Biochemical Pharmacology, Vol. 58, pp. 1657–1663, 1999.

ISSN 0006-2952/99/$–see front matter
PII S0006-2952(99)00235-X

Antiplatelet Effect of Amlodipine

A POSSIBLE MECHANISM THROUGH A NITRIC OXIDE-MEDIATED PROCESS

Tz-Chong Chou,† Chi-Yuan Li,‡ Mao-Hsiung Yen§ and Yu-An Ding‖*

*Graduate Institute of Medical Sciences, Departments of ‡Anesthesiology and §Pharmacology, Tri-Service General Hospital, National Defense Medical Center, and ‖Department of Cardiology, Veterans General Hospital-Taipei, Taipei, Taiwan, Republic of China

ABSTRACT. The effect of amlodipine, a novel calcium channel blocker of the dihydropyridine type, on rabbit platelet aggregation, and the possible antiaggregatory mechanisms of amlodipine, especially on the nitric oxide (NO) guanosine 3′,5′-cyclic monophosphate (cyclic GMP)-mediated pathway, were investigated. Other effects of amlodipine on thromboxane B_2 (TXB_2) formation in platelets also were examined. Amlodipine concentration-dependently inhibited rabbit platelet aggregation induced by collagen (10 μg/mL) or thrombin (0.1 U/mL) with an IC_{50} range of 32–69 μM. Along with this inhibition, our results also demonstrated that in the presence of L-arginine (100 μM), amlodipine (50 μM) increased nitric oxide synthetase (NOS) activity (from the resting activity of 2.05 ± 0.36 to 7.11 ± 0.95 pmol/mg protein/min) and NO release (by 80%), accompanied by an elevation of the cyclic GMP level (from the resting platelet level of 1.27 ± 0.12 to 6.21 ± 0.55 pmol/10^9 platelets) induced by collagen (10 μg/mL). However, the antiaggregatory effect of amlodipine (50 μM) could be attenuated significantly by oxyhemoglobin (5 μM), a NO scavenger, or N^G-nitro-L-arginine methyl ester (100 μM), a specific NOS inhibitor. In addition, the TXB_2 production in platelets induced by collagen or thrombin was concentration-dependently inhibited by amlodipine. Therefore, we propose that the antiaggregatory mechanisms of amlodipine might be mediated, in part, by a NO-cyclic GMP process accompanied by the inhibition of TXB_2 formation in platelets. BIOCHEM PHARMACOL **58**;10:1657–1663, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. amlodipine; nitric oxide; guanosine 3′,5′-cyclic monophosphate; thromboxane B_2; platelet aggregation

Platelet activation plays an important role in the initiation and maintenance of atherosclerosis and the thrombotic complications of atheroma [1–4]. In addition, platelet-mediated thrombi also are considered to be significant in coronary thrombosis, transient ischemic attacks, and some strokes [5]. In platelet activation and aggregation, the intracellular calcium concentration ($[Ca^{2+}]_i$) plays a crucial role [6]. During platelet activation, the increase of cytosolic Ca^{2+} as a result of either calcium influx or mobilization of intracellular stores [7] is fundamental in the platelet response to activation.

Calcium antagonists are among the most frequently prescribed medications for hypertension, angina, and coronary artery disease. Recently, the calcium antagonist amlodipine has been reported to have beneficial effects in nonischemic dilated cardiomyopathy [8]. In addition to the cardiovascular effects of calcium antagonists, several authors have demonstrated that these drugs, including amlodipine, a dihydropyridine, inhibit platelet aggregation *in vivo* and *in vitro* [9–12]. However, the mechanism of action of amlodipine on platelet function has not been clarified. It generally is accepted that the reduction of calcium influx by amlodipine is mediated through binding to L-type voltage-operated calcium channels [13]. However, it is noteworthy that platelets do not possess voltage-dependent calcium channels [14]. Therefore, the effect of calcium antagonists on platelet function may be mediated by other properties of amlodipine.

NO¶, produced from the conversion of L-arginine into L-citrulline and NO catalyzed by NOS, also has been proposed as an important mediator inhibiting platelet aggregation [15]. This effect is mediated via activation of intraplatelet soluble guanylate cyclase and leads to an increase of cyclic GMP [16]. Furthermore, it has been demonstrated that an L-arginine–NO pathway is present in human platelets. When platelets are stimulated with collagen, they release NO from L-arginine and down-regulate their response to collagen [17].

It has been reported that amlodipine and some dihydropyridines enhance the release of NO from canine coronary

† Corresponding author: Tz-Chong Chou, Ph.D., 5F, Research Building, Department of Medical Research, Tri-Service General Hospital, No. 8, Sec. 3, Ting-Chow Road, Taipei, Taiwan, ROC. Tel. (886) 2-23658577; FAX (886) 2-23654670; E-mail: tcchou@ms5.hinet.net

Received 12 February 1999; accepted 26 March 1999.

¶ *Abbreviations*: NO, nitric oxide; NOS, nitric oxide synthase; TX, thromboxane; oxy-Hb, oxyhemoglobin; AA, arachidonic acid; BrcGMP, 8-bromo-cyclic GMP; and L-NAME, N^G-nitro-L-arginine methyl ester.

microvessels and endothelial cells [18–20]. In addition, it has been suggested that a NO-mediated process may be involved in the antiaggregatory effect of nifedipine [21]. These results imply that amlodipine may be an effective antiaggregatory agent as a result of an action that enhances NO release. Thus, the aim of this study was to investigate the possible antiaggregatory mechanisms of amlodipine with respect to the NO-mediated pathway.

MATERIALS AND METHODS

Materials

Thrombin, collagen (type 1, equine tendon), bovine serum albumin, indomethacin, EDTA (disodium salt), L-arginine, L-NAME, methemoglobin, sodium dithionite, VCl_3, and a Dowex 50W column were purchased from the Sigma Chemical Co. TXB_2, cyclic AMP, and cyclic GMP EIA kits were purchased from the Cayman Chemical Co. Amlodipine was a gift from Pfizer Pharmaceuticals and was dissolved in DMSO.

Preparation of Platelet Suspension

Blood was withdrawn from a rabbit marginal ear vein, mixed with EDTA anticoagulant (100 mM; 14:1, v/v), and centrifuged at 160 *g* at 25° for 10 min to obtain platelet-rich plasma. A platelet suspension was prepared from the platelet-rich plasma according to previously described washing procedures [22]. Finally, the platelet pellet was suspended in Tyrode's solution of the following composition (mM): $CaCl_2$ (1), NaCl (136.8), KCl (2.7), $NaHCO_3$ (11.9), $MgCl_2$ (2.1), NaH_2PO_4 (0.4), and glucose (10) containing bovine serum albumin (0.35%). Platelet counts were done with a Coulter counter (model ZM), and concentrations were adjusted to 3.0×10^8 platelets/mL.

Platelet Aggregation

Aggregation was measured turbidimetrically at 37° with constant stirring at 1000 rpm, and the absorbance of Tyrode's solution was designated as 100% aggregation. The absorbance of a platelet suspension was designated as 0% aggregation. Following a 3-min equilibration at 37°, the suspension was incubated with amlodipine, L-arginine, L-NAME, or oxy-Hb for 3 min before the addition of an aggregation inducer. Platelet aggregation was recorded for 6 min, and the extent of aggregation was evaluated by measuring the maximum height reached by the aggregation curves. Data were shown as the percentage of maximal aggregation. Platelet aggregation was measured by an aggregometer (Chrono-Log Co., model 560) connected to two dual channel recorders. To eliminate the effect of the solvent on platelet aggregation, the final concentration of DMSO was fixed at 0.5%.

Preparation of Oxy-Hb

Oxy-Hb was prepared by a previously described method [23]. Briefly, methemoglobin crystals were dissolved in phosphate-buffered saline, pH 7.6, subsequently reduced with a 10-fold molar excess of the reducing agent sodium dithionite ($Na_2S_2O_4$), and thereafter gassed with oxygen for 15 min. Finally, the mixture was centrifuged at 1000 *g* for 5 min at 4°, and the supernatant was loaded onto a gel filtration column (Sephadex G-25 coarse, Pharmacia) and eluted with phosphate buffer. The purity of the oxy-Hb was determined spectrophotometrically.

Determination of Nitrate Level

After incubation of platelet suspension with amlodipine in the presence of L-arginine (100 μM) for 3 min followed by stimulation with collagen (10 μg/mL) for 6 min, the mixture was centrifuged at 5000 *g* for 5 min to collect the supernatant. The supernatant was deproteinized by mixing with 95% ethanol (4°) for 30 min, and the supernatant was collected by centrifugation. The nitrate measured in the supernatant was actually the total nitrite and nitrate concentrations. In this method, supernatant nitrite and nitrate were reduced to NO by adding a reducing agent (0.8% VCl_3 in 1 M HCl). Then the NO was drawn into a Sievers Nitrite Oxide Analyzer (Sievers 280 NOA, Sievers). Nitrate concentration was calculated by comparison with a standard solution of sodium nitrate (Sigma).

Determination of NOS Activity of Platelets

After incubation with or without amlodipine (50 μM) for 3 min, followed by the addition of collagen (10 μg/mL) for 6 min, the precipitated platelets were sedimented by centrifugation for NOS activity determination. NOS activity was determined by measuring the conversion of [^{3}H]L-arginine to [^{3}H]L-citrulline according to our previously published method [24]. In brief, platelets (10^8 cells), homogenized by an ultrasonic method, were incubated in 20 mM HEPES buffer (pH 7.5) containing 10 μM L-arginine and [^{3}H]L-arginine (3 μCi/mL), L-valine (60 mM), NADPH (1 mM), calmodulin (30 nM), tetrahydrobiopterin (5 μM), and calcium (2 mM) for 20 min at 37°. The reaction was stopped by adding 1 mL of ice-cold HEPES buffer (pH 5.5) containing EGTA (2 mM) and EDTA (2 mM); then the mixture was applied to Dowex 50W (Na^+ form) columns, and the amount of [^{3}H]L-citrulline eluted was quantitated by liquid scintillation (Beckman, LS3801, Beckman Instruments) for determination of NOS activity. Protein was determined using a dye-binding assay (Bio-Rad) with bovine serum albumin as a standard.

Platelet Cyclic AMP and Cyclic GMP Determination

Rabbit platelet suspension was incubated with amlodipine (50 μM) in the presence of L-arginine (100 μM) for 3 min

at 37°, followed by the addition or not of collagen (10 μg/mL) for 6 min. The incubation was stopped by adding 10 mM EDTA and immediately boiling for 5 min. After cooling to 4°, the precipitated protein was sedimented by centrifugation. The supernatants were used for the determination of cyclic AMP or cyclic GMP content with EIA kits, following acetylation of the samples as described by the manufacturer of the kits.

Measurement of TXB_2

After incubation with amlodipine for 3 min followed by the addition of collagen (10 μg/mL) or thrombin (0.1 U/mL) for 6 min, 2 mM EDTA and 50 μM indomethacin (a cyclooxygenase inhibitor) were added. Then the mixture was centrifuged at 10,000 *g* for 5 min to obtain the supernatant for TXB_2 determination using EIA kits according to the procedure described by the manufacturer.

Statistical Analysis

The experimental results are expressed as means ± SEM. Statistical analyses were performed with one-way ANOVA. Results were considered statistically significant at P less than 0.05.

RESULTS

Effect of Amlodipine on Platelet Aggregation

Amlodipine concentration-dependently inhibited platelet aggregation induced by collagen (10 μg/mL) or thrombin (0.1 U/mL), with IC_{50} values of 32.5 ± 9.6 and 68.4 ± 9.7 μM, respectively.

Effect of L-NAME on the Antiaggregatory Effect of Amlodipine

Preincubation with L-NAME (100 μM), a specific competitive inhibitor of NOS, in the presence of L-arginine (100 μM) for 3 min resulted in a significant attenuation of the amlodipine (50 μM) induced inhibition of platelet aggregation stimulated by collagen (10 μg/mL) to about a 60% reduction (Fig. 1). Furthermore, the addition of L-arginine (100 μM) alone, the physiological precursor of NO, enhanced the antiaggregatory effect of amlodipine about 15% (data not shown).

Effect of Oxy-Hb on the Antiaggregatory Effect of Amlodipine

To evaluate the role of NO release in platelets on the amlodipine-induced antiaggregatory effect, oxy-Hb (5 μM), a scavenger of NO, was used. As shown in Fig. 2, coincubation of oxy-Hb and amlodipine (50 μM) in the presence of L-arginine (100 μM) for 3 min followed by the addition of collagen (10 μg/mL) significantly inhibited the antiaggregatory effect of amlodipine to 40% of control. In addition, oxy-Hb itself slightly increased the aggregation.

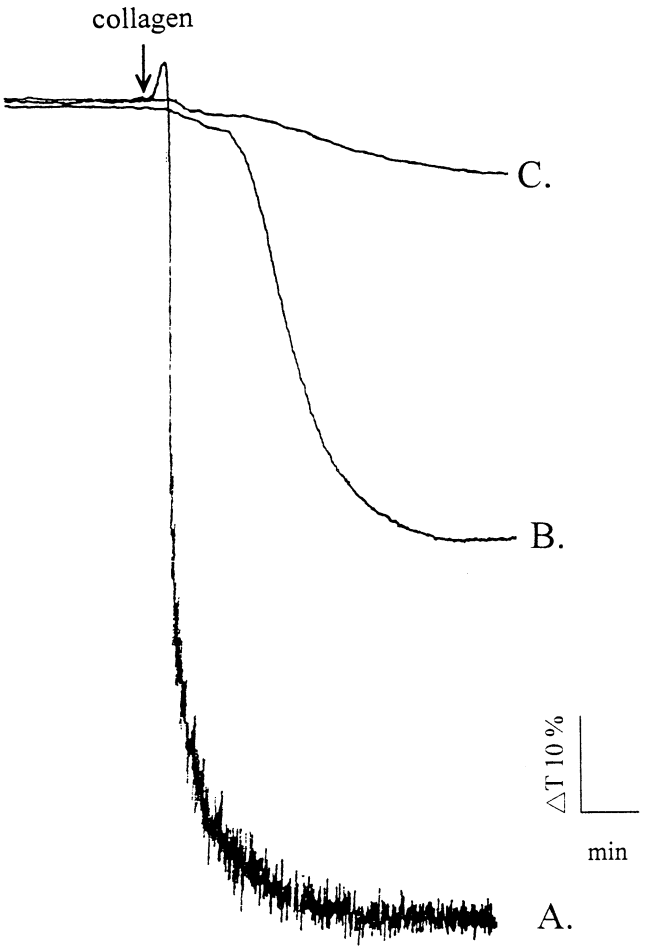

FIG. 1. Effect of L-NAME on the antiaggregatory effect induced by amlodipine. Typical aggregatory traces in the presence of L-arginine (100 μM) were induced by collagen (10 μg/mL) in the absence (control, A) or presence of amlodipine (50 μM) (C) or preincubation with L-NAME (100 μM) for 3 min followed by the addition of amlodipine (50 μM) (B). Amlodipine was added 3 min before collagen exposure. The traces are representative of five similar experiments.

Effect of Amlodipine on Nitrate Formation

In the presence of L-arginine (100 μM), stimulation with collagen (10 μg/mL) caused a significant increase of NO release in platelets compared with the control group. The addition of amlodipine (50 μM) in the presence of L-arginine (100 μM) for 3 min followed by collagen stimulation further enhanced NO release from platelets compared with collagen alone (Fig. 3). In addition, L-arginine (100 μM) itself did not result in the formation of a detectable amount of nitrate.

Effect of Amlodipine on NOS Activity in Platelets

To further clarify the effect of amlodipine on NOS activation, the NOS activity in platelets was determined. Our

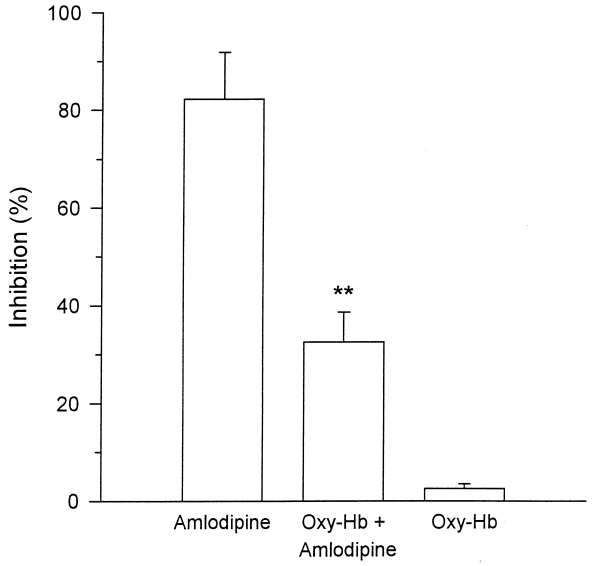

FIG. 2. Inhibition by oxy-Hb of the antiaggregatory effect of amlodipine. Platelets were incubated with amlodipine (50 μM) or DMSO (0.5%, v/v) or coincubation with oxy-Hb (5 μM) and amlodipine (50 μM) in the presence of L-arginine (100 μM) for 3 min followed by the addition of collagen (10 μg/mL). Key: (**) $P < 0.01$ as compared with the amlodipine group. Values are means ± SEM, N = 6.

data demonstrated that incubation with amlodipine (50 μM) for 3 min followed by the addition of collagen (10 μg/mL) for 6 min resulted in a significant increase of NOS activity in platelets compared with collagen alone (from

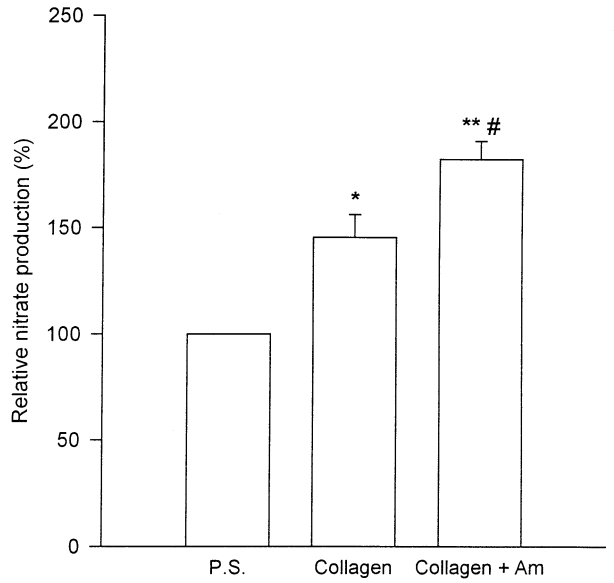

FIG. 3. Induction by amlodipine of nitrate production in collagen-activated platelets. Amlodipine (50 μM) or DMSO (0.5%, v/v) was added in the presence of L-arginine (100 μM) 3 min before stimulation with collagen (10 μg/mL) for 6 min. The supernatant was collected for nitrate measurement. The amount of nitrate in resting platelet suspension (PS) was designated as 100%. Key: (*) $P < 0.05$ and (**) $P < 0.01$ compared with the PS control; (#) $P < 0.05$ compared with the collagen group. Values are means ± SEM, N = 5.

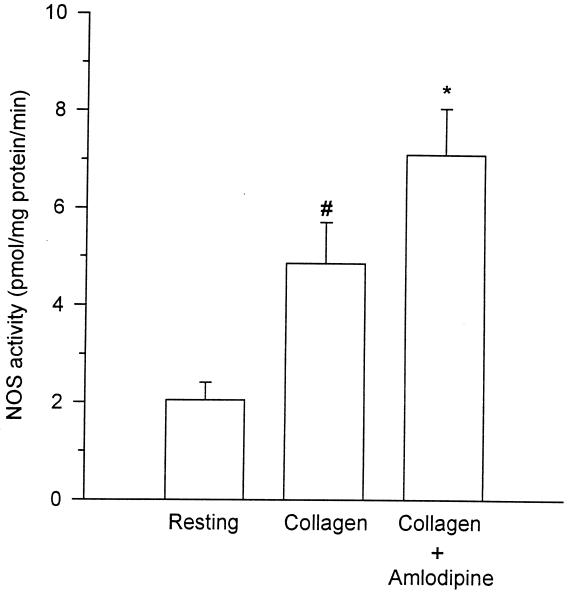

FIG. 4. Enhancement of platelet NOS activity by amlodipine. Platelets were incubated with or without amlodipine (50 μM) for 3 min, followed by the addition of collagen (10 μg/mL) for 6 min. The precipitated platelets were sedimented by centrifugation for NOS activity determination. Key: (*) $P < 0.05$ compared with the collagen alone, and (#) $P < 0.05$ compared with the resting platelets. Values are means ± SEM, N = 5.

4.86 ± 0.85 to 7.11 ± 0.95 pmol/mg protein/min, $P < 0.05$) (Fig. 4). Without stimulation of collagen, amlodipine only slightly increased the NOS activity in platelets. The NOS activity of resting platelets was 2.05 ± 0.36 pmol/mg protein/min.

Effect of Amlodipine on Platelet Cyclic GMP and Cyclic AMP Content

Amlodipine (50 μM) significantly increased the platelet concentration of cyclic GMP in the presence of L-arginine (100 μM) from the resting platelet level of 1.27 ± 0.12 pmol/10^9 platelets to 1.96 ± 0.12 pmol/10^9 platelets, $P < 0.05$. Collagen (10 μg/mL) resulted in a significant increase in the formation of cyclic GMP (to 3.21 ± 0.22 pmol/10^9 platelets) above basal levels. In addition, amlodipine (50 μM) further enhanced the cyclic GMP level significantly in the presence of L-arginine (100 μM) induced by collagen (to 6.21 ± 0.55 pmol/10^9 platelets, $P < 0.05$) compared with that of collagen alone. However, amlodipine had no significant effect on the cyclic AMP level in either resting or collagen-activated platelets.

Effect of Amlodipine on Platelet TXB_2 Production

Amlodipine (100 or 50 μM) also significantly inhibited TXB_2 formation induced by collagen or thrombin (Table 1). Amlodipine alone did not affect the TXB_2 level of platelets in the resting state.

TABLE 1. Effect of amlodipine on the TXB_2 formation of washed rabbit platelets induced by collagen and thrombine

	TXB_2 (ng/10^7 platelets) formation induced by:	
	Collagen (10 μg/mL))	Thrombin (0.1 U/mL)
Control	132.4 ± 15.5	185.4 ± 17.5
Amlodipine		
100 μM	33.1 ± 5.5*	46.3 ± 4.4*
50 μM	65.6 ± 8.3†	78.7 ± 7.7†

After amlodipine or DMSO (0.5%; control) preincubated with platelets at 37° for 3 min, the inducer was added for another 6 min. Then EDTA (2mM) and idomethacin (50 μM) was added to stop the aggregation and TXB_2 formation. Values are means ± SEM (N = 5).

*†Significantly different from control value: *$P < 0.01$, and †$P < 0.05$.

DISCUSSION

The release of NO from L-arginine by vascular endothelium provides a powerful mechanism for inhibiting platelet adhesion and aggregation, and causes disaggregation of platelets both *in vitro* and *in vivo* [25]. It has been reported that activation of human platelets is associated with the generation of NO, which down-regulates aggregation via a cyclic GMP-dependent mechanism [16]. Although many studies have reported that platelet aggregation is inhibited by dihydropyridines [9, 10, 26], the exact mechanisms of action of these drugs are still unclear. Our study showed that amlodipine significantly reduced rabbit platelet aggregation as well as TXB_2 formation in rabbit platelets induced by collagen or thrombin. In addition, amlodipine significantly enhanced the NOS activity, with a subsequent increase in NO release accompanied by elevation of cyclic GMP concentration in platelets stimulated with collagen. These results imply that the inhibitory effect on platelet aggregation of amlodipine may be associated with the release of NO. Various authors have reported that aggregation of platelets by collagen (1–15 μg/mL) but not by thrombin (0.1 U/mL) results in enhancement of NOS activity in platelets and concentration-dependent release of NO [27]. Thus, collagen (10 μg/mL) was used as an inducer for the remainder of the related NO studies, except where noted.

In our study, there is some evidence that confirms our initial hypothesis that amlodipine inhibition of platelet aggregation may occur through a NO-mediated process. First, the antiaggregatory effect of amlodipine was decreased significantly if NO was scavenged by oxy-Hb. Second, when NO synthesis was blocked with a selective competitive inhibitor of NO synthase, L-NAME (100 μM), the antiaggregatory effect of amlodipine was reduced significantly, to 60% (Fig. 1). The strongest evidence supporting our hypothesis is the direct measurement of NO release from platelets, demonstrating enhanced NO production by amlodipine (Fig. 3). Furthermore, we demonstrated that amlodipine significantly increased platelet NOS activity induced by collagen compared with that by collagen alone (Fig. 4). Based on these results, we demonstrated that amlodipine enhanced platelet NO release, especially when a high level of L-arginine was available. Therefore, the mechanisms by which amlodipine inhibits platelet aggregation may be mediated, in part, by NO synthesis.

It is noteworthy that L-arginine on its own did not result in the formation of detectable amounts of nitrate. The addition of amlodipine followed by collagen stimulation enhanced NO production further and inhibited platelet aggregation, but the inhibition was not observed in the group receiving collagen stimulation alone. These results indicate that in resting platelets, the L–arginine–NO pathway is not activated by exogenous L-arginine. However, upon platelet aggregation, the NO formation may be enhanced but not enough to inhibit platelet aggregation. When the NO production was increased further by the addition of amlodipine, the platelet aggregation was inhibited significantly. This may indicate that sufficient amounts of NO are needed to inhibit platelet aggregation.

Recently, cyclic GMP has been recognized as an important messenger in a number of biologic systems. In platelets, elevation of cyclic GMP levels induced by NO or NO-containing compounds such as sodium nitroprusside has been proposed as a mechanism for inhibition of platelet aggregation [28, 29]. Because our results showed that cyclic GMP levels increase with amlodipine treatment in both resting and collagen-induced platelets, it was postulated that the inhibitory effect of amlodipine on platelet aggregation may be mediated by cyclic GMP.

Furthermore, it has been demonstrated that preincubation with 0.4% NO reduces phosphatidic acid production, a sensitive index of phospholipase C activation, and phosphorylation of the 47-kDa substrate of protein kinase C in platelets induced by U-46619 or thrombin [28]. In addition, similar platelet inhibitory effects were also seen with the cell-permeant cyclic GMP analogue (8-BrcGMP) or with cyclic GMP elevating agents [29–31]. These observations suggest that the inhibition of platelet aggregation by NO can be attributed mainly to the NO-mediated rise in cyclic GMP, through the inhibition of agonist-evoked phospholipase C activation and subsequent Ca^{2+} mobilization. Accordingly, we propose that NO synthesis induced by amlodipine accompanied by elevation of cyclic GMP may be involved in the inhibition of platelet aggregation.

Sane *et al.* [32] reported that NO and 8-BrcGMP inhibited AA release from stimulated human platelets in a time- and -dependent manner. In parallel with the inhibition of AA release, the formation of AA metabolites such as TXB_2, the stable metabolite of TXA_2, was attenuated significantly by 8-BrcGMP [32]. It is well known that TXA_2 is also an important mediator of the release reaction and aggregation of platelets [33]. Thus, it appears that at least part of the antiplatelet effect of NO is mediated via elevation of cyclic GMP, which, in turn, inhibits AA release and subsequent metabolism of AA. Recently, it was postulated that inhibition of the phospholipase A_2/AA pathway is the preferential target of the action of

8-BrcGMP. This hypothesis is supported by the studies of Matsuoka *et al.* [34] and others [35], who found that agonist-induced AA release was inhibited by cyclic GMP but that the metabolism of exogenously added AA proceeded normally in collagen-stimulated rabbit platelets. Accordingly, the observation that amlodipine markedly inhibited the TXB_2 formation induced by collagen or thrombin (Table 1) may be associated with the NO-cyclic GMP mediated process.

However, the exact mechanism by which amlodipine enhances NO synthesis is still unknown. Recently, Zhang and Hintze [18] proposed that the release of NO from coronary vessels and the aorta by amlodipine is similar to that induced by angiotensin converting enzyme inhibitor—that is, modulation of the actions or formation of kinins. In addition, other properties of calcium antagonists, such as antioxidant effects and prevention of glutathione loss, which in turn suppresses free radical generation, have been reported [36, 37]. It has been demonstrated that oxygen-derived free radicals promote accumulation of TXA_2 and may be platelet proaggregatory agents [38, 39]. Accordingly, it is possible that the calcium antagonist amlodipine also may possess antioxidant activity, which may be associated, in part, with its antiaggregatory effect.

In conclusion, we demonstrated that amlodipine enhances NOS activity with a subsequent NO release accompanied by the elevation of cyclic GMP in rabbit platelets induced by collagen. This may be associated, in part, with the inhibition of platelet aggregation. We found strong evidence that the antiaggregatory effect of amlodipine was abolished by the NO scavenger oxy-Hb or the NOS inhibitor L-NAME. In addition, the reduction of TXB_2 formation in platelets by amlodipine also may be involved in the inhibition of platelet aggregation.

This study was supported, in part, by a research grant from the National Science Council of Taiwan, Republic of China (NSC 88–2314-B016–072). We thank Professor Joen-Rong Sheu (Graduate Institute of Medical Sciences, Taipei Medical College, Taipei, Taiwan) for his excellent comments.

References

1. Fuster V, Badimon L, Badimon JJ and Chesebro JH, The pathogenesis of coronary artery disease and the acute coronary syndromes. *N Engl J Med* **326:** 310–318, 1991.
2. Ross R, The pathogenesis of atherosclerosis: A perspective for the 1990's. *Nature* **362:** 801–809, 1990.
3. Mustard JF, The Gordon Wilson lecture: Function of platelets and their role in thrombosis. *Trans Am Clin Climatol Assoc* **87:** 104–127, 1976.
4. Packham MA and Mustard JF, The role of platelets in the development and complications of atherosclerosis. *Semin Hematol* **23:** 8–26, 1986.
5. Kerson LA and Olmos-Lau N, Review of ischemic cerebrovascular disease: Pathophysiology, clinical symptomatology, and their implications for therapy. *Cardiovasc Rev Rep* **5:** 227–239, 1984.
6. Kroll M and Schager A, Biochemical mechanisms of platelet activation. *Blood* **74:** 1185–1195, 1989.
7. Massini P and Luscher E, On the significance of the influx of calcium ions into stimulated human blood platelets. *Biochim Biophys Acta* **21:** 523–528, 1981.
8. Packer M, O'Connor CM, Ghali JK, Pressler ML, Carson PE, Belkin RN, Miller AB, Neuberg GW, Frid D, Wertheimer JH, Cropp AB and DeMets DL for the Prospective Randomized Amlodipine Survival Evaluation Study Group, Effect of amlodipine on morbidity and mortality in severe chronic heart failure. *N Engl J Med* **335:** 1107–1114, 1996.
9. Folts JD, Inhibition of platelet activity *in vivo* by amlodipine alone and combined with aspirin. *Int J Cardiol* **62:** S111–S117, 1997.
10. Hernandez R, Carvajal AR and Armas-de-Hernandez MJ, Amlodipine in hypertension: Its effects on platelet aggregation and dynamic exercise. *J Cardiovasc Pharmacol* **17:** S25–S27, 1991.
11. Pales J, Palacios-Araus L, Lopez A and Gaul A, Effect of dihydropyridines and inorganic calcium blockers on aggregation and on intracellular free calcium in platelets. *Biochim Biophys Acta* **1064:** 169–174, 1991.
12. Wallen NH, Held C, Rehnqvist N and Hjemdahl P, Platelet aggregability *in vivo* is attenuated by verapamil but not by metoprolol in patients with stable angina pectoris. *Am J Cardiol* **75:** 1–6, 1995.
13. Catterall W and Striessnig J, Receptor sites for Ca^{2+} channel antagonists. *Trends Pharmacol Sci* **13:** 256–262, 1992.
14. Doyle VM and Ruegg UT, Lack of evidence for voltage dependent calcium channels on platelets. *Biochem Biophys Res Commun* **127:** 161–167, 1985.
15. Mollace V, Salvemini D, Anggard E and Vane J, Studies on the importance of the proposed release of nitric oxide from platelets. *Thromb Res* **64:** 533–542, 1991.
16. Mellion BT, Ignarro LJ, Ohlstein EH, Pontecorvo EG, Hyman AL and Kadowitz PJ, Evidence for the inhibitory role of adenosine 3′,5′-monophosphate in ADP-induced human aggregation in the presence of nitric oxide and related vasodilators. *Blood* **57:** 946–955, 1981.
17. Radomski MW, Palmer RMJ and Moncada S, An L-arginine/nitric oxide pathway present in human platelets regulates aggregation. *Proc Natl Acad Sci USA* **87**: 5193–5197, 1990.
18. Zhang X and Hintze TH, Amlodipine releases nitric oxide from canine coronary microvessels: An unexpected mechanism of action of a calcium channel-blocking agent. *Circulation* **97:** 576–580, 1998.
19. Gunther J, Dhein S, Rosen R, Klaus W and Fricke U, Nitric oxide (EDRF) enhances the vasorelaxing effect of nitrendipine in various isolated arteries. *Basic Res Cardiol* **87:** 452–460, 1992.
20. Vilaine JP, Biondi ML, Villeneuve N, Feletou M, Peglion JL and Vanhoutte PM, The calcium channel antagonist S 11568 causes endothelium-dependent relaxation in canine arteries. *Eur J Pharmacol* **197:** 41–48, 1991.
21. Berkels R, Klaus W, Boller M and Rosen R, The calcium modulator nifedipine exerts its antiaggregatory property via a nitric oxide mediated process. *Thromb Haemost* **72:** 309–312, 1994.
22. Chou T-C, Hsu L-Y, Yen M-H and Ding Y-A, The inhibitory effect of 2-thienyl 2′-hydroxyphenyl ketone (C85) on platelet thromboxane formation. *Thromb Res* **84:** 83–95, 1996.
23. Zhou Q, Hellermann GR and Solomonson LP, Nitric oxide release from resting human platelets. *Thromb Res* **77:** 87–96, 1995.
24. Chou T-C, Yen M-H, Li C-Y and Ding Y-A, Alterations of nitric oxide synthase expression with aging and hypertension in rats. *Hypertension* **31:** 643–648, 1998.
25. Radomski MW and Moncada S, The biological and pharmacological role of nitric oxide in platelet function. *Adv Exp Med Biol* **344:** 251–264, 1993.

26. Takahara KK, Kuroiwa A and Matsushima T, Effects of nifedipine on platelet function. *Am Heart J* **109:** 4–8, 1985.
27. Malinski T, Radomski MW, Taha Z and Moncada S, Direct electrochemical measurement of nitric oxide released from human platelets. *Biochem Biophys Res Commun* **194:** 960–965, 1993.
28. Nguyen BL, Saitoh M and Ware JA, Interaction of nitric oxide and cGMP with signal transduction in activated platelets. *Am J Physiol* **261:** H1043–H1052, 1991.
29. Hawkins DJ, Meyrick BO and Murray JJ, Activation of guanylate cyclase and inhibition of platelet aggregation by endothelium-derived relaxing factor released from cultured cells. *Biochim Biophys Acta* **969:** 289–296, 1988.
30. Nakashima S, Tohmatsu T, Hattori H, Okano YA and Nozawa Y, Inhibitory action of cyclic GMP on secretion, polyphosphoinositide hydrolysis and calcium mobilization in thrombin-stimulated human platelets. *Biochem Biophys Res Commun* **135:** 1099–1104, 1986.
31. Geiger J, Nolte C and Walter U, Regulation of calcium mobilization and entry in human platelets by endothelium-derived factors. *Am J Physiol* **267:** C236–C244, 1994.
32. Sane DC, Bielawska A, Greenberg CS and Hannun YA, Cyclic GMP analogs inhibit gamma thrombin-induced arachidonic acid release in human platelets. *Biochem Biophys Res Commun* **165:** 708–714, 1989.
33. Armstrong RA, Jones RL and Wilson NH, Ligand binding to thromboxane receptors on human platelets: Correlation with biological activity. *Br J Pharmacol* **79:** 953–964, 1983.
34. Matsuoka I, Nakahata N and Nakanishi H, Inhibitory effect of 8-bromo cyclic GMP on an extracellular Ca^{2+}-dependent arachidonic acid liberation in collagen-stimulated rabbit platelets. *Biochem Pharmacol* **38:** 1841–1847, 1989.
35. Billah MM, Lapetina EG and Cuatrecasas P, Phospholipase A_2 activity specific for phosphatidic acid. A possible mechanism for the production of arachidonic acid in platelets. *J Biol Chem* **256:** 5399–5403, 1981.
36. Hishikawa K and Luscher T, Felodipine inhibits free-radical production by cytokines and glucose in human smooth muscle cells. *Hypertension* **32:** 1011–1015, 1998.
37. Mak IT and Weglicki WB, Antioxidant activity of calcium channel blocking drugs. *Methods Enzymol* **234:** 620–630, 1994.
38. Shook LA, Pauly TH, Marple SL, Horstman SJ, Tai H-H, Bowdy BD and Gillespie MN, Group B streptococcus promotes oxygen radical-dependent thromboxane accumulation in young piglets. *Pediatr Res* **27:** 349–352, 1990.
39. Salvemini D, Radziszewski W, Mollace V, Moore A, Willoughby D and Vane J, Diphenylene iodonium, an inhibitor of free radical formation, inhibits platelet aggregation. *Eur J Pharmacol* **199:** 15–18, 1991.