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Inhibitory mechanisms of gabapentin, an antiseizure drug, on platelet aggregation

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Abstract

Gabapentin (Neurontin) is an analogue of gamma-aminobutyric acid (GABA) that is effective against partial seizures. Gabapentin has been reported to modulate serotonin release from platelets, but the effects of gabapentin on platelet activation have not been explored. In this study, gabapentin concentration-dependently (60–240 μM) inhibited platelet aggregation in washed platelets stimulated by collagen (1 μg mL^{-1}), ADP (20 μM) and arachidonic acid (60 μM). Gabapentin (120 and 240 μM) also concentration-dependently inhibited collagen (1 μg mL^{-1})-induced phosphoinositide breakdown, intracellular Ca^{2+} mobilization, thromboxane A_2 formation, and p38 MAPK phosphorylation in human platelets. In conclusion, the most important findings of this study suggest that gabapentin inhibits platelet aggregation, at least in part, through the phospholipase C–inositol 1,4,5-trisphosphate–thromboxane A_2–Ca^{2+} pathway. Thus, it is possible that gabapentin treatment, alone or in combination with other antiplatelet drugs, may induce or potentiate inhibition of platelet aggregation, which may affect haemostasis in-vivo.

Introduction

The antiseizure drug gabapentin (1-(aminomethyl)cyclohexane acetic acid) was originally developed as a gamma-aminobutyric acid (GABA)-mimetic compound to treat spasticity, and was shown to have a potent anticonvulsive effect (Satzinger 1994). Gabapentin is also effective in the treatment of chronic pain syndromes, especially neuropathic pain (Rose & Kam 2002), postherpetic neuralgia, diabetic neuropathy and trigeminal neuralgia (Magnus 1999). Clinical use of gabapentin has been associated with several side-effects, but it is generally well tolerated (Ramsey 1995).

Intravascular thrombosis is one of the generators of a wide variety of cardiovascular diseases. Initiation of intraluminal thrombosis is believed to involve platelet adherence and aggregation. In normal circulation, platelets cannot aggregate by themselves. However, when a blood vessel is damaged, platelets adhere to the disrupted surface, and the adherent platelets release biologically active constituents and aggregate (Sheu et al 1997). Thus, platelet aggregation may play a crucial role in the atherothrombotic process. Indeed, antiplatelet agents (e.g. ticlopidine and aspirin) have been shown to reduce the incidence of stroke in high-risk patients (Hass et al 1989).

Some GABA agonists, for example the sedative and anaesthesia induction agent triazolam, have been demonstrated to inhibit platelet aggregation (Chesney et al 1987). Karaseva et al (1998) also reported that gidazepam and phenazepam inhibit aggregation of platelets in rats through inhibition of lipid peroxidation. Recently, we have found that the antiplatelet activity of midazolam may initially relate to induction of conformational changes in platelet membranes (Sheu et al 2002). The effects of gabapentin on platelets have rarely been compared with those of other GABA agonists. Rao et al (1988) presented data indicating that gabapentin modulates the release of serotonin from blood platelets. However, the mechanisms underlying the gabapentin signalling pathways remain obscure. Platelets are anucleate, do not differentiate or proliferate, and thus are a good model for studying the signal pathways of gabapentin and its functions.

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Materials and Methods

Gabapentin (MW 171.24), collagen (type I, bovine achilles tendon), luciferin–luciferase, fluorescein sodium, Dowex-1 (100~200 mesh; X_8, chloride form), myoinositol, prostaglandin E_1 (PGE_1), arachidonic acid (AA), ADP and bovine serum albumin (BSA) were purchased from Sigma (St Louis, MO, USA); Fura 2-AM from Molecular Probe (Eugene, OR, USA), the thromboxane (TX) B_2 enzyme immunoassay (EIA) kit from Cayman (Ann Arbor, MI, USA), anti-phospho-p38 mitogen-activated protein kinase (MAPK) (Ser^{182}) monoclonal antibody and SB203580 from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and anti-p38 MAPK from Cell Signaling (Beverly, MA USA). The Hybond-P PVDF membrane, myo-2-[^{3}H] inositol, enhanced chemiluminesence (ECL) and western blotting reagents and analysis system, and horseradish peroxidase-conjugated sheep anti-mouse IgG were purchased from Amersham (Buckinghamshire, UK). Triflavin, an $\alpha_{IIb}\beta_3$ integrin antagonist purified from *Trimeresurus flavoviridis* snake venom (Sheu et al 1999), was purchased from Latoxan (Rosans, France).

Preparation of human platelet suspensions

Human platelet suspensions were prepared as described previously (Hsiao et al 2005). In brief, blood was collected from healthy human volunteers who had taken no medication during the preceding 2 weeks. Volunteers gave informed consent, and the study was approved by the Institutional Review Board of Taipei Medical University and conformed to the principles outlined in the Helsinki Declaration.

Blood was mixed with acid/citrate/glucose (ACD) (blood: ACD 9:1 v/v). After centrifugation, the supernatant (platelet-rich plasma; PRP) was supplemented with PGE_1 (0.5 μM) and heparin (6.4 IU mL^{-1}), incubated for 10 min, then centrifuged at 500 *g*. The washed platelets were finally suspended in Tyrode's solution containing BSA (3.5 mg mL^{-1}). The final concentration of Ca^{2+} in Tyrode's solution was 1 mM.

Platelet aggregation

Platelet aggregation was measured using the turbidimetric method (Hsiao et al 2005) using a Lumi-Aggregometer (Payton, Scarborough, Ontario, Canada). Platelet suspensions (0.4 mL, 3.6×10^8 platelets mL^{-1}) were pre-warmed to 37 °C for 2 min; gabapentin (60–240 μM) or an equivalent volume of vehicle (Tyrode's solution) was then added 3 min before addition of agonists. The reaction was allowed to proceed for at least 6 min, and the extent of aggregation was expressed in light transmission units. When measuring adenosine triphosphate (ATP) release, 20 μL luciferin–luciferase mixture was added 1 min before addition of the agonists, and ATP release was compared with that of the control.

Labelling of membrane phospholipids and measurement of the production of [^{3}H]-inositol phosphates

The method was carried out as described previously (Sheu et al 2000). Briefly, citrated PRP was centrifuged and the pellets suspended in Tyrode's solution containing [^{3}H]-inositol (75 μCi mL^{-1}). Platelets were incubated for 2 h followed by centrifugation, and were finally resuspended in Ca^{2+}-free Tyrode's solution (5×10^8 mL^{-1}). Gabapentin (120 and 240 μM) or the solvent control (Tyrode's solution) was preincubated with 1 mL loaded platelets for 3 min, and collagen (1 μg mL^{-1}) was then added to trigger activation. The reaction was stopped by adding ice-cold trichloroacetic acid (10% w/v), and samples centrifuged for 4 min. The inositol phosphates of the supernatants were separated in a Dowex-1 column. Only [^{3}H]-inositol monophosphate (IP) was measured as an index of total inositol phosphate formation.

Measurement of platelet $[Ca^{2+}]_i$ mobilization by Fura 2-AM fluorescence

Citrated whole blood was centrifuged at 120 *g* for 10 min. The supernatant was incubated with Fura 2-AM (5 μM) for 1 h. Human platelets were prepared as described above. The external Ca^{2+} concentration of the platelet suspensions were adjusted to 1 mM. The rise in intracellular calcium ion concentration $[Ca^{2+}]_i$ was measured using a fluorescence spectrophotometer (CAF 110, Jasco, Tokyo, Japan) at excitation wavelengths of 340 and 380 nm, and an emission wavelength of 500 nm.

Measurement of thromboxane A_2 formation

Washed human platelets (3.6×10^8 mL^{-1}) were preincubated with gabapentin (120 and 240 μM) for 3 min before the addition of collagen (1 μg mL^{-1}). Six min after addition of agonists, EDTA (2 mM) and indometacin (50 μM) were added to the reaction suspensions. The vials were then centrifuged for 3 min. The TxA_2 levels of the supernatants were measured using an EIA kit, according to the manufacturer's protocol.

Flow cytometric analysis

Fluorescence-conjugated triflavin and collagen were prepared as described previously (Sheu et al 2000). The final concentrations of fluorescein isothiocyanate (FITC)-conjugated triflavin and collagen were adjusted to 1 mg mL^{-1}. Washed platelets were prepared as described above. Aliquots of platelet suspensions (3.6×10^8 mL^{-1}) were preincubated with gabapentin (120 and 240 μM) for 3 min, followed by the addition of 2 μL FITC-conjugated triflavin (2 μg mL^{-1}). The suspensions were incubated for another 5 min, and the volume was adjusted to 1 mL per tube with Tyrode's solution. The suspensions were then assayed for fluorescein-labelled platelets using a flow cytometer (Becton Dickinson, FACScan Syst., San Jose, CA, USA). Data were collected from 50 000 platelets per experimental group. All experiments were repeated at least four times to ensure reproducibility.

Quantitative immunoblotting

Washed platelets (1.2×10^9 mL^{-1}) were preincubated with gabapentin (120 and 240 μM) for 3 min, followed by the addition of collagen to trigger platelet activation. The reaction was stopped by the addition of EDTA (10 mM), suspensions

were centrifuged at 3000 *g* for 5 min and then immediately resuspended in 200 mL lysis buffer (50 mM HEPES, 5 mM EDTA, 50 mM NaCl, 1% Triton X-100, 10 μg mL^{-1} aprotinin, 1 mM phenylmethanesulfonyl fluoride, 10 μg mL^{-1} leupeptin, 10 mM NaF, 1 mM sodium orthovanadate and 5 mM sodium pyrophosphate). Collected lysates were centrifuged at 10 000 *g* for 5 min, after which the supernatants were dissolved in β-mercaptoethanol (5%) and bromophenol blue (0.1%). Samples containing 80 μg protein were separated by SDS-PAGE (12%); the proteins were electrotransferred to a Hybond-P ploy vinylidene difluoride membrane by semidry transfer (Bio-Rad, Hercules, CA, USA). Blots were blocked with TBST (10 mM Tris-base, 100 mM NaCl, 0.01% Tween 20) containing 5% BSA for 1 h. The blots were then probed with the primary antibodies (anti-p-p38 MAPK and anti-p38 MAPK [diluted 1:1000 in TBST]) for 2 h. Membranes were washed and then incubated with horseradish-peroxidase-linked anti-mouse IgG (diluted 1:3000 in TBST) for 1 h. Immunoreactive bands were detected by chemiluminescence using the ECL system. Ratios of quantitative results were obtained by scanning reactive bands and quantifying the optical density using videodensitometry (Bio-1D version 99 imaging software).

Statistical analysis

Data are expressed as mean ± s.e.m. and are accompanied by the number of observations. Data were analysed using analysis of variance. If this analysis indicated significant differences between group means, groups were compared using the Newman–Keuls test. A P value of less than 0.05 was considered statistically significant.

Results

Effects of gabapentin on agonist-induced platelet aggregation in human platelets

Gabapentin (60–240 μM) inhibited platelet aggregation stimulated by collagen (Figure 1A) and AA in both washed human platelets (Figure 1B) and PRP (data not shown) in a concentration-dependent manner. Gabapentin also inhibited ADP-induced aggregation of washed platelets in the presence of fibrinogen (200 μg mL^{-1}) (Figure 1B). Furthermore, gabapentin (60–240 μM) inhibited ATP release when stimulated by agonists (i.e. collagen) (Figure 1A). IC50 values of gabapentin for platelet aggregation induced by ADP, collagen and arachidonic acid were estimated to be approximately 102, 120 and 50 μM, respectively (Figure 1B). When platelets were preincubated with a higher concentration of gabapentin (360 μM) or Tyrode's solution for 10 min, followed by two washes with Tyrode's solution, there were no significant differences between the aggregation curves of either platelet preparation stimulated by collagen (1 μg mL^{-1}), indicating that the effect of gabapentin on inhibition of platelet aggregation occurs in a reversible manner (data not shown). In subsequent experiments we used collagen as the agonist to explore the inhibitory mechanisms of gabapentin on platelet aggregation.

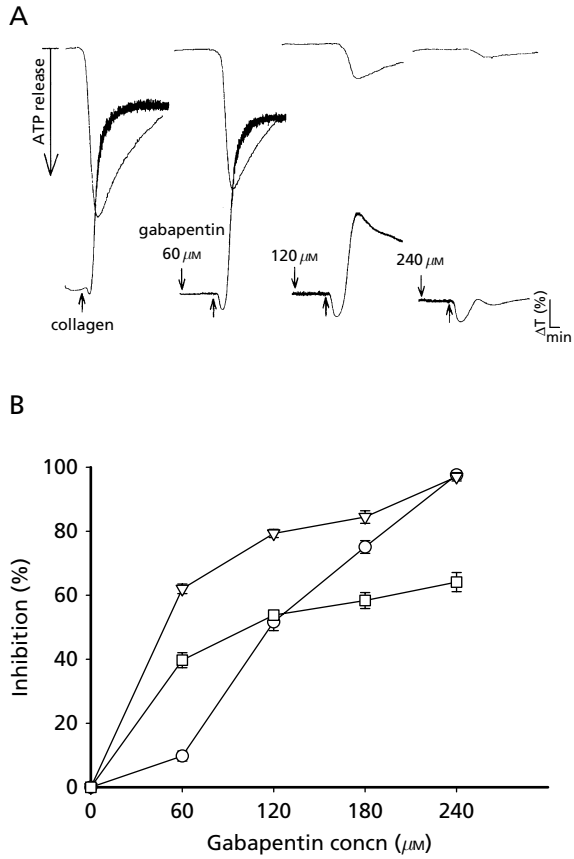

Figure 1 A Effect of gabapentin on collagen-induced platelet aggregation. B Concentration-dependent effect of gabapentin in inhibiting platelet aggregation induced by collagen (1 μg mL^{-1}, circles), arachidonic acid (60 μM, triangles) or ADP (20 μM, squares) in washed human platelets. Platelets (3.6×10^8 mL^{-1}) were preincubated with gabapentin (60–240 μM) for 3 min; agonists were then added to trigger aggregation (lower tracings) and ATP release (upper tracings) (A). Data in B are percentage of the control, mean ± s.e.m., n = 4.

Effects of gabapentin on binding of FITC-triflavin to washed platelets

Triflavin inhibits platelet aggregation through direct interference with fibrinogen binding to $\alpha_{IIb}\beta_3$ integrin (Sheu et al 1999). There is now a wealth of evidence suggesting that the binding of fibrinogen to $\alpha_{IIb}\beta_3$ integrin is the final common pathway for agonist-induced platelet aggregation. We therefore decided to evaluate whether gabapentin inhibits agonist-induced platelet aggregation by binding directly to platelet $\alpha_{IIb}\beta_3$ integrin. In this study, the relative fluorescence intensity of FITC-triflavin (2 μg mL^{-1}) bound directly to collagen-activated platelets was 124.3 ± 7.9 (n = 4), and was markedly reduced in the presence of 5 mM EDTA (negative control, 31.7 ± 2.7, n = 4, $P < 0.001$). Gabapentin (120 and 240 μM) did not significantly interfere with FITC-triflavin binding to the $\alpha_{IIb}\beta_3$ integrin in platelet suspensions (120 μM: 123.3 ± 6.5; 240 μM: 123.1 ± 7.5, n = 4), indicating that the inhibitory effect of gabapentin on platelet aggregation is not mediated via binding to the $\alpha_{IIb}\beta_3$ integrin.

Effects of gabapentin on $[Ca^{2+}]_i$ mobilization and phosphoinositide breakdown in human platelets

The free cytoplasmic Ca^{2+} concentration in human platelets was measured by the Fura 2-AM loading method. As shown in Figure 2, collagen (1 μg mL^{-1}) evoked a marked increase in $[Ca^{2+}]_i$, and this increase was markedly inhibited in the presence of gabapentin (120 μM: 35.3 ± 2.0%; 240 μM: 90.6 ± 2.6%).

Phosphoinositide breakdown in platelets is activated by many different agonists. In this study, we found that collagen (1 μg mL^{-1}) induced the rapid formation of radioactive IP, inositol diphosphate and inositol 1,4,5-trisphosphate (IP_3) in human platelets loaded with [^{3}H]-inositol. We measured only [^{3}H]-IP formation as an index of total inositol phosphate formation. As shown in Figure 3, the addition of collagen (1 μg mL^{-1}) resulted in a rise in IP formation of about 4.6-fold compared with that of unstimulated (resting) platelets (4.6 ± 1.0 vs 21.1 ± 5.0 × 10^3 cpm). In the presence of gabapentin, the radioactivity of IP formation in collagen-stimulated human platelets was markedly decreased (120 μM: 8.3 ± 3.1 × 10^3 cpm; 240 μM: 5.1 ± 2.1 × 10^3 cpm) (Figure 3).

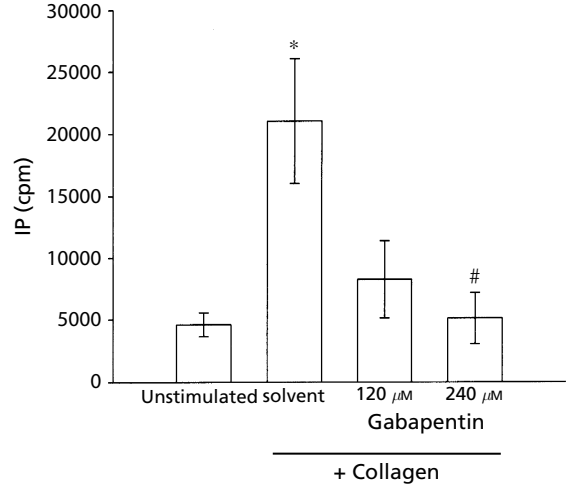

Figure 3 Effect of gabapentin on collagen-induced inositol monophosphate (IP) formation in human platelets. [^{3}H]-Inositol-labelled platelets were preincubated with the solvent control (Tyrode's solution) or gabapentin (120 and 240 μM), followed by the addition of collagen (1 μg mL^{-1}) to trigger platelet aggregation. Data are mean ± s.e.m. (n = 5). *$P < 0.01$ vs unstimulated platelets; #$P < 0.05$ vs activated platelets (solvent control).

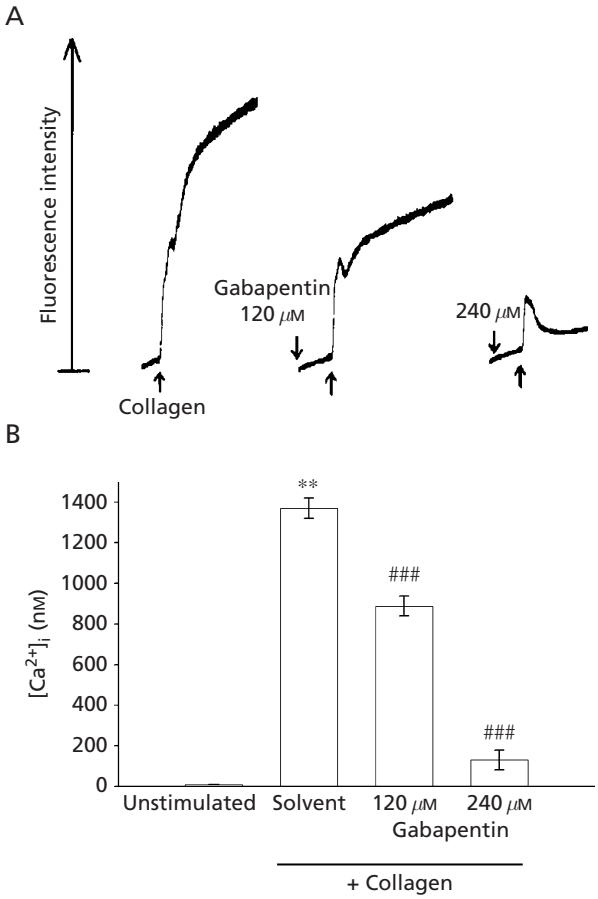

Figure 2 Effect of gabapentin on collagen-induced intracellular Ca^{2+} mobilization in human platelets. Platelets were preincubated with Fura 2-AM (5 μM) followed by the addition of collagen (1 μg mL^{-1}) in the absence or presence of gabapentin (120 and 240 μM). Profiles in A are representative examples of six similar experiments. Data in B are mean ± s.e.m. (n = 6). **$P < 0.001$ vs unstimulated platelets; ###$P < 0.001$ vs activated platelets (solvent control).

Effect of gabapentin on thromboxane A_2 formation

As shown in Figure 4, unstimulated platelets produced relatively little TxA_2 compared with collagen-activated platelets (7.0 ± 1.0 vs 29.2 ± 2.0 ng mL^{-1}). Gabapentin markedly inhibited TxA_2 formation in collagen-stimulated platelets (120 μM: 12.5 ± 0.9 ng mL^{-1}; 240 μM: 11.0 ± 0.5 ng mL^{-1}) (Figure 4).

Effect of gabapentin on collagen-induced p38 MAPK phosphorylation

To further investigate the mechanisms by which gabapentin inhibited collagen-induced platelet activation, we measured p38 MAPK, which provides a key signal necessary for the aggregation of agonist-stimulated platelets. An initial study was performed to determine the effects of SB203580, a p38 MAPK inhibitor, on platelet aggregation. Platelets were stimulated with sufficient collagen (1 μg mL^{-1}) to cause full aggregation, which was strongly inhibited in the presence of SB203580 (20 μM) (Figure 5A), with concomitant inhibition of ATP secretion (data not shown). Immunoblot analysis revealed that treatment with collagen (1 μg mL^{-1}) produced more marked p38 MAPK phosphorylation than in unstimulated platelets (Figure 5B). Gabapentin (120 and 240 μM) inhibited collagen-induced p38 MAPK phosphorylation in a concentration-dependent manner (Figure 5B).

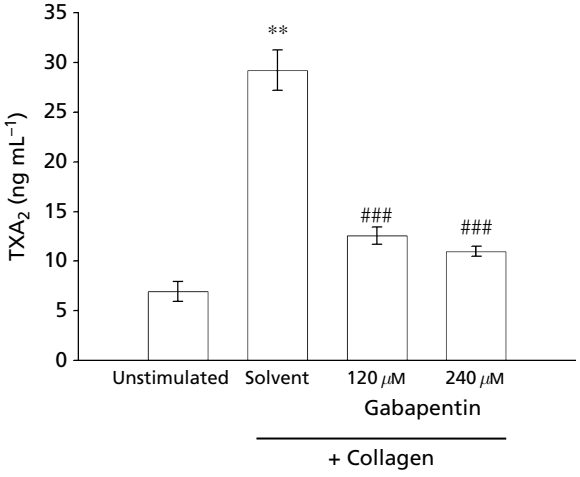

Figure 4 Effect of gabapentin on thromboxane A_2 (TXA_2) formation stimulated by collagen in washed platelets. Platelet suspensions (3.6×10^8 mL^{-1}) were preincubated with solvent control (Tyrode's solution) or gabapentin (120 and 240 μM), followed by the addition of collagen (1 μg mL^{-1}) to trigger TxA_2 formation. $^{**}P<0.001$ vs unstimulated platelets; $^{###}P<0.001$ vs activated platelets (solvent control).

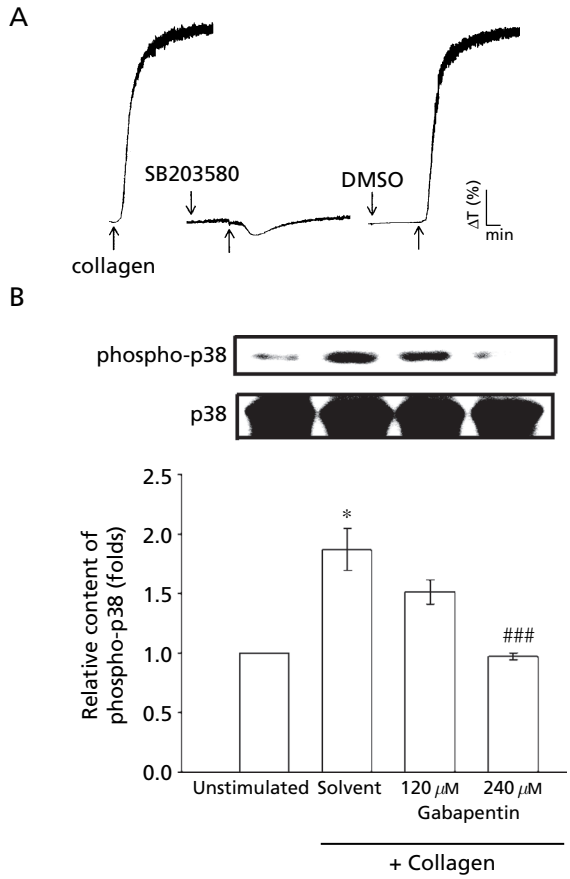

Figure 5 Effects of SB203580, a p38 MAPK inhibitor, on platelet aggregation (A) and gabapentin (B) on p38 MAPK phosphorylation stimulated by collagen in washed human platelets. (A) Platelets (3.6×10^8 mL^{-1}) were pretreated with solvent control (0.5% DMSO) or SB203580 (20 μM), followed by the addition of collagen (1 μg mL^{-1}) to trigger platelet aggregation. (B) Platelets (1.2×10^9 mL^{-1}) were pretreated with solvent control (Tyrode's solution) or gabapentin (120 and 240 μM), followed by the addition of collagen (1 μg mL^{-1}) to trigger p38 MAPK phosphorylation. Cells were collected, and subcellular extracts were analysed for p38 MAPK phosphorylation by Western blotting. Profiles in A are representative examples of four similar experiments. The bar graph in B depicts the ratios of quantitative results obtained by scanning the reactive bands of anti-p-p38 MAPK against anti-p38 MAPK. Data are mean ± s.e.m. (n = 4). $^{*}P<0.01$ vs unstimulated platelets; $^{###}P<0.001$ vs activated platelets (solvent control).

Discussion

As a relatively new anticonvulsant, gabapentin was investigated for potential use in the treatment of bipolar disorder, anxiety disorders and behavioural dyscontrol (Satzinger 1994; Taylor 1995). It is widely prescribed to relieve pain associated with peripheral nerve injuries, diabetic neuropathy and cancer (Taylor 1995; Attal et al 1998; Gu & Huang 2001).

Our results show that gabapentin inhibits platelet aggregation, a finding that has not been described previously. The principal objective of this study was to describe the mechanisms by which gabapentin inhibits platelet activation. The inhibition was directly proportional to the concentration of gabapentin used. Vollmer et al (1989) reported that peak plasma concentrations of gabapentin of 2.7–2.99 mg L^{-1} are achieved 3–3.2 h after ingestion of a single 300 mg capsule. Gabapentin is usually effective at 900–1800 mg daily in three doses, although 3600 mg may be required in some patients to achieve reasonable seizure control (Taylor 1995). In this study, gabapentin was used at a concentration of approximately 120 μM, which inhibited platelet aggregation induced by agonists. This concentration of gabapentin is reasonably close to the blood concentration of gabapentin achieved with oral dosing.

In this study, platelet aggregation induced by collagen appeared to be affected by gabapentin, indicating that gabapentin may affect release of Ca^{2+} from intracellular stores (i.e. dense tubular systems and dense bodies), in accordance with the concept that intracellular Ca^{2+} release is responsible for platelet aggregation.

Although the mechanisms of action by which various agonists such as collagen, ADP and arachidonic acid stimulate platelet aggregation differ, gabapentin significantly inhibited platelet aggregation stimulated by all of them. This implies that gabapentin may block a common step shared by these inducers. These results also indicate that the site of action of gabapentin is not at the receptor level of individual agonists. Triflavin acts by binding to $\alpha_{IIb}\beta_3$ integrin on the platelet surface membrane, which interferes with the interaction of fibrinogen with its specific receptor (Sheu et al 1992). We found that gabapentin did not significantly affect FITC-triflavin binding to $\alpha_{IIb}\beta_3$ integrin, indicating that the antiplatelet activity of gabapentin is not by direct interference with the binding of fibrinogen to its specific receptor on the platelet membrane.

Stimulation of platelets by agonists such as collagen results in phospholipase C (PLC)-catalysed hydrolysis of the minor plasma membrane phospholipid, phosphatidylinositol 4,5-bisphosphate, with concomitant formation of IP_3 and diacylglycerol (Mangin et al 2003). There is strong evidence that IP_3 induces the release of Ca^{2+} from intracellular stores (Sala et al 2005). Diacylglycerol activates protein kinase C, inducing protein phosphorylation and a release reaction (Figure 6). In this study, phosphoinositide breakdown of collagen-activated platelets was inhibited by gabapentin, suggesting that inhibition of platelet aggregation by gabapentin is related to inhibition of PLC activation. Moreover, TxA_2 is an important mediator of the release reaction and aggregation of platelets. Formation of collagen-induced TxA_2, a stable metabolite of TxA_2, was markedly inhibited by gabapentin. Phosphoinositide breakdown has been shown to induce TxA_2 formation via release of free AA from membrane phospholipids catalysed by diglyceride lipase or endogenous phospholipase A_2 (Nakamura et al 1996). Thus, it seems likely that a key aspect of the inhibition of collagen-induced platelet aggregation by gapapentin involves inhibition of the PLC–IP_3–TxA_2–Ca^{2+} cascades (Figure 6).

MAPKs are a family of serine–threonine kinases activated by many stimuli, including growth factors and hormones, in proliferative cells (Bugaud et al 1999), which regulate a number of transcription factors, cytoplasmic proteins and downstream kinases. This family consists of three major subgroups: the ERKs (p44 ERK1 and p42 ERK2) are involved in proliferation, adhesion and cell progression (Bugaud et al 1999); p38 MAPK and JNK or stress-activated protein kinase (SAPK), which includes the 46-kDa JNK1 and 55-kDa JNK2 isoforms, seem to be involved in apoptosis (Bugaud et al 1999). In platelets, three MAPKs, ERKs (especially ERK2), JNK and p38 MAPK have been identified (Bugaud et al 1999). The roles of JNK and ERK2 in platelets are unclear, but they have been suggested to suppress $\alpha_{IIb}\beta_3$ activation or negatively regulate platelet activation (Hughes et al 1997). On the other hand, p38 MAPK provides a crucial signal that is necessary for aggregation caused by collagen or thrombin (Figure 5A; Saklatvala et al 1996). Among the numerous downstream targets of p38 MAPK, the most physiologically relevant one in platelets is cytosolic phospholipase A_2 which catalyses AA release to produce TxA_2 (Coulon et al 2003). Thus, p38 MAPK appears to provide a TxA_2-dependent platelet aggregation pathway (Figure 6). Gabapentin significantly inhibits TxA_2 formation, at least in part, via inhibition of p38 MAPK phosphorylation.

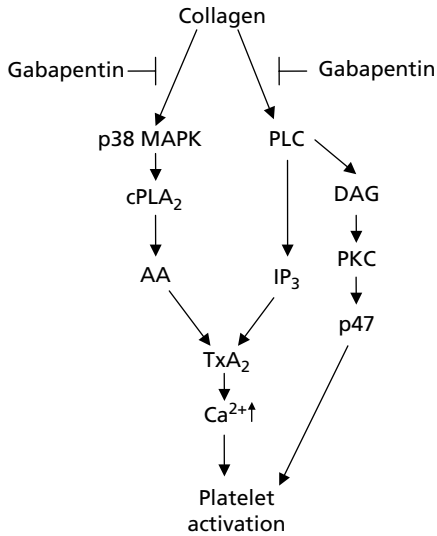

Figure 6 Hypothesis for the inhibition of platelet aggregation by gabapentin in human platelets stimulated by collagen. Gabapentin may inhibit the activation of phospholipase C (PLC), which catalyses conversion of phosphatidylinositol 4,5-bisphosphate into 1,2-diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). DAG stimulates protein kinase C (PKC), followed by the phosphorylation of p47. IP_3 induces the release of Ca^{2+} and triggers platelet activation. Gabapentin also inhibits p38 MAPK phosphorylation. p38 MAPK can activate cytosolic phospholipase A_2 ($cPLA_2$), which catalyses release of arachidonic acid (AA) to form thromboxane A_2 (TxA_2).

Conclusion

The most important findings of this study suggest that gabapentin markedly inhibits agonist-induced platelet aggregation, which may involve the following mechanisms. Gabapentin may inhibit the activation of PLC and p38 MAPK phosphorylation, followed by inhibition of phosphoinositide breakdown and TXA_2 formation, and thereby leading to inhibition of intracellular Ca^{2+} mobilization. Thus, it is possible that gabapentin treatment, alone or in combination with other antiplatelet drugs, may induce or potentiate inhibition of platelet aggregation, which may affect haemostasis. Whether this effect is of clinical importance, particularly in critically ill patients or patients with haematological diseases, remains to be determined.

References

Attal, N., Brasseur, L., Parker, F., Chauvin, M., Bouhassira, D. (1998) Effects of gabapentin on the different components of peripheral and central neuropathic pain syndromes: a pilot study. *Eur. J. Neurol.* **40**: 191–200

Bugaud, F., Nadal-Wollbold, F., Levy-Toledano, S., Rosa J. P., Bryckaert, M. (1999) Regulation of c-jun-NH2 terminal kinase and extracellular-signal regulated kinase in human platelets. *Blood* **94**: 3800–3805

Chesney, C. M., Pifer, D. D., Cagen, L. M. (1987) Triazolobenzodiazepines competitively inhibit the binding of platelet activating factor to human platelets. *Biochem. Biophys. Res. Commun.* **144**: 359–366

Coulon, L., Calzada, C., Moulin, P., Vericel, E., Lagarde, M. (2003) Activation of p38 mitogen-activated protein kinase/cytosolic phospholipase A_2 cascade in hydroperoxide-stressed platelets. *Free Radic. Biol. Med.* **35**: 616–625

Gu, Y. P., Huang, L. Y. (2001) Gabapentin actions on N-methyl-D aspartate receptor channels are protein kinase C-dependent. *Pain* **93**: 85–92

Hass, W. K., Easton, J. D., Adams, H. P., Pryse-Philips, W., Molony, B. A., Anderson S., Kamm, B. A. (1989) Randomized trial comparing ticlopidine hydrochloride with aspirin for the prevention of stroke in high-risk patients. *N. Engl. J. Med.* **321**: 501–507

Hsiao, G., Lin, K. H., Chang, Y., Chen, T. L., Tzu, N. H., Chou, D. S., Sheu, J. R. (2005) Protective mechanisms of inosine in platelet activation and cerebral ischemic damage. *Arterioscler. Thromb. Vacs. Biol*. **25**: 1998–2004

Hughes, P. E., Renshaw, M. W., Pfaff, M., Forsyth, J., Keivens, V. M., Schwartz, M. A., Ginsberg, M. H. (1997) Suppression of integrin activation: a novel function of a Ras/Raf-initiated MAP kinase pathway. *Cell* **88**: 521–530

Karaseva, T. L., Belikova, M. V., Pavlovskii, V. I., Andronati, K. S., Kabanova, T. A. (1998) In vitro effect of 1,2-dihydro-3H-1,4-benzodiazepine-2-one derivatives on the platelet aggregation and lipid peroxidation in rats. *Ukr. Biokhim. Zhurnal*. **70**: 81–85

Magnus, L. (1999) Nonepileptic uses of gabapentin. *Epilepsia* **40**: S66–S74

Mangin, P., Yuan, Y., Goncalves, I., Eckly, A., Freund, M., Cazenave, J. P., Gachet, C., Jackson, S. P., Lanza, F. (2003) Signaling role for phospholipase C gamma 2 in platelet glycoprotein Ib alpha calcium flux and cytoskeletal reorganization. Involvement of a pathway distinct from FcR gamma chain and Fc gamma RIIA. *J. Biol. Chem*. **278**: 32880–32891

Nakamura, F., Minshall, R. D., Le Breton, G. C., Rabito, S. F. (1996) Thromboxane A_2 mediates the stimulation of inositol 1,4,5-trisphosphate production and intracellular calcium mobilization by bradykinin in neonatal rat ventricular cardiomyocytes. *Hypertension* **28**: 444–449

Ramsey, R. E. (1995) Gabapentin: toxicity. In: Levy RH, Mattson R. H., Meldrum B. S. (eds) *Antiepileptic drugs*. Raven Press, New York, pp 857–860

Rao, M. L., Clarenbach, P., Vahlensieck, M., Kratzschmer, S. (1988) Gabapentin augments whole blood serotonin in healthy young men. *J. Neural. Transm*. **73**: 129–134

Rose, M. A., Kam, C. A. (2002) Gabapentin: pharmacology and its use in pain management. *Anaesthesia* **57**: 451–462

Saklatvala, J., Rawlinson, L., Waller, R. J., Sarsfield, S., Lee, J. C., Morton, L. F., Barnes, M. J. Farndale, R. W. (1996) Role for p38 mitogen-activated protein kinase in platelet aggregation caused by collagen or a thromboxane analogue. *J. Biol. Chem*. **271**: 6586–6589

Sala, C., Roussignol, G., Meldolesi, J., Fagni, L. (2005) Key role of the postsynaptic density scaffold proteins Shank and Homer in the functional architecture of Ca^{2+} homeostasis at dendritic spines in hippocampal neurons. *J. Neurosci*. **25**: 4587–4592

Satzinger, G. (1994) Antiepileptics from gamma-aminobutyric acid. *Arzneimittelforschung* **44**: 261–266

Sheu, J. R., Teng, C. M., Huang, T. F. (1992) Triflavin, an RGD-containing antiplatelet peptide, binds to GP IIIa of ADP-stimulated platelets. *Biochem. Biophys. Res. Commun*. **189**: 1236–1242

Sheu, J. R., Yen, M. H., Hung, W. C., Lee, Y. M., Su, C. H., Huang, T. F. (1997) Triflavin inhibits platelet-induced vasocontraction in deendothelialized aorta. *Arterioscler. Thromb. Vasc. Biol*. **17**: 3461–3468

Sheu, J. R., Hung, W. C., Wu, C. H., Ma, M. C., Kan, Y. C., Lin, C. H., Lin, M. S., Luk, H. N., Yen, M. H. (1999) Reduction in lipopolysaccharide-induced thrombocytopenia by triflavin in a rat model of septicemia. *Circulation* **99**: 3056–3062

Sheu, J. R., Lee, C. R., Lin, C. H., Hsiao, G., Ko, W. C., Chen, Y. C., Yen, M. H. (2000) Mechanisms involved in the antiplatelet activity of *Staphylococcus aureus* lipoteichoic acid in human platelets. *Thromb. Haemost*. **72**: 617–621

Sheu, J. R., Hsiao, G., Luk, H. N., Chen, Y. W., Chen, T. L., Lee, L. W., Lin, C. H., Chou, D. S. (2002) Mechanisms involved in the antiplatelet activity of midazolam in human platelets. *Anesthesiology* **96**: 651–658

Taylor, C. P. (1995) Gabapentin: mechanisms of action. In: Levy R. H., Mattson R. H., Meldrum B. S. (eds) *Antiepileptic drugs*. Raven Press, New York, pp 829–841

Vollmer, K. O., Anhut, H., Thomann, P., Wagner, F., Jahncken, D. (1989) Pharmacokinetic model and absolute bioavailability of the new anticonvulsant gabapentin. *Adv. Epileptol*. **17**: 209–211