ƒ ‹F | Œfia‹ ˙'e›ß• |–M^D‹ ª¤s›p e ·`⁄⁄¶i« ‡ł§i

Amyloid-beta ⁄ ¸¤ «e¯X‟«APP ƒb´ ¯Ø⁄ ¸‹ ¡¯Ø °‟«„Œ¯ ⁄⁄„

ƒ ⁄p‟O‹ ¡⁄˘†£¥˝⁄£ƒP§@¥ ¸‟” ´ –· Q(1/3)

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›p e‰ ¸s„¡GNSC92-2321-B-038-001-

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› p „ " ^ § O ¡ G / fl°(«‹ ª ª¤s‹›p„"

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**Abstract**

Aging is a major risk factor for neurodegenerative disorders, such as Alzheimer's disease (AD). The pathological hallmarks of AD brains include cortical atrophy, neurofibrillary tangles and senile plaques. The amyloid-β (Aβ) is the major constituent of senile plaques. The Aβ is present in a soluble, non-toxic form in all human brains, mainly as a 39-43 amino acid sequence. Deposition of Aβ in senile plaques and in the walls of cortical and leptomeningeal blood vessels is a hallmark of AD. Aβ is derived from proteolytic cleavage of amyloid precursor protein (APP). APP is abundantly expressed in the platelet α-granule. The $A\beta_{1-40}$ is the dominant form associated with blood vessel amyloid; whereas the longer species $A\beta_{1-42}$, which are less soluble and more cytotoxic, are the dominant forms found in senile plaques. Because neurons secrete substantial amounts of Aβ, intracerebral deposition of Aβ may occur locally. However, cerebral vascular amyloid deposits may be derived in part from circulating Aβ. Indeed, human platelets contain high levels of membrane-associated and soluble forms of APP, which upon platelet activation, the cleaved APP is readily released by platelets and may contribute to more than 90% of circulating APP. The resting plasma concentration of APP is low (¡ 60 pM), assuming a physiological concentration of $3 \times 10^8$ platelet/ml of blood, degranulation is reported to contribute about 30 nM of APP in blood. In addition, platelets may also be a source of Aβ detected in whole blood. The Aβ, like APP, is also released upon platelet activation. Therefore, much higher localized concentrations of APP and Aβ could be achieved when platelets accumulate at the site of vascular injury. The platelet therefore represents an efficient means of targeting the release of high doses of APP and Aβ into a generating thrombus. To date, little is known concerning the effect of APP and/or Aβ in platelets in *in vitro* and *in vivo* studies. In our studies, we found that the sAPPα (3-15 nM) can effectively inhibit the platelet aggregation. sAPPα dose-dependently inhibited the aggregation induced by collagen, ADP, and AA. Contrariwise, Aβ (0.5-2 μM) potentiated platelet aggregation and ATP release by collagen (0.5 μg/ml) and ADP (4 μM) in human platelet suspensions. Furthermore, Aβ also potentiated $[Ca^{2+}]i$ mobilization in human platelet suspensions stimulated by collagen (0.5 μg/ml). Our studies also consist with the previous results with sAPPα and Aβ exhibit opposite effects in platelet activation.

Therefore, we want to further evaluate the tyrosine phosphorylation ($pp72^{SYK}$, $pp125^{FAK}$, $pp60^{C\text{-}SRC}$ and JAK family kinase), free radical and NO formatioins; (2) quantitative and qualitative analysis of APP and Aβ in platelets by HPCE, TEM, and confocal microscope. Through these studies, we will obtain a better insight into the detailed different mechanisms and activities of APP and Aβ in platelets, and further understand physiologically and pathologically relevant functions for APP and Aβ as feedback regulator during platelet activation.

**Keywords** ¡GAlzheimer's disease, β-amyloid, amyloid precursor protein, platelets.

## ⁄G¡B†‡Si⁄"fie

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• H⁄Y"¥ › S ¡ ƒ ~ ˜ ⁄£"™"ƒ⁄W⁄fi ¡ A † o ¯ ª ¥ S ›⁄W¶v"¥¤Þ⁄AJff]ff„⁄˜ ƒ ƒ ~ H‹""fl e fl f – S ¤@fl° ⁄ j † † ⁄ ¸ ´^™"⁄^⁄H›‡P› « ‡ ¡ C " · fi qflg (Alzheimer's disease)· N ‹ O ⁄ @ " – ' ¤ £ " " ƒ ƒ ~ fl e fl f ¡ A ¥ B ‹ ƒƒ~¥¢·...flg"" › ⁄ H – ' ¥ X † { ¿ S † " æ o ¥ ˝ " " ¤ ˘ ¡ B L " k ƒ n ⁄ Ø – ¥˝‹ ¡´†‡ ""¤˘ • P ¡ B ¥ ¢ ‰S(Ff´p⁄S) ¿ ⁄l)x¡BS˘ › ¤ " "Hfi (¯ "" &k¡B† ·...' ¸ x')˜ ¥¥¡ AN, ^ ‰‹OS _ – w ⁄ F "·flfg AdJ „ »¶i@¤B ¶E´_» P " v‡f¡C ¥i» ‹O, £ fl « g•"1f¥D ƒ ]Af , £ fl ‹g"•"1fi† " G « K • | † ƒ¤fl"g¡·F fl" q·fiflg¥Q› n o¥f˝ b £†"¡"⁄ j , ¥ %Ł (cerebral cortex) ¡Bfi ¤j(hippocampus) ⁄ ¸ S ⁄fl(amygdala) † S¸ƒ ¤ ‰, ḡ ß, ' (neurofibrillary tangles) 'Mf ƒ~· ¶ (senile plaques) (Chartier-Harlin et al., 1991)¡ F ‰, ḡ " ß »P⁄ @ " ‹ tau""† ¥ %Ł£¥¿–' ¿ fi†¡ˆFf ƒ~· ¶ ¥D› fi] amyloid-β (Aβ) ⁄j¶q"" ¤I¿n' "cf¤¡Af Aβ‹ O @"ƒsƒb' ⁄Hˆ ,£⁄⁄¥i• » ,ffl Æ Ł†¥B¤ªƒ†fl ‹g‹ ‡°"" i »˜ (Glenner and Wong, 1984)¡C

"æ·Xƒ~¤ „ ' " · fi flgS¸ƒ¤" " ›ƒJ†¤ › ¥D› ‰" †»¡¡G⁄@› ‹O amyloid †» ¡A†o › †»¡»{‹ fl ‹g""¶¸fi' (SY" ·fi qfl g " "o¥D›n¤ ƒ ' amyloid precursor protein (APP)⁄£¥¿– ""¥ N´¡Af ¥ƒAβ ""¶q⁄ W ¡A¤ˆ¥B ›PAβ⁄j¶ qI¿n' ,£⁄⁄ƒ S¸ƒ¤senile plaques¡A¶ ‡ « ¤˘" · fi fl g " "o¥˝ƒ ' fl ‹g† › M'‹[ ¶¸fi' †» ¡A†o †»¡»{‹ " · fi fl g " "o¥˝ƒ ' fl ‹g† › M'"S ¯ (Braak and Braak, 1991)¡C¥ flf† z ˜[ƒ ¤¥¡A" · fi flgƒ b ‰, fgf† z´⁄W¤ªƒ†fl fi "˝¯ ⁄˘¡A¥ ƒ †¥~˘[ ⁄W¥i¤£¤ ⁄ j , ¥ %Ł `Y¡B,£""• j⁄]¯ ...e¥B,£« ´X⁄ j F‡fb ´ ⁄`⁄W¥ ¥i¤£¤ fl ‹g† ›M.. ˘ ¥ ' ēª¡·A⁄⁄¤ ƒbø fi›'Mˆ Þ›¡ ¶E´_" · fi fl g"†› ‹» flf† z «...⁄@‹ƒ ƒ† (senile plaques, SP) ⁄ @ fl ‹g¯ "ß˜æ † (neurofibrillary tangles, NFT)¡ F„¥~' |ƒ†` ß † "¯"w ¯ '° (granulovacuolar degeneration) ¥¯ ⁄˘ (Suh and Checler, 2002)¡C

Senile plaques ¥D› nOAβ ""¤I ' † ¤¡ASP""... ¥ • U ƒ H ¸¥ flf–wS ¥ifl ‹O" ·fi qfl g"–w" ¡Af SPƒb‹ ¥·¥¿–"" " ƒ⁄H¸⁄@¤·,£¶¸«Æ" " ffl⁄¸H ‡¡⁄⁄¥ • |‹ ¤ ¡ A]ƒ„†{ƒb S ¶i⁄@¤B–NSP ›‹ ˜ "J''°" " ƒƒ~† (diffuse plaques)'Mfl ‹g"¢''°ƒ ƒ~·†(neuritic plaques)¡ F ‰, g¢''°ƒ ƒ~·†« " ˝O¥Hamyloid‹ ⁄⁄ ¡A' Þ ƒ†⁄@¤· `Y'°""fl ‹g¶ b ¯ ⁄˘¡Af ˜ "J''°" " ƒƒ~†« ¥uf†† fl´""amyloid¤I¿n¡C†{ƒb»{‹ fl ‹g"¢''°ƒ ƒ~· †M" ·fi qflg⁄ æßƒ†‹ ^ ''°¡Afb¥¿– ' ƒ ⁄H›⁄¤£¤ ""⁄j†£‹O˜ "J''°ƒ ƒ~·†(Hardy and Higgins, 1992)¡C

Neurofibrillary tangles (NFT) ‹ O @"ƒ ' fl ‹g† ›M⁄"™"¯ "ß¤I¿n¡ANFT""ƒ¤¥ ¥D›n ‹O„ L «`»Œ˘⁄˘™""tau † ¥ (hyperphosphorylated tau protein)¥H´ Ø †"o» ŒXƒ ƒ¤ (paired helical filaments)¡ANFT¥ »» „ fl ‹g† ›M¤ªƒ†‹ ‡°¥B• ¶i⁄@¤ B † y ¥flk‹,¥Mƒ ⁄ ¡Ctau† ¥ ‹ O @"^ ›w† ›M⁄"•L† ⁄ ß «h»P† ›M⁄" i¥ ™"„ ŒØ ƒ ª ¡A• tau†Q`O»˘⁄˘«ÆSY† ¥¢^ › ¥•‡L ⁄ ß "⁄"fl ⁄ O Ąffl ¤˘–o•L† ⁄ ß ¤ ¤ fl }a¡A¶i ƒ … vT† ›M"⁄¿–'¥\fl ¡Cf†¤· ·" »{‹ " · fi fl g ‴flf¥D› nO¥ NFTƒbø fi»t † ¥ ¶} ' ‡S«ÆfA´Xfii¤ ¤ ¥L,£ ˘¡A¤ˆ¥Hf„¤ ‹ " · fi fl g"⁄ ·`¡A¥i‹Of†¤·'" · fi fl g ffl⁄H " ffl†z¯ ⁄˘⁄ ¸¡ANFT¤ˆ£' ēª¡Af † fl´¥HSP‹ ¥D(Braak and Braak, 1991)¡C

¥ ⁄ ⁄l¥˝™« ·"⁄"˜ [If ¤¥¡A" · fi flg"⁄ ⁄l¥˝™« ·‹ ªs¥D› nO¥ ⁄@¤·¤ªƒ†¿ ¶·'° fia– "⁄"" · fi fl g fl £Hk⁄ªsƒ ¤ ¡F¥ «e,ß† M¡"⁄"¥ ¸Aamyloid precursor protein (APP) ƒ ]Af ' †˜21„ ‹ V ƒ¯ØFpresenilin 1 (PS1)¡Af ' †˜14„ ‹ V ƒ¯ØF presenilin 2 (PS2)¡A

ƒ ' † ˜ ⁄ @ „ ‹ V ƒ ¯ØŠPC«e‰amyloidŠ”Y ©¥APP⁄ , ƒ¤APPÆƒ † ¤ ”¥™fl ¥ N ` ‡~fiÅ⁄@‹ O†Qα-secretase⁄ , ¡Å h ⁄ £ • †£¥ffl¤ª« , gr™”amyloid ¡F⁄@‹ O†Q β-secretase⁄ , ¡ÆA¥ γ-secretase¶i⁄@¤B⁄ , ƒ ¤ ÐfÎ™Aβ ¡C APP ƒ⁄W‹ Y ¤ ‹ •⏐…W ¥[APP'„ β-secretase¥ Ñ´ƒ … ₩[Aβ ¤ ¿n¡ Æ PS1»PPS2«h†Q» ‹ »P γ-secretase,ßƒ†^ «Y¡CAPP‹ O⁄@” † › M ‰†¥Ð‡Q⁄T” ⁄£ƒP™”† J ¥ »^Œl−¡B β−¡B γ−secretase' ⁄ , ¡A ¥ ‹ O¶ ¡ −†ƒy¤™ · fi ™Œf¥Œƒ]¡F·N† ¥ ”fcƒ ¤¥¡AAPP¥ ‹Acystein-rich, heparin binding domain, Zn binding domain, acidic domain, KPI domain (Kunitz-type serine protease inhibitor), RERNS trophio domain, glycosylation ⁄¸ Aβ ¡BCT$_{26}$ ¡BCT$_{31}$ ¥¡CAPP¥ ‡Q β−secretase⁄ †¸ƒ¤¤ › †⁄ ¡ANH$_2$” ‹ ⁄@› ¥i•»' °™”· ´ APP (β−APPs)¡ Æ COOH” «h ‹ O⁄@› ¥ 99 › i »˜' † ƒ¤™”† ¥ ‰ Ł ø‹(ŒCT$_{99}$)¡F⁄ †¸«Æ™”CT$_{99}$⁄ · flƒb M‰⁄W¡A‹ O⁄@ › ⁄£· ´ ™”† ¥ ¡A›YCT$_{99}$˜ ~ †Q γ−secretase⁄ , « h¶i⁄@¤BS ¸ƒ¤⁄@” 4 kDa™”Aβ (†yƒ¤ ™ · fi ™Œf¥Œ›n† ¥ )¡Cƒ ¥⁄~¡AAPP›Y†Q α−secretase⁄ , ¡¥A ' ¤ ⁄ , ™”†¡⁄ › ÆŁnƒ ƒbAPP ”fc⁄W™”Aβ region (β regionƒ ƒbAPP$_{695}$⁄W† ˜597¤ 639™” i »˜⁄W)¡Aƒ]ƒ„• ⏐⁄ , †£ ¥˝¤ › † ¥ ¡A⁄@› ‹ APPαs¥t⁄@› ‹ Št83 › i »˜™”† ¥ ‰ Ł ø‹(ŒCT$_{83}$)¡ACT$_{83}$ ›Y˜ ~ †Q γ−secretase⁄ , « h⏐Š¸ƒ¤⁄@› ⁄ £⏐⁄ _™ · fi ™Œf¥Œg ¥ ‰ Ł ø‹(ŒCT$_{57-59}$ (Suh and Checler, 2002)¡C

™ æé~¤ „ ' ™ · fi ™Œfkgª ¤ s › « ƒbA'ßæ'MAβ ' † ¥˝⁄ Š ^ fl « , gr⁄W¸A¥B»{ ‹ APP™” ƒ ¸ ‹¯O†yƒ¤Aβ ƒb,£⁄... ₩[™”¥D›n› ƒ] (Suzuki et al., 1994)¡FAβ¥ »× O APP¥ ¿ '¥˝† zU' † ¥˝™”¥ Ñ´†£™« (β regionƒ ƒbAPP$_{695}$⁄W† ˜597¤ 639™” i »˜⁄W) (Golde et al., 1992)¥B⁄ j ‰…ᵉ † ¥˝™”Aβ ¥i•»' ƒ …',¿ffl Æ Ł†Œ⁄ (Seubert et al., 1992)¡F‹ ªs ф{90 % ¥H⁄W™”Aβ‹ Ø 40 › i »˜' † ƒ¤™”† ¥ ⁄ øœ(Aβ$_{40}$)¡A¥uf†⁄ ' 10 % ‹ Ø 42-43 › i »˜' † ƒ¤™”† ¥ ⁄ øœ(Aβ$_{42}$)¡FAβ$_{42}$⁄æAβ$_{40}$,ß¤ª† ¤ '°ƒ]ƒ„Aβ$_{42}$,ß' ƒb,£⁄Š¸ƒ¤cerebral plaques (Iwatsubo et al., 1994)¡C

Amyloid† ¥ ⁄⁄†Q» ‹ »PŠ¸ƒ¤™ · fi ™Œfkgª« ^ «Y™”† ¥ ¥ ‹AAβ'MCT (APP™”C” † ¥ )¡A¶ † ¸⁄WAβ ⁄Š' ¥H†Q» ‹ »P™ · fi ™Œfkgª ...h™”^ `p' °¥D›n‹ Of]Aβ ™”† ¿n•⏐ †yƒ¤fl ‹g¶ fi¸' (Sisodia et al., 1990)¡CAβ™” fi ¶ „L⁄¥D›n† ¸ILcross-β pleated-sheetƒ † ƒ¤⁄@› †Q» ‹ ‹ O†yƒ¤™ · fi ™Œfkgª†z ”fc (Dumery et al., 2001)¡C¤ „Œ⁄Wk ªs ф{Aβ ƒb¥... ¶ « e „'fl ‹g† ›M‹ K¤ª ƒ†‹ £°¡Aƒ]ƒ„ ¤M™ · fi ™Œfl g ^ D » ¥ ⁄¬qAβ™” Š¸ƒ¤ ⁄ (McLean et al., 1999)¡Cƒ ¥⁄~amyloid' †yƒ¤™”fl « , gr¤', † ´ ƒ ⁄·‹ O› `...¡Aƒ ⁄j›P⁄W¥i† ¥˝⁄Uƒ C · X ¶⁄¥ ‹ª¡ G « ª ˇ¥ƒ ⁄j¶q† ¿n¡B¶t´ ⁄l⁄£¥ ¿ '˜ 'æ¡B o ™ ¢ ˇ ⁄+¸... ₩[† ›M⁄”⁄£¥ ¿ ™” ffiŠ¶ · » ... Š¥ ¸¥¡Cƒ ¥ ⁄j¶q† ¿n⁄@™‰¥H¤ †Q» ‹ ‹ O†yƒ¤ ,£fl ‹g¶ fi¸' ›P™ · fi ŠfÎfg™”›«›n› ƒ]⁄Š⁄@ (Markesbery, 1997)¡Fƒ ¥ ™”Š¸ƒ¤¥D ›n‹ O† zILAβ'M† M‰⁄Š¶¡™”¥ ⁄‹Š Ø¸ƒ ƒ¤¡Aƒ ⁄ ⁄·⁄£† M¡¡ C@fl°» ‹ Aβ ¥i· ¸J neuronal'Mglial´ø..h‰⁄⁄¡A¤ˆ¥B,g¥ fl ‰Ł„lfi æ˘(lipid peroxidation)'M† ¥ ‰Łfi æ˘(protein oxidation)Š @ ¥ «Pfiæ˘ ^ '°™”ƒ ¥ ¥˝ƒ¤˜ 'æ(Varadarajan et al., 2000)¡FAβ' ⁄ o™”ƒ ¥ ¶ fi¸'• ⏐ ›P† ›M™ ″@¤·¥\fl ' ¥¢¡A¥ ‹AŠ ¤ ATPase¡B¥¢¥h¶t´ ⁄l‰ −−fl ⁄O¡BŠ ¤ glial† ›Mneuronal excitatory NMDA¤ ¯ Ø″Na$^+$-dependent glutamate uptake¡B† ¥¢† ¥ ‰Ł„B ¿Øfl ⁄O¡B† ⁄ † ›M⁄”‹ ¥ · ffiŠ¶ · » ... Š¥™ o¥˝¤ˆ¥B¥i¥H‹ ¡⁄˘fi ´ ¿ ƒ]⁄l (nuclear transcription factor ) 'M⁄ _† ›M› ⁄'(apoptotic) , fi⏐¡C† ™ æ ª¤ª ф{¡AAβ ¥i† ¸IL(1) voltage-gate¶t´ ⁄l† ‹gD(N'ML-«‹† ‹gD)... ₩[¶t´ ⁄l™”˜ 'æ¡F(2) Aβ'M⁄@› ¿ '°™”¶Š´ ⁄l † ‹gD †ƒX¡A‹ P ¤¶t ⁄l¶i⁄J† M‰¡F(3) › Š`´ ⁄l»PNMDA¤ ¯Ø †ƒX¡A‹ P ¤¶t ⁄l™” ¶i⁄J¡F(4) Š ¤ „[´ ⁄l† ‹gD'MNa$^+$/Ca$^{2+}$¥ ·«¡A.. ₩[¶t´ ⁄l¿ @ ¡A ›Pfl ‹g† ›Mƒ”⁄ ' (Suh and Checler, 2002)¡Cƒ ¥⁄~¡AAβ « ª ˇfl ‹g† ›Mƒ”⁄ ™”ƒPfi ¥ •⏐« ª ˇ¤ ¥L† ›M† ¥¢› ƒ†⁄Š

¥ \ fl ƒ ¥ [ ‡ t ” · fi qflgflf–¡·c/ˇ¡C

¥ t ¥ ~ Ą‹mięcroglia 'Mastrocytes¥ H / ¸ / _ o ” ¢ˇ'M¶¥ ¸‡Ł›»¤{ ¢·” ””·fi fłgf ‡ (Chong et al., 2001)¡F/ _Ś K 'M o ” ¢ˇŚ ## Q ›‹¥ i ›.¯™T · fi ™ffłgf ¡ (Dumery et al., 2001)¡Cƒ ] ƒ „ ł,g'o°₵”„ L {ƒb · fi ™ffłgf† z Wƒfl‡›« › fiaƒ (Calingasan et al., 2002)¡Cƒ‡‹ ª ¤ « ƒ¥X” · fi qflgfłf/»”microglia• | ”†¥XAβ¤ ›” c @¤· ð¢” « ‰ ¥]ćytokines¡B chemokines¡B growth factors¡B complements'M¤ ¥L o ”¢‹ ^ '°” ‰Ł(Lue et al., 2001)¡F ¶i/@¤B‹ ªsŚ o†{¡AfbAβ¥ … fi ¶«e¤w{ƒ† prointerleukin-1β ¡Binterleukin-6 ¡Btumor necrosis factor-β ¡Bmonocyte chemoattractant protein-1 ¡Bmacrophage inflammatory peptide-1β ¡Binterleukin-8 'Mmacrophage colony-stimulating factor (Lue et al., 2001) ¥ ð¢‹ ^ ” ‰Ł† ¥˝¡F¥ ƒ„¥i” o ” ¢ˇ„ ‡ fl ‹g¶¸ fi ' ›℗ · fi fłgfłQ/ – K`” ” «Y¡Cƒ„¥ †AAβ ¥ ¥i‹ ƒˇ¤ ¥L T fi Ś ¶ »…¡A¤fi‡p MAPK pathway (mitogen-activated protein kinase)¡A ƒpAβ ƒbfi ‡^fl ‹g (hippocampal neurons) ƒb« ℗ˇtau` ß/~/ˇfi «K• |/ cMAPK pathway (Rapoport and Ferreira, 2000)¡C

ƒ p”O ƒˇ‹ O ¯ Ø / ” ƒ ””fłfø¥¿†z¥ Ń ”/”¥†›n/ ˇ‡¡Ef ”¤¶¸”” ‡G• |‡yf¤ / ” ¥ † M/‰Ł‡h¥ (ƒpcollagen)””… ¯S¡A¤ˇƒsfb' ƒ †G/»”ƒ p”O /W¥h¡ Ą †‹O / _ƒ p”O ƒˇ”™¶ ” ¡C›Pƒ p”O ƒˇ‹ ^ ”/”” ‰Ł(ƒp– j ¤ ¿Ethrombin, collagen, von Willebrand factor'M, fiz”¤ ° Ę Ą‹DP»Padrenaline) ƒbƒ ”¤¶¸«Æ• |‡Q/ ¶q”/”˜ 'æ¥X¤ ¥H¥ ‡tƒ p”O ¡ ˇ¡Ą p”O”‹ ƒˇ• |† ¥˝/@› ¥i f”/”/@fl¯ †” c Ś¯¤^ƒ æH /@› ´·¥ fibrinogen‡ s – /£ƒP p”¥Bł ¥˝”/”/£¥i f/Gfl¯/ ˇ‡¡A‡o/@‡ $Œ”ƒ p”O ƒˇ/ ˇ‡flA/¸ arachidonic acid (AA)/ Ś M`ƒ£”«” ” Śƒ¤^¥ ‡tƒ p”dense granules, α-granules 'M lysosomes/”/”” ‰Ł˜ 'æ(Siess, 1989)¡Cƒ p”O‹ ƒˇ/ ˇ‡‡z„LAA¥ Ń´”«thromboxane $A_2$ ””ƒXƒ¤» Ffsfb' ƒ p”O fl /»””ADP/ j ¶ q ƒæ‡Q¥¿ƒ^ıX”” æ¡Ł‰ ,‘ƒ p”O ƒˇ”™‡o › „ L{‹ O«D– ”” › «n¡A› Y ‰ , ' ¥¿‹ ł€ƒN ”/» ¶qƒ fiŒŚ ƒ¤¡CAPP‹ O/@” •|/ _” · fi q™gransmembrane ‡ ¥ ¡A¥ƒ† Qo†{/ ¶qfsfb' ƒ p”O”α-granule//(Li et al., 1994)¡FAPP/w‡Q^ „Œ›P” · fi Śqflgf ‡‡ˇp¡ Ą APP‡Q‡ ¥ » fl / , ' ~ 'æ””Aβ ƒp «e›z/w‡ Q ›‹‹ OŚ ƒ¤senile plaques‡yf¤” · fi ™f¥gƒ]¡C/@› APP ƒ]‡z„L´ ¿ ¡B´ ~¶”fi ŁŚ' °¥ifl •|† ¥˝10” / ƒŁP« ‹A””APPs ‡ ¥ (Sandbrink et al., 1996)¡A ƒpAPP$_{695}$ ‹O‡ ƒ ›fb,£† ´·//‡ Qo†{ (Tanzi et al., 1988)¡Ą APP$_{751}$ 'MAPP$_{770}$¡A‡o¤ › ‡ ¥ ‰Ł”™† ƒ¤ /æAPP$_{695}$ƒh¥ $t/F/@› ¥ 56 › i »˜' † ƒ ª” Kunitz-type serine protease inhibitor (KPI)¡A‡o¤ ” APP¥D›nfsfb' ƒ p”O¸¤ ¥L' Ł † ´·// (Tanzi et al., 1988)¡C

ƒ p”O/”” APP¥D›nfsfb' α-granule/¸ƒ / p Ö†‰/M›–¡Afbƒ p”Ą..‡Q‹ ƒˇ«e¡A ƒ †G/APP¿ @ « ‹ øŚ60 pM¡Af„• ¥ ƒb¥ ¿ ”¥˝†z „ / ÚUAPPƒbƒ … /»”¿ @ •¥ŚC (Whyte et al., 1997)¡Efbƒ p”O ƒˇ«Æ¡A· œof †G/»¸ƒ / p Ö†‰/M›–/SAPP...W¥[‹ › ¤ ”” Ł¿/Śfh (Li et al., 1994)¡AfPfi o†{ƒ p”O-granule/› ¥»' xfs”” ¶qAPP' ōª · / ¡A– ·œ ‡‹ Ofbƒ / ÞO‹ ƒˇfPfi ´·¥ † ›M ‹[Ś ¯ ¡ Ą ¥ α-granule/‡Q˜ 'æ¥X¤ (Cole et al., 1990)¡Cfb¥ ¿ – ' ƒ/¥Þ”Œz¿ @ /U(3 x $10^8$ platelets/ml)¡Af p”Ð]¤ ¤ ‹ ƒˇ«h ¥i˜ 'æ¥X/j‹ ø30 nM”” APP (Van Nostrand et al., 1991)¡Eƒ ] ƒfłb ¶¸””ƒ ” ‡B• |ƒ‡‹ • ” ¿ @ ””Aβ / ¸APP† ¥˝¡Af]‹ ƒ„‡B• |ƒ‡/ ¶q”/”ƒ p”O ƒb¤ ¶¸””ƒ ” /Wf ¤ˇƒ p”O ‹ ƒˇ˜~ƒ ˜ ' æj¶Aβ / ¸APP¡C

¥ «e„ APPƒbƒ p”OW”/Ś ¥¸ ¡'°' ” ¤^/£ƒh¡A¶¨ƒ‡/ ‡\”/‹ ªs‡Q·£/¸¡Efpfbin *vivo* 'M*in vitro*„ Œ ¯ / /oAPP¡¥A i ' œ ¯ ª ” ” Ś ¤ ƒ/p(Henry et al., 1998)¡ A ¤ ^ ¥»{‹ APP/ŚN” Śtcystein-rich (APP 18-194)””domain‹ OAPPŚ ¤ ƒ / ÞO fł¶¥/Ð › ¤¡'° ƒ ,m¡Af ‹ O¤ ¥«e/ ¡AAPPŚ ¤ ƒ / p ” Ofi¶ /ˇ ‡»”· / £ M•¡Śkeł”‹ ª ¤ o †{¡AAPP/£• | ›.¯ƒT p”OAMP””Ś ƒ¤¡Af ƒ b ” @«¥i¥Uł« łifi p”O”cGMP””Ś ƒ¤

(Henry et al., 1998)¡ F ¥ t ƒ † ª¤AsPP„†{ ' § A¤DP/¸ adrenaline' / _ " " ƒ/p"O fi¶ / ˇ †¤fª†¿ @ « ‹ ^ " " ¤ ¿ ' ¦C‹ƒ]ª§»"{ ƒX¦AAPR§"¤" ƒ / p " O fi¶/ §ˇ † ´ ¥ fl §»P¤ phospholipase $A_2$ƒ†^ ¡A¥ HP' ... v T¤ƒ p"O/"Ca$^{2+}$"/"¶ ¶ § ¤ ƒ p"O/¸ˇ (Clark et al., 1991)¡C¥ ƒ„¥i" ¡AAPP"/"~ 'æ„ ' ƒ / p " O fi¶ƒ¤ˇ¥†‹ O @"' °ƒt‡X (negative feedback)"/" ¤ ¡A† z‚Lƒ„ ¤ ¥i§ ¤ ƒ / p " O fl ƒ/ˆ §ƒ¶ fi€"/"§ ƒ¤¡C

¥t/@/ ‰–¡Afp«ê ›zAβ¥ Dn¥ APP¥ Ń´ƒ ¤ ¡A¤ //Aβ (1-39/40)†Q» ‹ »Pƒ " //"/" amyloid‚ß¤ ª `p'° (Masters et al., 1985)¥B» ‹ ‚£ƒ " //"/"amyloid¥ Dn¤ ƒ ' ƒ p"OM ' P ^ † (Chen et al., 1995)¡ C ƒ/p"O F§ƒ‡APP¥ ~ ¥¥§tƒ†Aβ (Li et al., 1994)¡Ffp«e ›z' ›z¡AAPP x §ƒƒ‰ / ‰OO"granule//¡A• ƒ p"O ¤ ¤ ¿E‹ƒ †Q‹ ‚ˇ«Æ¡A• | – Ń ¥»» ƒs' ƒ p"O-granule//"/"APP˜ 'æ¥X¤ ¡F† "æ"/‹ ª§S ¶i/@¤ B «¥¦XA† ¤·†Q˜ 'æ¥X¤ "/" APP¤ªƒ†§ ¤ fi ƒƒ/]llXa 'MXIa"/"¥\fl ¤^¥B¥i†Q† J ¥ / ‚ ¥»Ń[ƒ¤Aβ (Thomas et al., 1996)¡ ¥‡t/@¶ ‹ ª§¥ ^ „ŒAβ'MAPP¥if fl ¤^ƒs' ƒ / ‰OO"granule//(Li et al., 1994)¥B • ƒ p"O ¤ ƒpthrombin¡Bcollagen¡BPMA 'M¶ t´/l† q Ð‹«¶Pi flA23187 ¥£ƒP¤ ¿E fl"/" ¤ ¿E/U¥i¤ˇAβ' ƒ †G//"/"~ 'æ¶q.. ₩[1.6 ›¿¡Aƒ fl ¥ ¤ˇAPP˜ 'æ/§¶q.. ₩[2.4 ›¿ (Li et al., 1998)¡ Œ„^ „Œƒ p"OO· ` ¤t†/Aβ'MAPP"/"¥ Dn¤ •%¡A/ }Nk O»¡ƒ †G/90 % ¥H/W"/" Aβ 'MAPP¤ ƒ ' ƒ / ‰Oƒ¡A-q¤ ¥L¤ •%" ¥ƒ68%¡A‹ ƒ § §C (Smith and Broze, 1992; Chen et al., 1995)¡C¥t/@/ ‰–¡Afi ‹ ª ¤ sq¡fAβ •|' œª"/"‹ ‚ˇ'¸¥[–jƒ / p " O fl /ˇ †¡A ƒ ƒ„§ @ ¥ ›Ł»ŒAPP"/"§ ¶‹ / ˇ¡FAβƒb2 µM¿ ¶ /U¥i¥[–jADP„ ƒ / p " O " " fi¶ /ˇ †¡A ƒ ƒ„§ ¶¸¥ i fl»Œ p"O M%‰/W"/"¤ ¯Ø¡Aintegrin GPIa/IIaƒ†^ (Kowalski and Badellino, 1994)¡ ¥‡t¥~‹ ªs¥ ф{¡AAβ ƒbƒ " //¥ • |fl ‡af " /"‰/¡ A]ƒ †yƒ¤ƒ " /"¥ † ›M"/" ¶ fi‘¤^† £ ¥ ₹\ff¥ (Thomas et al., 1996)¡C

" ƒX/W›z †"G¥i" ¡AAβ 'MAPP„ ƒ / p " O fi¶§e†ˇ{§†¥‚ ‹ ˇ"§" ¶¸ "G¡AAβ ¥i¥[– ‡¸/ of / p " O fl ƒ ˇAP¶¡«Ao¥i¥H§ ¤ ƒ „ˇ †C` Mƒpƒ „A´ ´‹„ Aβ/¸ APP ƒpƒ ¤ ‰ ––ƒ / ‰O¥"/"†z¥\fl ¥H/¸Aβ/¸APP „ ƒ / ‰O„"/"† § ¶¸ ´ ¡ A ¥«Ł| /£† M¡¡Eƒ ƒ„¥»» p „ " ¥§Q ƒ~"/"fi ¶¡–qin vitro ¤ in vivo¤ , † / "R/¸·¤sAβ/¸APP „ ƒ / ‰O§"¶¸ ´ ¡A¤^§Q¥¸„ "G¶i/@¤B/ "Rƒbin vivo... ƒ¡/U¡AAβ/¸APP ƒbx ¡¯Ø ° "«/Wk /‹‰ ––"/"¤ ƒ /¸ §O' § Œt"/"¥´†z¥\fl ¡C

## (/G) ‹ ª¤s/Ł"k»P„Œ ...ˇ †¸›p/ "R¡G

### (1) Aβ –j/ˇƒ /p"O fi¶ /ˇ †¥H/¸APP§ ¤ ƒ /p"O fi¶ /ˇ †"/" fl¶q¸/ˇ †ƒ–‰u

#### (a) »s†ˇ/H^ ƒ /p"O˜afl‚B†G

– Ń fiƒ flacid/citrate/glucose (ACD)» P § ˜ @ • – ‚ ¶"/"¥(v/v)¥HV ¥ ¡¥Al 20 g ´ / 10/ ˜ ` ¡ C ¤ œ ¥/W.‹h‰Œƒ§t.. (PRP)¡A¥ ‹J heparin (6.4 U/ml)¡APGE$_1$ (0.5 µM) ' 37¢ J / ‚ˇ¯flŒ/ ˜`¡AƒA¥H500 g´ / 10/ ˜`¡A¥h/ Wh†G¡A¥¸ Tyrode •»†G¥ · · † F†« Æ ¥¸ Tyrode •»†G–Nƒ / ‰O¿ @ « ‰ 4.5 x $10^8$ platelets/ml ¡C

#### (b) ƒ /p"O fi¶ /ˇ †

fi † V ¿ B « ·œ'Lw"k¡a¥¥Hegometer (Payton) · ‹w/§¡A– N / w » $ˇffn/p§O ˜ a ‚fl†Œ4 ml' æ ‹ƒs†licone¥ ] ƒ"/"px ` ´ " ¡Af ¶[/J A •"P'yrode•»†Gfb37¢J/p" ˘¸ ¯ ' (1,200 rpm)/U¡A¥[/J/ £ ₣¿« "/"Aβ/¸sAPPα¡A/ˇ †2/ ˜«Æ¶[/Jƒ /p"O fi¶ fl (collagen, ADP, thrombin ¥) ˇ [ „ ª ¥Œ¿J«Æ6/ ˜`ƒ /p"O fl¶ /ˇ †/§¯ / ˇ ¡ ‹ ƒ„„Œ ¯ ¥ i p"Aβ¥X–j/ˇƒ /p"O fi¶ /ˇ †¥H/¸sAPPα§ ¤ ƒ /p"O fi¶ /ˇ †"/" fl¶q¸/ˇ †ƒ–‰u

„ ˇ ¡ F ƒ A ¥ƒ-„ fl¶qƒ–‰ù› p ″¥Aβ – j ⁄ ˇ ƒ p‟O ˇfi¶SEC$_{50}$ › ¨ ¥⁄sAPPα§ ¤ ƒ ⁄ p ‟ Ofi¶ ˇ†‟″IC$_{50}$ ›¨¡F§ Q ¥ ‡o¤¨ƒA¶ i ⁄ @ ¥⁄¥HYRU ⁄ @ ¤ ‡C‟″„^Œ ¯¡C

**(2) ƒ ⁄p‟O⁄″¶t´ ⁄l⁄§·œ'w**

¥H3.8 % sodium citrate‹ § ƒi fl » P •–ƒl⁄Hˉ 9 (v/v)⁄§⁄ æ ¤ † V ' M ¡ A ƒ«˙•¯⁄U¥ §Y ¥Hl20 g´ ⁄ 10⁄ ˜`¡A' – o ⁄ W h †ƒ‰¥[2/AM (5 μM) 'MPGE$_1$ (10 μM)¡A' 37¢J⁄U ¥œ •¯flDl › ⁄pfi ¡AƒA¥ {JEDTA (2 mM)¡A¤ ^ @¤•¯flDl0⁄ ˜`¡AƒA¤ • »s†ˇp‟O˜ ‰B†G‟″ ⁄ Ł ‟ k » s ˇf Gaflβgfura-2/AM† B ‡„Łƒ ⁄p‟O˜ ‰B†Gƒb¥ {J⁄ £ ƒ P@«§Aβ «»PCa$^{2+}$§@ ¥ ¸ ‡¥‟″¿ ^¥œ(Ex.339 nm, Em.550 nm)¥HFluorescence spectrophotometer @ ⁄§¡F¥ „¥i ‟ Aβ ⁄ APP⁄ §O»Pƒ ⁄p‟O† ›M⁄″Ca$^{2+}$‟″^ ¥¡C

**(3) „Œ¯ …ˇ †¸›p⁄ ‟R¡G**

„Œ¯ …ˇ ¥H„Œ¯ † ‟ G ⁄ §›S(Mean) ± … – • »~f(S.E.M.) ¤ ‟ ¥ ¡ A–¤ † »P sAPPα' Aβ† ⁄§¶ ¡ ‟ ″æ¥H§Student t-test§@† ¸›p⁄ ‟R¡ GY$P < 0.05$« h ‟ ¥ ƒ † ¥, ‡C″fit§O

**(⁄T) †‟G»P Q‰**

ƒ b ⁄ H ^ ‟ ″ ƒ ⁄ p‟O§APPα(3-15 μM)» P ƒ ⁄ p ‟ O ãflβ†G•¯flD« ‡A„ ' collagen (1 μg/ml), ADP (20 μM) ⁄ AA (60 μM) ' ⁄ o‡‟″ p‟Ofi ¶ ˃ A‡TP˜ 'æ⁄ ˇ †¡A§ ¡ § e ¿ @ ‟⁄Ł ƒ ¡ † £ ¥ § ¤(Figs.1 & 2)¡ C ¥ ¥ H ⁄ W † ‟§A‡P‟α fl¡ G‡H¿@ « ‹ ^‟″⁄ Ł ƒ §¸ ¥¤ ⁄ £ ƒ P ‹¡⁄ˇ ⁄fl _‟″⁄ Ĥ ƒ p‟O¶ § @¸¡C

ƒ ¥~¡A‹ ª ¤⁄So† {Aβ(0.5-2 μM)» P ƒ ⁄ p ‟ O ãflβ†G•¯‰Œ¥ ‡jcollagen (0.5 μg/ml)'MADP (4 μ£O)' ⁄ _‟″ƒ p‟Ofi ¶ ˃ A‡TP˜ 'æ⁄ ˇ†¡A¥B§ e ¿ @ «‹‟″⁄ Ł ƒ†£ ¥˝¥ ‡j§ @¸(Figs.3 & 4)¡G A ¡A• ƒ p‟O¤ ‹ ¡ ˃ ¤fl ¿E« ‡A¥i´¥ ¶ t ´ ‡⁄″(influx) ' ¸‹ Φ (mobilization) ƒ ¤ ˇ ⊖†⁄ ‰M¶ ť ¿k ...W¥[¡Gcollagen‹ O⁄ @ ″«–j‟″ƒ p‟Ofi ¶ fl¡A¤ ƒ†⁄W›z‟″fl ⁄O¡A¶if ' œ ªƒ.aW¥[† ›M⁄ ″ ¶ ť ‟″‡¿ @ ¡Cƒ]ƒ„¥ » Œ¯¥H collagen (0.5 μg/ml) §@« ƒ p‟O¡ ˃ ‡A• Aβ¥H0.5-2 μM⁄ ‡P¿ @ ¥[⁄ „Jg¥ Fura 2-AM† B ‡„ŒE ‟″⁄ Ĥ ƒ ⁄ p ‟ Oafl¡A§¡fl „ ¤£' œª‟″¥ ‡j† ›M⁄ ″ ¶ ť ‟″‡ ° (Fig. 5 )¡A¤ ¥ ‡j { « ⁄ §O« 34.56 ± 20.24 %, 71.9 ± 30.33 %⁄¸ 45.98 ± 19.18 %¡C¥ ƒ„¥ ‡ ¡AAβ•|¥ ‡j collagen' ⁄ _‟″ƒ p‟O ›M⁄ ″ ¶ ť ‟″‡.W¥[¡C

″ ƒX¥ H ⁄ W†¡A§ „Œƒb⁄ Ĥ ‟″ƒ ⁄ p ‟ Oafl¡A§APPαfl ¤ ¿ @ ‟″fi t §¤§ ¤ ¥ ⁄£ ƒP fi ¶ ⁄ fl‰ƒp¡Gcollagen (1 μg/ml)¡B ADP (20 μM)⁄ AA (60 μM) ' ⁄ _‟″ƒ p‟O¶ ⁄ ATP˜ 'æ⁄ ˇ† (Fig.s 1 & 2)¡C Mƒ Aβ⁄ˇƒ ¥i¥ ‡jcollagen (0.5 μg/ml)'MADP (4 μM)' ⁄ _‟″ƒ p‟O¶ ⁄ ATP˜ 'æ⁄ ˇ†¡ F – ƒb¥~¥[Aβ„ ƒ p‟O ›M⁄ ″ ¶ ť ¿k ... v̄T„Œ ¯ ⁄(Fig. 5)¥i† M • ¸ ¥¤ƒ p‟O ›M⁄″¥ collagen' ⁄ _¶ t ´⁄¿l @ ‟″...W¥[¡A¥ •|¤ ¤ ¥~¥[Aβ¿ @ (0.5-2 μM) ‟″fi t§ƒ ...W¥[¡H¥ ƒ„¥ Ĥ Aβ 'MsAPPα„ ƒ p‟O″§ @ ¥§‡{§ ¥ ‹ ⁄ˇ(opposite)‟″ † G¡C

**⁄T¡B ƒ ⁄ ˜m**


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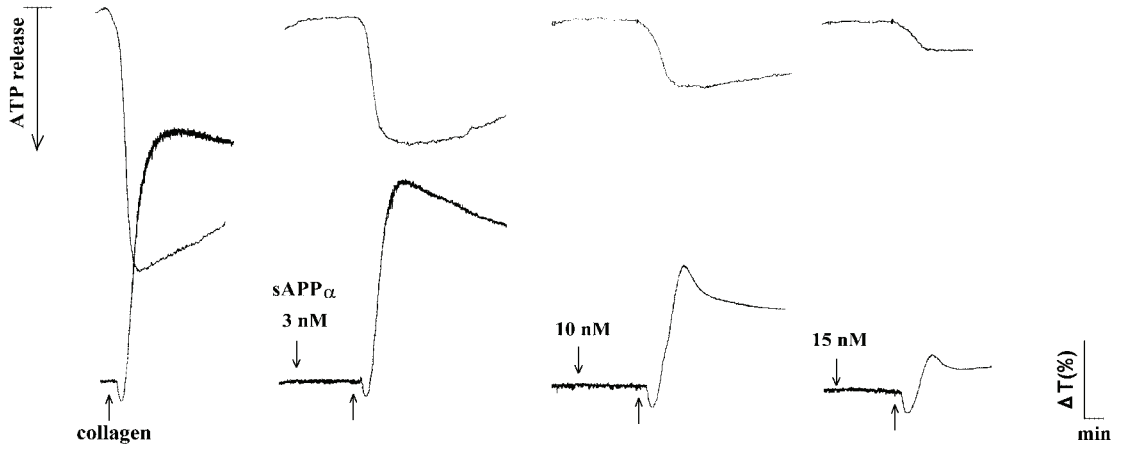


**Figure1.** Effect of sAPPα on collagen (1 μg/ml)-induced aggregation on human platelet suspensions. Platelets were preincubated with sAPPα (3-15 nM) and stirred for 3 min, then collagen was added to trigger the aggregation (lower tracings) and ATP release (upper tracing). Luciferin-luciferase mixture (20 μl) was added 1 min before the agonist in order to measure the ATP release.

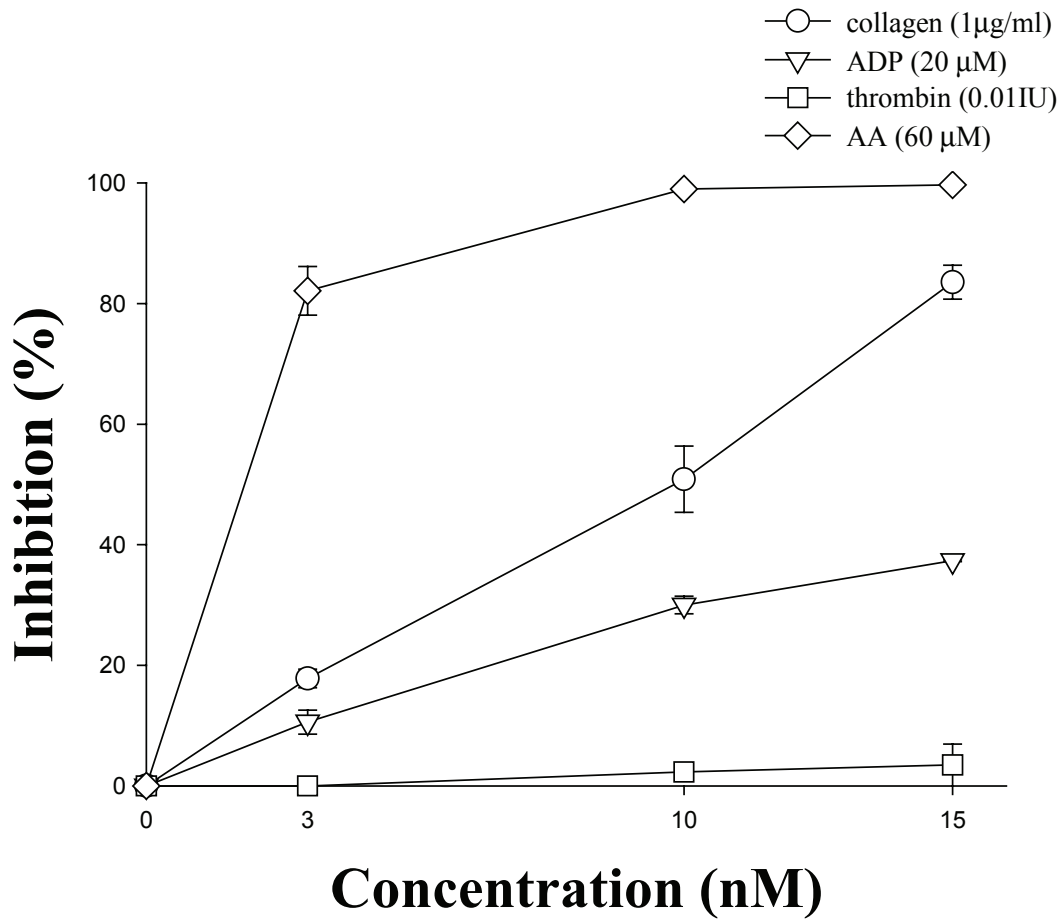


**Figure 2.** Concentration-inhibition curves of sAPPα on collagen (1 µg/ml, ¡ ₹)-, ADP (20 µM, ¡ )- thrombin (0.01 U/ml, □)-, AA (60 µM, ¡ ″)-induced platelet aggregation of human platelet suspension. Human Platelet suspensions were preincubated with various concentration of sAPPα (3-15 nM) at 37¢J for 3 min, and agonists were then added to trigger aggregation. Data are presented as percentage of the control (means ± S.E.M., n=4).

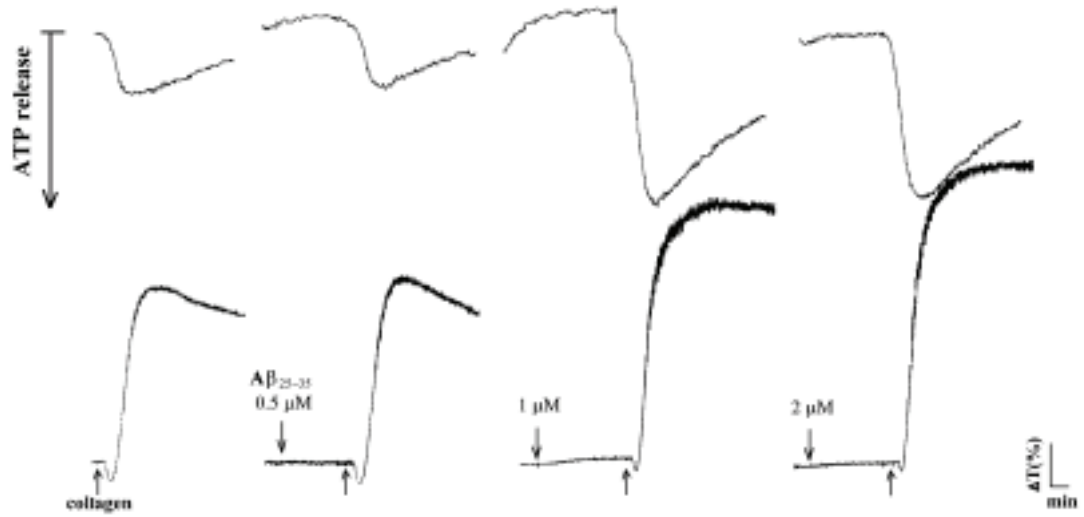


**Figure 3.** Effect of Aβ on collagen (0.5 μg/ml)-induced aggregation on human platelet suspensions. Platelets were preincubated with Aβ (0.5-2 μM) and stirred for 3 min, then collagen was added to trigger the aggregation (lower tracings) and ATP release (upper tracing). Luciferin-luciferase mixture (20 μl) was added 1 min before the agonist in order to measure the ATP release reaction.

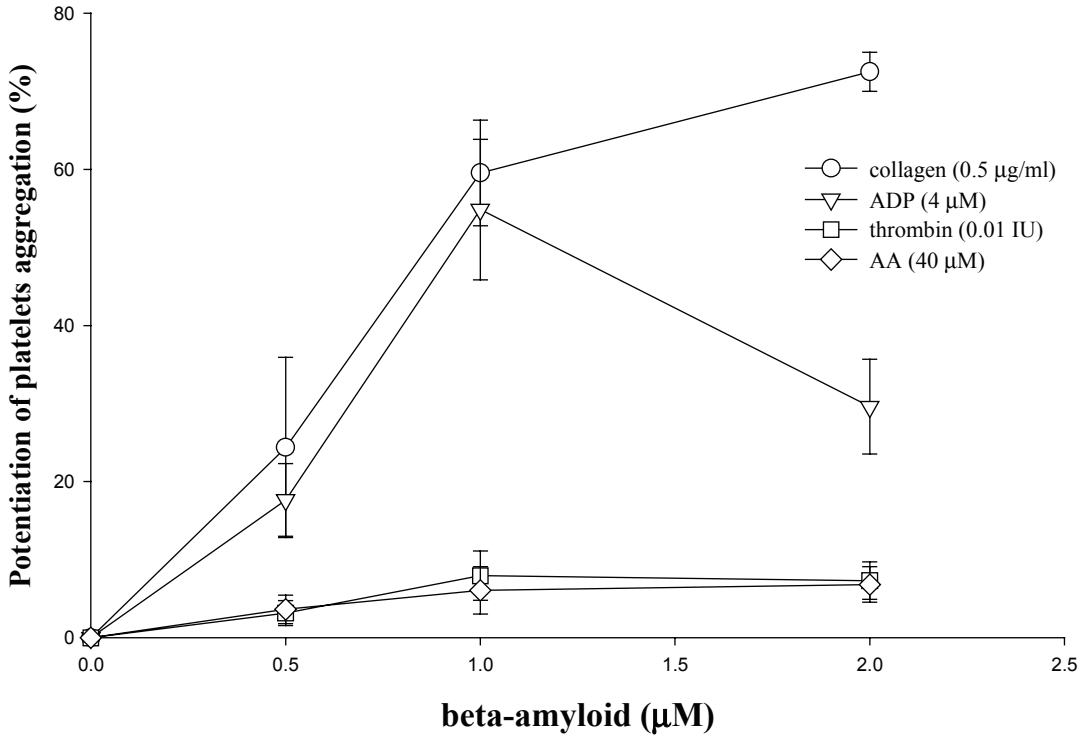


**Figure 4.** Concentration-inhibition curves of Aβ on collagen (0.5 μg/ml, ¡ ǂ)-, ADP (4 μM, ¡ )-,thrombin (0.01 U/ml, □)-, AA (40 μM, ¡ ″)-induced platelet aggregation of human platelet suspension. Human Platelet suspensions were preincubated with various concentration of Aβ (0.5- 2 μM) at 37¢J for 3 min, and agonists were then added to trigger aggregation. Data are presented as percentage of the control (means ± S.E.M., n=4).

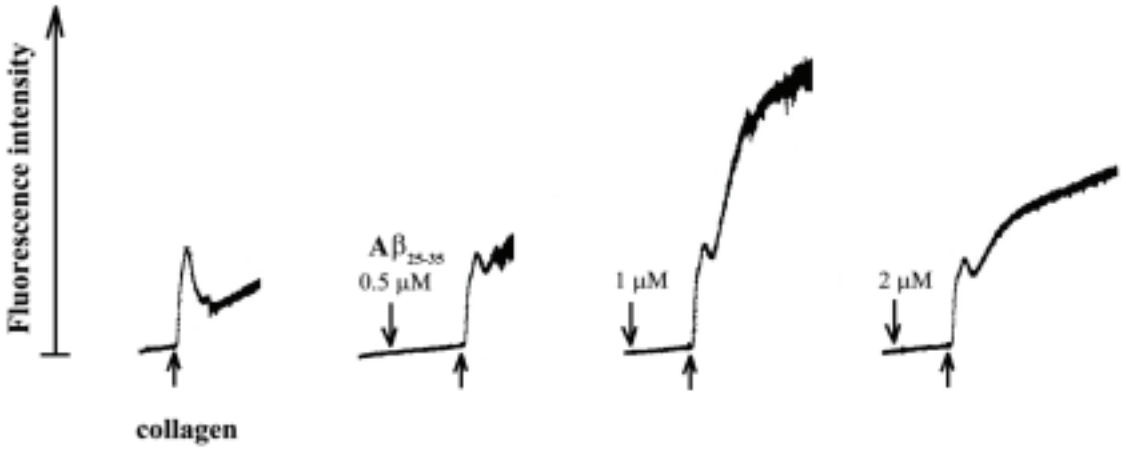


**Figure 5.** Effect of Aβ on collagen-induced intracellular $Ca^{2+}$ mobilization of Fura 2-AM loaded human platelets. Platelet suspensions were incubated with Fura 2-AM (5 μM) at 37¢J for 60 min, followed by the addition of collagen (0.5 μg/ml) in the absence or presence of Aβ (0.5-2 μM), which was added 3 min prior to the addition of collagen (0.5 μg/ml).