984; Masters et al, 1985a). As Aβ(1–42) appears to be the determining species in amyloid deposition and plaque formation, we have also studied whether platelets are able to generate Aβ(x-42) species using Aβ42 end-specific monoclonal antibodies. Aβ(x-42) was not detected in our study; however, this may be because of the limit of detection with the present method, as Aβ(x-42) levels in plasma/CSF are usually 5- to 10-fold lower than those of Aβ(x-40) (Scheuner et al, 1996).

We have previously shown that the APP contained in platelet and released upon activation is C-terminally truncated (Li et al, 1994, 1995). The detection of APP in platelet releasate by mAb W02 (epitope at residues

5 to 8 of Aβ) suggests that the C-terminally truncated APP, which is referred to as sAPPα, is generated by cleavage at or near the α-secretase site. We were unable to detect the p3 fragment, corresponding to sequential cleavage by α- and γ-secretases, because of the lack of a sensitive immunoblotting antibody reacting to Aβ(17–39/43) fragment. The presence of sAPPα and Aβ in the platelets suggests that platelets contain the α-, β-, and γ-secretase activities and are an accessible peripheral system to study the processing of APP and Aβ.

The presence of Aβ within unstimulated platelets is consistent with its intracellular occurrence in cultured cells (both normal and transfected) (Fuller et al, 1995; Hartmann et al, 1997; Iizuka et al, 1996; Tienari et al, 1997; Turner et al, 1996; Wild-Bode et al, 1997). Studies in cultured cells indicate that Aβ is cleaved from APP in at least two distinct subcellular sites in the late secretory and endocytic/lysosomal pathways (Busciglio et al, 1993; Haass et al, 1995; Koo and Squazzo, 1994; Shoji et al, 1992; Xu et al, 1997). Recent studies by Wild-Bode et al (1997) and Hartmann et al (1997) suggest that Aβ42 can be generated within the endoplasmic reticulum and later within the Golgi. However, the precise intracellular compartment of platelet Aβ production and storage remains unclear. The fact that platelet Aβ can be released by a process of regulated secretion suggests that it could be localized in α-granules, where sAPP is also stored (Li et al, 1994; Van Nostrand et al, 1990).

The release of Aβ and sAPP upon platelet degranulation with thrombin, collagen, PMA, or calcium ionophore A23187 is consistent with a 1.6-fold increase in Aβ levels in serum and a concomitant 2.4-fold increase of sAPP, relative to plasma. These results support the view that platelets are the primary source of circulating Aβ and sAPP, because platelets contain > 90% of Aβ and APP immunoreactivities in blood, and plasma only contains 6% or less of these APP products (Bush et al, 1990; Chen et al, 1995; Cole et al, 1990; Smith and Broze, 1992; Van Nostrand et al, 1991). Platelets from subjects who show early signs of probable AD were also found to secrete Aβ (Q-X Li et al, unpublished results). Over time and under certain pathologic circumstances, the blood Aβ could contribute to the amyloid deposits in the cerebrovasculature of AD brain. In fact, a recent study indicates that soluble Aβ can be internalized into lysosomes by the cerebrovascular smooth muscle cells (Urmoneit et al, 1997). Degeneration of the smooth muscle cells could leave fibrillar Aβ or nonfibrillar Aβ within the extracellular space of the vessel wall, which could contribute to the cerebral amyloid angiopathy.

Platelet activation leads to secretion of a variety of proteins from the granules including thrombospondin, fibrinogen, von Willebrand factor, coagulation factor V, and platelet derived growth factor. These molecules participate in platelet aggregation, coagulation, and vascular repair. The secretion of Aβ peptide upon platelet activation may have a physiologic role in normal platelet function. It has been shown that aggregated Aβ(1–40) induces platelet aggregation and adhesion, possibly through an interaction with integrin receptors (Kowalska and Badellino, 1994). Physiologic concentrations of Aβ(1–40) are shown to augment platelet aggregation induced by adenosine diphosphate (B Wolozin, personal communication). It has also been reported that soluble Aβ mediates vasoactivity and may cause vascular endothelial damage via free radical production (Thomas et al, 1996). Other studies have shown that Aβ plays a role in neurodegeneration by altering Ca^{2+} homeostasis (Mattson et al, 1993), inducing neurotoxic responses (Roher et al, 1996; Yankner et al, 1990), or by inhibiting potassium channels (Etcheberrigaray et al, 1994). Platelet Aβ may therefore regulate hemostasis by influencing vascular function and by promoting platelet aggregation. In contrast, sAPP released by platelets is known to inhibit the coagulation cascade by inhibiting factors IXa and XIa (Schmaier et al, 1993; Smith et al, 1990) and has been detected within vascular thrombi (Lang et al, 1997). sAPP is also an inhibitor of weak agonist-induced platelet aggregation (A Henry et al, submitted for publication). Aβ may therefore have antagonistic effects on sAPP in the circulation. The agonist function of Aβ may assist the formation of the platelet plug, whereas sAPP may act to regulate its extension and associated degree of clot formation. Further knowledge of the function of Aβ and APP in platelets may help in understanding what their roles are in the central nervous system, where both Aβ and APP are present, and how they contribute to the development of Alzheimer's disease.

Materials and Methods

Materials

Monoclonal antibody (mAb) 22C11, which recognizes a denatured epitope near the N-terminus of APP (Hilbich et al, 1993; Weidemann et al, 1989), was obtained from Boehringer-Mannheim GmbH (Mannheim, Germany). mAb W02 recognizes residues 5 to 8 of Aβ (Ida et al, 1996). G210 and G211 recognize the C-terminal residues of x-40 (Aβ40) or x-42 (Aβ42), respectively (Ida et al, 1996). Monoclonal antibody 3B5 was raised to Aβ35–43 and recognizes the C-terminus of Aβ42 (R Jayasena et al, unpublished data). mAb 1E8 was raised against peptides 12 to 28 of Aβ; its defined epitope is approximately Aβ(18–22) (Allsop et al, 1997). [^{125}I]-Protein A was from ICN Biochemicals (Irvine, California). Thrombin purified from human plasma was obtained from Boehringer-Mannheim GmbH. Collagen, calcium ionophore A23187, and PMA were from Sigma (St. Louis, Missouri). Lactate dehydrogenase was measured using a kit from Boehringer-Mannheim GmbH. Q-Sepharose resin and Protein A Sepharose were from Pharmacia Biotech (Uppsala, Sweden).

Preparation of Platelet Lysate and Immunoprecipitation

Human platelets were isolated from fresh blood of normal laboratory volunteers collected into anticoagulant and washed in the presence of PGE_1 to minimize

activation as previously described (Li et al, 1995). Briefly, platelets from each individual were purified by differential centrifugation, washed, resuspended in Tyrode's buffer at 1×10^9/ml, and incubated with PGE_1 (3 μM), thrombin (1 U/ml), collagen (20 μg/ml), PMA (1 μM), or calcium ionophore A23187 (1 μM), for 15 minutes at room temperature, as described (Li et al, 1995). Platelets were pelleted by centrifugation at 1,500*g* for 15 minutes, and the supernatant was respun at the same speed and taken as "platelet releasate," which was subsequently adjusted to contain 0.2% NP-40, 50 μg/ml leupeptin, and 1 mM phenyl-methyl-sulfonyl-fluoride (PMSF). To prepare platelet lysates, pellets (corresponding to 1×10^9 platelets) were homogenized in STEN buffer (Tris-HCl [pH 7.4], 150 mM NaCl, 5 mM EDTA, 0.2% NP-40 [Nonidet P-40]), containing 50 μg/ml leupeptin, 1 mM PMSF, and 0.1% SDS, and were spun at 10,000*g* to remove insoluble materials. Human brain homogenate was prepared as described previously (Li et al, 1994). To immunoprecipitate Aβ, platelet lysates, releasates, or brain homogenates were incubated with mAb W02, G210, G211, 1E8, or 3B5, and Protein A Sepharose saturated with rabbit anti-mouse IgG. After washing the beads three times with STEN and three times with STE buffer (Tris-HCl [pH 7.4], 150 mM NaCl, 5 mM EDTA), the immunoprecipitated products were solubilized by adding sample buffer (42 mM Tris-HCl [pH 6.8], 2% SDS [w/v], and 5% [v/v] β-mercaptoethanol), and separated on 10% Tris-Tricine SDS-PAGE followed by immunoblotting.

Detection of Aβ from Human Plasma and Serum

Four separate plasma and serum samples were collected from each of three healthy young subjects. Plasma was prepared from blood collected into a syringe containing a mixture of PGE_1 (3 μM), heparin (17 U/ml), and theophylline (1 mM), and processed at 4° C to minimize platelet activation (Whyte et al, 1997). Serum was prepared from blood collected into tubes without anticoagulant. Both plasma and serum were obtained by centrifugation of the blood samples at 1500*g* for 30 minutes. To analyze Aβ, 100 μl of the samples were further centrifuged through Centricon 50 (Amicon, Beverly, Massachusetts) as directed by the manufacturer. The filtrate was collected and analyzed by 10% Tris-Tricine SDS-PAGE and Western blotted using mAb W02. To determine sAPP, 0.125 ml of plasma or serum was loaded onto Q-Sepharose resin (0.025 ml bed volume) equilibrated with TB buffer (50 mM Tris-HCl, pH 7.4, containing 350 mM NaCl), and eluted with TB buffer containing 1 M NaCl as described (Whyte et al, 1997). Eluates (containing equal amounts of protein) were analyzed by immunoblotting using mAb 22C11 or mAb WO2. Each blot contained specimens from the same initial blood collection sample (matched plasma and serum), which allowed direct comparison of each experimental condition upon the same blot in a repeat measures design.

SDS-Polyacrylamide Gel Electrophoresis (PAGE) and Immunoblotting

Samples were dissolved in reducing sample buffer, boiled, and separated on 10% Tris-Tricine polyacrylamide gels in the presence of SDS (for Aβ detection). The proteins were transferred electrophoretically to nitrocellulose (Bio-Rad, Hercules, California) as described (Ida et al, 1996). Nonspecific binding of primary antibody was blocked with 0.5% (w/v) hydrolyzed casein, and the membranes were incubated with primary antibody W02 (1 μg/ml) for 2 hours at room temperature. For enhanced chemiluminescence detection of platelet Aβ, a secondary anti-mouse IgG conjugated to horseradish peroxidase (1:3000; Amersham, Buckinghamshire, United Kingdom) was used to detect the bound primary antibody. Peroxidase substrate solution for ECL detection was obtained from Pierce (Rockford, Illinois). For quantitative measure of Aβ in plasma and serum, the membrane was incubated with mAb W02; next, rabbit anti-mouse Ig was added for 1 hour followed by incubation with [^{125}I]-Protein A. For immunoblot analysis of sAPP in plasma and serum, an 8.5% Tris-glycine PAGE and mAb 22C11 (1 μg/ml) were used, followed by incubation with alkaline-phosphatase-conjugated secondary antibody (1:10,000). The signals were visualized by using Fast-Red/Naphthol AS-MX phosphate as substrates, as described previously (Li et al, 1995).

Data Analysis

Quantitative measure of Aβ bands of plasma and serum was carried out using a Fuji Phosphorimaging system FUJIX BAS 1000 with MacBAS V.1.01 software (Fuji, Miyamodai, Japan). Quantitative measure of sAPP bands of plasma and serum and of platelet Aβ and sAPP was carried out by densitometry using the NIH Image software (V. 1.57; National Institutes of Health, Bethesda, Maryland). Data in text and figures are expressed as mean ± SEM. The values were analyzed statistically using the nonparametric Wilcoxon test, or Friedman ANOVA followed by individual comparisons with a two-tailed repeat measures *t* test as appropriate. $p < 0.05$ was regarded as statistically significant.

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