Journal of Cardiovascular Pharmacology™
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Morphine-Potentiated Agonist-Induced Platelet Aggregation Through α_2-Adrenoceptors in Human Platelets

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Summary: Morphine dose-dependently (0.6, 1, and 5 μ*M*) potentiated platelet aggregation and ATP release stimulated by agonists (i.e., collagen and U46619) in washed human platelets. Furthermore, morphine (1 and 5 μ*M*) markedly potentiated collagen (1 μg/ml) evoked an increase of intracellular Ca^{2+} mobilization in fura 2-AM loading human platelets. Morphine (1 and 5 μ*M*) did not influence the binding of fluorescein isothiocyanate–triflavin to platelet glycoprotein IIb/IIIa complex. Yohimbine (0.1 μ*M*), a specific α_2-adrenoceptor antagonist, markedly abolished the potentiation of morphine in platelet aggregation stimulated by collagen. Moreover, morphine (0.6–5 μ*M*) markedly inhibited prostaglandin E_1 (10 μ*M*)-induced cAMP formation in human platelets, and yohimbine (0.1 μ*M*) significantly reversed the inhibition of cAMP by morphine (0.6 and 1 μ*M*) in this study. Morphine (1 and 5 μ*M*) significantly potentiated thromboxane B_2 formation stimulated by collagen in human platelets, and yohimbine also reversed this effect of morphine in this study. In addition, morphine (1 and 5 μ*M*) did not significantly affect nitrate production in human platelets. Morphine may exert its potentiation in platelet aggregation by binding to α_2-adrenoceptors in human platelets, which leads to reduced cAMP formation and subsequently to increased intracellular Ca^{2+} mobilization; this, in turn, is followed by increased thromboxane A_2 formation and finally potentiates platelet aggregation and ATP release. **Key Words:** Adrenoceptors—cAMP—Morphine—Platelet aggregation—Thromboxane A_2.

The narcotic analgesics, of which morphine is the prototype, produce a large variety of pharmacologic responses by interacting with the opioid receptors in the nervous system.

The cardiovascular effects of morphine have been extensively studied (1). IV administration of morphine to anesthetized rats or dogs produces dose-dependent hypotension. The cardiovascular action of morphine in humans depends on the physiologic state of the subject. IV administration of 10–30 mg of morphine to supine, normal subjects does not change blood pressure consistently (1). However, a similar dose given to normal subjects during head uptilting produces a fall in blood pressure and bradycardia and, in some subjects, syncope (1). Fur-

Received November 27, 2001; accepted April 18, 2002.

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This work was supported by a grant from the National Science Council of Taiwan (NSC 89-2320-B-038-002-M53).

thermore, in conscious patients undergoing coronary artery catheterization, morphine can decrease the cardiac index, stroke volume, and myocardial contractility, sometimes causing a fall in blood pressure, even while subjects are supine (2).

Conversely, studies of morphine in platelets are relatively rarely compared with those in blood vessels. Thus, platelet aggregation may play a crucial role in the atherothrombotic process. Gryglewski et al. (3) presented data to indicate that morphine antagonizes prostaglandin E_1–mediated inhibition of human platelet aggregation; the antagonism between prostaglandin E_1 and morphine is similar to that described in rat brain homogenate and may also be demonstrated in human platelets (4). They also speculated that adenylate cyclase may be the common target for prostaglandin E_1 and morphine in platelets as it is in neurons (4). Ballesta and Orts (5) also demonstrated that morphine and semisynthetic antagonist derivatives inhibit the binding of [^{3}H] clonidine to human platelet α_2-adrenoceptors, indicating that morphine binds to human platelets through α_2-adrenoceptors. However, the detailed mechanisms underlying the morphine signaling pathways in platelets remain obscure. We therefore systematically examined the influence of morphine on washed human platelets in this study and used the findings to characterize the mechanisms involved in this influence.

METHODS

Platelet aggregation

Human platelet suspensions were prepared as previously described (6). The washed platelets were finally suspended in Tyrode solution containing bovine serum albumin (3.5 mg/ml) and adjusted to a concentration of 4.5×10^8 platelets/ml. The final concentration of Ca^{2+} in Tyrode solution was 1 m*M*.

The turbidimetric method was applied to measure platelet aggregation (7), using a Lumi-Aggregometer (Payton, Canada). Platelet suspensions (0.4 ml) were preincubated with morphine for 3 min followed by the 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. While measuring ATP release, 20 μl of luciferin/luciferase mixture was added 1 min before the addition of the agonists, and ATP release was compared with that of the control.

Analysis of the platelet surface glycoprotein IIb/IIIa complex by flow cytometry

Triflavin, a specific fibrinogen receptor (glycoprotein IIb/IIIa complex) antagonist, was prepared as previously described (8,9). Fluorescein isothiocyanate (FITC)-conjugated triflavin was also prepared as previously described (10). The final concentration of FITC-conjugated triflavin was adjusted to 1 mg/ml. Human platelet suspensions were prepared as described here. Aliquots of platelet suspensions (4.5×10^8/ml) were preincubated with morphine (1 and 5 μ*M*) for 3 min, and collagen (1 μg/ml) was then added for 3 min, followed by the addition of 2 μl of FITC-triflavin. The suspensions were incubated for another 5 min, and the volume was adjusted to 1 ml/tube with Tyrode solution. The suspensions were then assayed for fluorescein-labeled platelets with a flow cytometer (FACScan System, Becton Dickinson, Franklin Lakes, NJ, U.S.A.). Data were collected from 50,000 platelets per experimental group. All experiments were repeated at least five times to ensure reproducibility.

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 protected from light and incubated with fura 2-AM (5 μ*M*) at 37°C for 1 h. Human platelet suspensions were then prepared as described previously. Finally, the external Ca^{2+} concentration of the platelet suspensions was adjusted to 1 m*M*. The $[Ca^{2+}]_i$ rise was measured using a fluorescence spectrophotometer (CAF 110, Jasco, Tokyo, Japan) of excitation wavelengths at 340 and 380 nm, emission wavelength at 500 nm, respectively. $[Ca^{2+}]_i$ was calculated from the fluorescence, using 224 n*M* as the Ca^{2+}-fura 2 dissociation constant (11).

Estimation of platelet cAMP

The method of Karniguian et al. (12) was followed. Platelet suspensions (4.5×10^8/ml) were preincubated with morphine (0.6, 1, and 5 μ*M*) or morphine (0.6 and 1 μ*M*) combined with yohimbine (0.1 μ*M*) for 3 min followed by the addition of prostaglandin E_1 (10 μ*M*) for 6 min at 37°C. The incubation was stopped by the addition of 10 m*M* of ethylenediamine tetra-acetic acid (EDTA) and immediately boiling the mixture for 5 min. The reaction mixtures were cooled to 4°C, and the precipitated protein was collected as sediment after centrifugation in an Eppendorf centrifuge (Sigma-Aldrich). The supernatant (400 μl) was freeze-dried and the residue was dissolved in 100 ml of distilled water. To determine the cAMP concentration, 50 μl of the reconstituted supernatant was acetylated and used for enzyme immunoassay as described by the manufacturer (Cayman, Ann Arbor, MI, U.S.A.).

Measurement of thromboxane B_2 formation

Platelet suspensions (4.5×10^8/ml) were preincubated with morphine (0.6, 1, and 5 μM) or morphine (1 and 5 μM) combined with yohimbine (0.1 μM) for 3 min followed by addition of collagen (1 μg/ml) at 37°C. Six minutes after the addition of collagen, EDTA (2 mM) and indomethacin (500 μM) were added to the reaction suspensions. The vials were then centrifuged in an Eppendorf centrifuge (model 5414) for 3 min at 14,000 rpm. The thromboxane B_2 levels of the supernatants were measured using an enzyme immunoassay kit (Cayman) according to the manufacturer's instructions.

Measurement of prostaglandin E_2 formation

Morphine (1 and 5 μM) was preincubated for 3 min in the presence of imidazole (600 μM) in washed human platelets (0.4 ml, 4.5×10^8/ml). Six minutes after the addition of arachidonic acid (60 μM), EDTA (10 mM) and indomethacin (500 μM) were added to the reaction suspensions. The vials were then centrifuged, and the prostaglandin E_2 levels of the supernatants were measured using an enzyme immunoassay kit (Cayman) according to the manufacturer's instructions.

Estimation of nitrate in human platelet suspensions

Nitric oxide was assayed in platelet suspensions using a sensitive and specific chemiluminescence detection method as described previously (13). In brief, platelet suspensions (0.4 ml, 1×10^9/ml) were preincubated with collagen (10 μg/ml), morphine (1 and 5 μM), or morphine (5 μM) combined with collagen (10 μg/ml) for 6 min at 37°C, respectively, followed by centrifugation. The supernatants were deproteinized by incubation with 95% ethanol at 4°C for 30 min as described previously (13). Finally, the amount of nitrate in the platelet suspensions (10 μl) was measured by Sievers NO Analyzer (280 NOA, Sievers, Boulder, CO, U.S.A.).

Materials

Collagen (type I, bovine Achilles tendon), sodium citrate, luciferin-luciferase, indomethacin, prostaglandin E_1, nitroglycerin, apyrase, heparin, arachidonic acid, morphine, and yohimbine were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Fura 2-AM and FITC were purchased from Molecular Probes (Eugene, OR, U.S.A.). U46619 was purchased from Biomol Research Laboratories (Plymouth Meeting, PA, U.S.A.). VCl_3 was obtained from Aldrich (Milwaukee, WI). *Trimeresurus flavoviridis* venom was purchased from Latoxan (Rosans, France). cAMP, thromboxane B_2, and prostaglandin E_2 enzyme immunoassay kits were purchased from Cayman.

Statistical analysis

The experimental results are expressed as the means ± SEM and are accompanied by the number of observations. Data were assessed with analysis of variance. If this analysis indicated significant differences among the group means, then each group was compared by the Newman-Keuls method. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Effect of morphine on platelet aggregation in washed human platelets

Morphine dose-dependently (0.6–5 μM) potentiated platelet aggregation stimulated by collagen (1 μg/ml) and U46619 (0.5 μM), a prostaglandin endoperoxide analogue compound, in washed human platelet suspensions (Fig. 1). It also similarly potentiated ADP (2 μM)-induced platelet aggregation in the presence of fibrinogen (200 μg/ml) (data not shown). Furthermore, morphine also potentiated the ATP release reaction when stimulated by agonists (i.e., collagen) (Fig. 1). At the same concentrations (0.6, 1, and 5 μM), morphine showed a lower activity of potentiation for U46619-induced platelet aggregation (Fig. 1B).

Effect of morphine on collagen-induced glycoprotein IIb/IIIa complex exposure in human platelets

In this study, the relative intensities of fluorescence of FITC-triflavin (2 μg/ml) bound directly to collagen (1 μg/ml)-activated platelets was 61.9 ± 3.4, and it was markedly reduced in the presence of 5 mM of EDTA (negative control, 8.3 ± 0.6) (data not shown). At a concentration of 1 μM, morphine did not significantly increase FITC-triflavin binding to the glycoprotein IIb/IIIa complex in platelet suspensions (66.2 ± 2.7), even at a higher concentration (5 μM) (62.4 ± 3.2) (data not shown).

Effect of morphine on $[Ca^{2+}]_i$ mobilization in human platelets

Free cytoplasmic Ca^{2+} concentrations in human platelets were measured by the fura 2-AM loading method. As shown in Fig. 2, collagen did not significantly evoke an increase of $[Ca^{2+}]_i$ in human platelets (35.8 ± 5.7 nM) at a lower concentration (1 μg/ml). However, morphine (1 and 5 μM) markedly potentiated collagen evoking an increase of $[Ca^{2+}]_i$ in human platelets (1 μM, 92.5 ± 8.7 nM; 5 μM, 187.4 ± 15.3 nM; $n = 4$). In contrast, morphine (10 μM) alone did not significantly evoke an increase of $[Ca^{2+}]_i$ (data not shown).

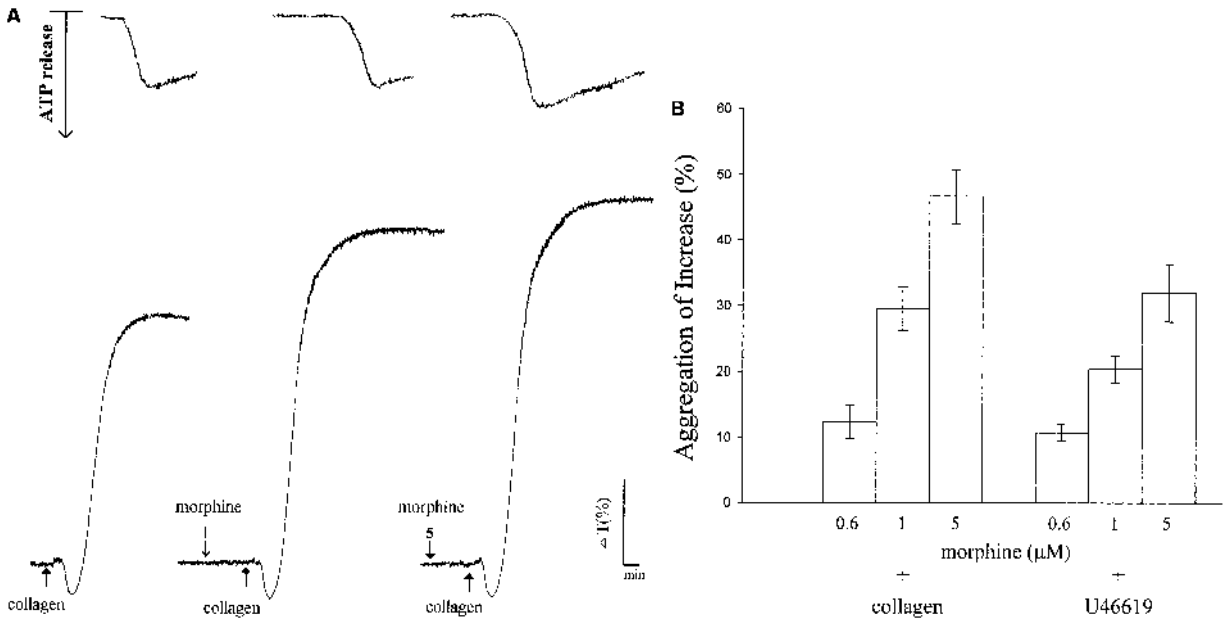

FIG. 1. Typical patterns of the potentiation by morphine of collagen (1 μg/ml)-induced platelet aggregation and ATP release (**A**) and concentration-potentiation histogram of morphine on collagen (1 μg/ml)- and U46619 (0.5 μ*M*)-induced platelet aggregation in washed human platelet suspensions (**B**). Platelets (4.5×10^8/ml) were preincubated with morphine (0.6, 1, and 5 μ*M*) at 37° for 3 min, then agonists were added to trigger aggregation (lower tracings) and ATP release (upper tracings) (A). Data are expressed as percent aggregation of increase as compared with the individual agonist group (in the absence of morphine) and presented as mean ± SEM (n = 7).

Effect of yohimbine in morphine-potentiated platelet aggregation

As shown in Fig. 3A, yohimbine (0.1 μ*M*), a specific α_2-adrenoceptor antagonist (14), markedly abolished the activity of potentiation induced by morphine (1 μ*M*) (Fig. 3A); however, in itself it did not significantly affect the aggregation stimulated by collagen (Fig. 3A).

Effect of morphine on cAMP level in human platelets

The level of cAMP in unstimulated platelets was low (41.7 ± 6.9 pmol/ml). Addition of prostaglandin E_1 (10 μ*M*) increased the cAMP level to 217.5 ± 19.5 pmol/ml (Fig. 3B). When platelet suspensions were preincubated with various concentrations of morphine (0.6, 1, and 5 μ*M*) for 3 min followed by the addition of prostaglandin E_1 (10 μ*M*), we found that morphine markedly inhibited prostaglandin E_1–induced cAMP formation in human platelets (Fig. 3B). Yohimbine (0.1 μ*M*) significantly reversed the inhibition of cAMP formation by morphine (0.6 and 1 μ*M*) in the presence of prostaglandin E_1 (10 μ*M*). We also performed similar studies measuring cGMP responses. The level of cGMP in unstimulated platelets was very low (4.7 ± 0.5 pmol/ml, n = 4), but when nitroglycerin (10 μ*M*) was added to the platelet suspensions, cGMP level increased about 4.3-fold as compared with resting platelets (20.1 ± 3.5 pmol/ml, n = 4). However, morphine (5 μ*M*) did not significantly inhibit nitroglycerin-induced cGMP formation in human platelets (19.7 ± 3.2 pmol/ml, n = 4).

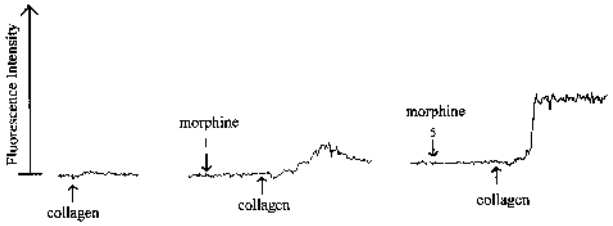

FIG. 2. Effect of morphine on collagen-induced intracellular Ca^{2+} mobilization in fura 2-AM-loaded human platelets. Platelet suspensions were preincubated with fura 2-AM (5 μ*M*) at 37° for 30 min, followed by the addition of collagen (1 μg/ml) in the absence or presence of morphine (1 and 5 μ*M*), which was added 3 min prior to the addition of collagen. The profiles are representative examples of four similar experiments.

Effect of morphine on thromboxane B_2 formation

As shown in Fig. 4, resting platelets produced relatively little thromboxane B_2 compared with collagen-activated platelets. Furthermore, results obtained using various concentrations of morphine (0.6, 1, and 5 μ*M*) indicated that morphine (1 and 5 μ*M*) significantly potentiated thromboxane B_2 formation in platelets stimu-

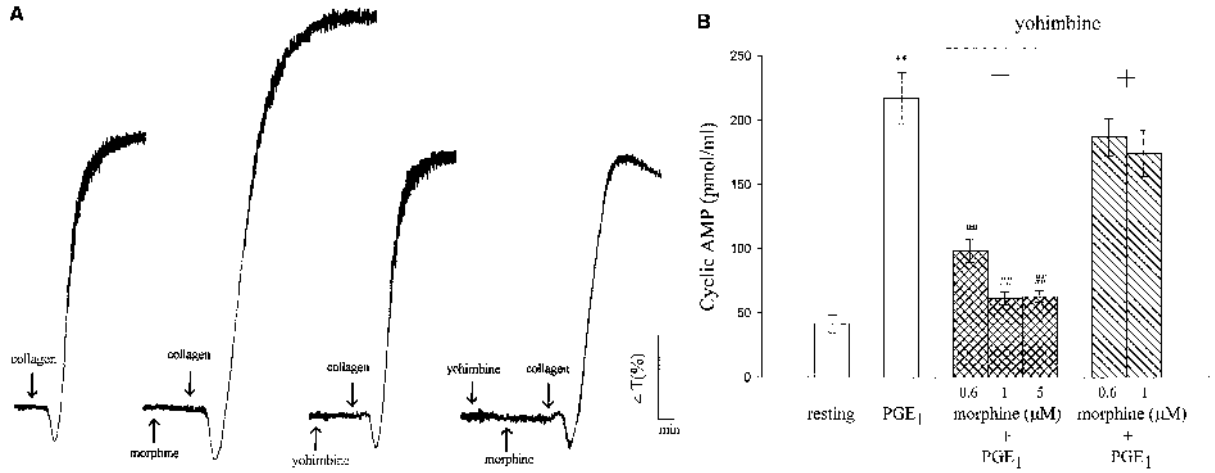

FIG. 3. Effect of yohimbine on (**A**) morphine-potentiated collagen-induced platelet aggregation and (**B**) morphine inhibition of prostaglandin E_1-induced cAMP formation in washed human platelets. **A**. Platelet suspensions (4.5×10^8 /ml) were preincubated with yohimbine (0.1 μ*M*) for 3 min, followed by addition of morphine (1 μ*M*) and stirring for 3 min; collagen (1 μg/ml) was then added to trigger aggregation. **B**. Platelet suspensions (4.5×10^8/ml) were preincubated with morphine (0.6, 1, and 5 μ*M*; cross-hatched bars) or morphine (0.6 and 1 μ*M*) combined with yohimbine (0.1 μ*M*; hatched bars) for 3 min followed by the addition of prostaglandin E_1 (10 μ*M*) for 6 min at 37°. Addition of prostaglandin E_1 in platelet suspensions was represented as positive control (open bar). The data are presented as mean ± SEM (n = 7). **p < 0.001 as compared with the resting group. ##p < 0.001 as compared with the prostaglandin E_1 group.

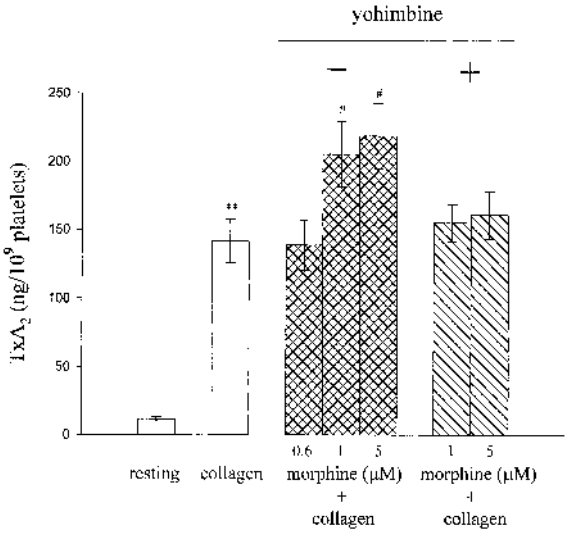

FIG. 4. Effect of morphine on collagen (1 μg/ml)-induced thromboxane B_2 formation in washed human platelets. Morphine (0.6, 1, and 5 μ*M*; cross-hatched bars) or morphine (1 and 5 μ*M*) combined with yohimbine (0.1 μ*M*; hatched bars) was added to washed human platelets (4.5×10^8/ml) 3 min before the addition of collagen (1 μg/ml) as described in "Methods." Addition of collagen (1 μg/ml; open bar) in platelet suspensions was represented as positive control. Data are presented as mean ± SEM (n = 7). **p < 0.001 as compared with the resting group. #p < 0.05 as compared with the collagen group.

lated by collagen (1 μg/ml); concurrently, yohimbine (0.1 μ*M*) reversed this effect of morphine on potentiation of thromboxane B_2 formation (Fig. 4). In addition, morphine (5 μ*M*) alone did not significantly increase thromboxane B_2 formation in the absence of collagen as compared with resting platelets (data not shown).

Effect of morphine on prostaglandin E_2 formation

As shown in Table 1, exogenous arachidonic acid (60 μ*M*) induced relatively high levels of prostaglandin E_2 formation in washed human platelets in the presence of imidazole (600 μ*M*) as compared with resting platelets. Furthermore, results obtained using various concentrations of morphine indicated that morphine (1 and 5 μ*M*)

TABLE 1.

Effect of morphine on arachidonic acid (60 μM)-induced prostaglandin E_2 formation in washed human platelets

Treatment	Prostaglandin E_2 (pg/ml)
Resting	244.8 ± 17.6
Arachidonic acid	2249.4 ± 471.8**
+ Morphine (1 μ*M*)	2313.1 ± 420.1**
+ Morphine (5 μ*M*)	1807.9 ± 446.4**

Morphine (1 and 5 μ*M*) was added to washed human platelets (4.5×10^8/ml) 3 min before the addition of arachidonic acid (60 μ*M*) in the presence of imidazole (600 μ*M*) as described in "Methods." Data are presented as mean ± SEM (n = 4). **p < 0.001 as compared with the resting group.

did not significantly inhibit prostaglandin E_2 formation in platelets stimulated by arachidonic acid (Table 1).

Effect of morphine on nitrate production in human platelets

In this study, nitric oxide was quantified using a sensitive and specific ozone redox-chemiluminescence detector. As shown in Table 2, nitrate production increased in collagen (10 μg/ml)-stimulated platelets. Collagen (10 μg/ml) caused about a 1.7-fold rise in nitrate formation, compared with that in resting platelets (4.5 ± 0.6 μ*M* versus 7.6 ±0.7 μ*M*). In the presence of morphine (1 and 5 μ*M*), nitrate production in washed human platelets did not significantly change, even at a higher concentration (10 μ*M*) (data not shown). Furthermore, morphine did not affect the increase of nitrate production in collagen-stimulated platelets (Table 2).

DISCUSSION

The principal objective of this study was to describe the detailed mechanisms of morphine in the potentiation of agonist-induced human platelet aggregation. The most important finding in this study was the demonstration that morphine exerts a potentiation role on agonist-induced platelet aggregation that has not been described previously. This potentiation by morphine was demonstrable with the use of various agonists: collagen and U46619. The potentiation was also directly proportional to the amount of morphine used. Matos et al. (15) reported that the minimal extracellular morphine concentration in spinal cord required to produce a significant increase in the nociceptive threshold is approximately 100 pg/25 μl, which corresponds to a tissue concentration of about 10 mg/g of morphine. In this study, morphine was used at concentrations that potentiated platelet aggregation induced by agonists at concentrations of about 0.6–5 μ*M*. These results indicate that the concentrations of morphine used to potentiate platelet aggregation in vitro are more reasonable than those of blood concentrations obtained during morphine-induced analgesia in vivo studies. In this study, both platelet aggregation and the ATP release reaction induced by agonists (i.e., collagen) appeared to be potentiated in the presence of morphine. Therefore, we can infer that morphine may increase $[Ca^{2+}]_i$ release from intracellular Ca^{2+} storage sites (i.e., dense tubular systems or dense bodies) (Fig. 2), which is in accord with the concept that $[Ca^{2+}]_i$ release is responsible for the ATP release reaction (16).

Although the action mechanisms of various platelet aggregation agonists, such as collagen, ADP, and U46619, differ, morphine significantly potentiated platelet aggregation stimulated by all of them. This implies that morphine may activate a common step shared by these inducers. These results also indicate that the site of action of morphine is not at the receptor level of individual agonists on platelet surface membrane. In this study, we found that morphine did not significantly increase FITC-triflavin binding to the glycoprotein IIb/IIIa complex, indicating that the potentiation of platelet aggregation of morphine might not be directly due to increase in the exposure of glycoprotein IIb/IIIa complex on the platelet membrane surface.

Ballesta and Orts (5) demonstrated that morphine interacts with human platelet via α_2-adrenoceptors. Local interactions between α_2-adrenoceptors and an opioid agonist or antagonist have also been reported (17,18). In this study, we found that morphine-potentiated agonist-induced platelet aggregation can be abolished by pretreatment with yohimbine. Furthermore, we also found that yohimbine (0.1 μ*M*) significantly reversed the inhibition of cAMP formation by morphine (0.6 and 1 μ*M*) in the presence of prostaglandin E_1 (10 μ*M*) (Fig. 3B). These results indicate that the potentiation activity of morphine may be due to binding with α_2-adrenoceptors in human platelets, resulting in potentiation of platelet aggregation. In many systems, α_2-adrenoceptors are coupled to the inhibition of adenylate cyclase through an inhibitory G protein termed G_i (19). Therefore, reductions in intracellular concentrations of cAMP thereby increase the activation of platelet. This inverse relationship of cAMP in modulating platelet reactivity is well established. In addition to inhibiting most platelet responses, elevated levels of cAMP decrease $[Ca^{2+}]_i$ with uptake of Ca^{2+} into the dense tubular system (20). The data presented in this study suggest that the potentiation of morphine is due, at least partly, to lowering the level

TABLE 2.
Effect of morphine and collagen on nitrate formation in washed human platelets

Treatment	Nitrate (μ*M*)
Resting	4.5 ± 0.6
Collagen (10 μg/ml)	7.6 ± 0.7*
Morphine (1 μ*M*)	5.1 ± 0.4
Morphine (5 μ*M*)	4.7 ± 0.6
Collagen (10 μg/ml) + Morphine (5 μ*M*)	7.9 ± 0.8*

Washed human platelets (1×10^9/ml) were preincubated with collagen, morphine, or collagen combined with morphine for 6 min at 37°C. Addition of collagen (10 μg/ml) to platelet suspensions served as a positive control. Data are presented as mean ± SEM (n = 6). *$p < 0.01$ as compared with the resting group.

of cAMP (Fig. 3B), resulting in increased $[Ca^{2+}]_i$ mobilization (Fig. 2).

In this study, collagen-induced formation of thromboxane B_2, a stable metabolite of thromboxane A_2, was markedly potentiated by morphine (1 and 5 μM) (Fig. 4). Thus, it seems likely that thromboxane B_2 formation plays an important role in the mediation of the potentiation of morphine in platelet aggregation. Yohimbine significantly reversed the potentiation of thromboxane A_2 formation by morphine in the presence of collagen, indicating that this potentiation was mediated by α_2-adrenoceptors in human platelets. In addition, morphine is not capable of increasing arachidonic acid–induced synthesis of prostaglandin E_2 in the presence of imidazole (Table 1), indicating that morphine-stimulated thromboxane A_2 formation in human platelets is not through increasing the activity of cyclooxygenase. Conversely, our preliminary study revealed that morphine (1 μM) significantly increased the arachidonic acid release in [^{3}H] arachidonic acid–labeled platelets stimulated by collagen (unpublished data). This result implies that the increase of thromboxane A_2 formation stimulated by morphine may be through activation of phospholipase A_2.

Morphine has recently been strengthened with a new opiate receptor subtype, designated mμ 3. This opiate receptor is coupled to nitric oxide release in human endothelial cells, granulocytes, and monocytes (21). Whether morphine potentiates platelet aggregation through a change of nitric oxide release in human platelets, however, remains to be determined. In this study, morphine caused no significant changes in nitric oxide formation in either resting or collagen-activated platelets (Table 2). Furthermore, we found that pretreatment with morphine did not affect the cGMP formation stimulated by nitroglycerin (10 μM) in human platelets. Signaling by cGMP somehow interferes with the agonist-stimulated Ca^{2+}-mobilizing second messengers (22). This result of cGMP study is in accord with the result of nitric oxide formation, because the nitric oxide being produced is biologically active; most cellular actions of nitric oxide occur via stimulation of intracellular soluble guanylate cyclase, leading to an increase in cGMP and thereby producing vasodilation, inhibiting platelet aggregation, and inhibiting protein kinase C activation (22,23).

In conclusion, the observations of this study suggest that morphine potentiates agonist-induced human platelet aggregation. This may involve the following mechanisms: morphine activation of α_2-adrenoceptors in human platelets, with a resulting inhibition of adenylate cyclase, thereby reducing intracellular concentration of cAMP. This leads to elevated $[Ca^{2+}]_i$ followed by stimulation of thromboxane A_2 formation, finally resulting in potentiation of platelet aggregation. Results of this study imply that the abuse of morphine may increase the risk of thromboembolic-related disorders or that patients undergoing analgesic regimens with morphine may experience increased morbidity of arterial thrombotic complications. However, the physiologic relevance of a direct proaggregatory effect of morphine is unclear and remains to be further studied.

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