Phosphodiesterase isoenzymes in equine platelets and their influence on platelet adhesion

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Objective—To determine the phosphodiesterase (PDE) isoenzymes in equine platelets and evaluate their influence on platelet adhesion.

Sample Population—Platelets obtained from healthy New Forest Pony geldings that ranged from 12 to 20 years of age (mean ± SEM, 17.3 ± 1.1 years).

Procedures—PDE isoenzyme activity in equine platelets was determined by use of a 2-step radioactive assay. Functional importance of PDE isoenzymes was established by use of selective inhibitors in a colorimetric adhesion assay.

Results—PDE1, PDE2, PDE3, and PDE5 and small amounts of PDE4 were found in equine platelets. Inhibition of PDE3 abolished platelet adhesion almost completely, whereas inhibition of PDE4 and PDE5 had little effect.

Conclusions and Clinical Relevance—Function of equine platelets can be influenced by inhibition of PDE3. Selective PDE3 inhibitors may be clinically useful to regulate platelet function. They offer the advantage of increased potency with fewer adverse effects, compared with those for nonselective PDE inhibitors. (*Am J Vet Res* 2007;68:1354–1360)

A role for platelets as inflammatory cells is being increasingly recognized in multiple diseases of humans.[1] Thus, it has been suggested that pathologic platelet activation may contribute to atherosclerosis; ischemic heart disease; diabetes; ulcerative colitis; and several respiratory tract disorders, including asthma, cystic fibrosis, and acute respiratory distress syndrome.[2] Therefore, influencing platelet function may offer an alternative therapeutic approach for these diseases. As more is learned about their biological processes, the role of platelets as inflammatory cells in diseases of horses is also becoming more apparent,[3-7] and platelet activation has been described in several diseases of horses.[8-14]

Although aggregation is an important functional response that has been studied extensively, other platelet functions, such as adherence, are likely to be important in inflammation. Therefore, it is of interest to investigate ways to modulate such responses. The intracellular cyclic nucleotides, cAMP and cGMP, are second messengers involved in regulating several functional responses of platelets, including cytoskeletal rearrangement, activation of fibrinogen receptors, degranulation, and expression of proinflammatory signaling molecules.[2] Increases in intracellular concentrations of nucleotides can be achieved by activating adenylyl cyclase or guanylyl cyclase or by inhibiting the PDE enzyme responsible for their breakdown.

Abbreviations

cGMP	Cyclic guanosine monophosphate
PDE	Phosphodiesterase
BSA	Bovine serum albumin
DMSO	Dimethyl sulfoxide
IC_{50}	Half maximal inhibitory concentration
CRC	Concentration-response curve
IBMX	Isobutyl methylxanthine
PAF	Platelet-activating factor

Phosphodiesterases are a continuously expanding enzyme family currently comprising 11 isoenzymes with > 50 isoforms[15] that are differentially expressed in almost all mammalian tissues. Therapeutic success for the use of PDE inhibitors has been greatly enhanced by the development of inhibitors selective for specific isoenzymes, thereby increasing potency and reducing the undesirable adverse effects that result from use of nonselective inhibitors.[16,17] Isoenzyme-selective compounds for specific isoenzymes are currently being used for the treatment of humans with conditions such as erectile dysfunction and pulmonary hypertension and are under active investigation as anti-inflammatory and antiplatelet drugs.[15,16] In veterinary medicine, milrinone and pimobendan, which inhibit PDE3, are used in the treatment of animals with cardiovascular disease.[18,19]

Nonselective PDE inhibitors have been used with varying success as therapeutics in horses with common diseases, such as recurrent airway obstruction, laminitis, and systemic inflammatory response syndrome,[20-23] and examination of the role of isoenzyme-

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selective compounds in this species will be facilitated by knowledge of the isoenzymes in various inflammatory cells. The PDE isoenzyme profile of equine neutrophils and lymphocytes has been reported,[24,25] but that of equine platelets has yet to be established, although it has been suggested[26] that their profile contains PDE3 and that inhibition of this isoenzyme prevents in vitro aggregation.

To test the hypothesis that the function of equine platelets can be influenced by selective PDE inhibition, the study reported here was conducted to determine the PDE isoenzyme profile in equine platelets. An assay of platelet adhesion to serum-coated plastic was then used to examine the effects of nonselective and PDE isoenzyme–selective inhibition because adhesion of human platelets to protein-coated plastic is dependent on cAMP.[27]

Materials and Methods

Sample population—Blood samples were obtained from healthy New Forest Pony geldings. Ponies were 12 to 20 years old (mean ± SEM, 17.3 ± 1.1 years). Ponies were housed on pasture and regularly dewormed and vaccinated against tetanus. The study was performed under a Home Office license approved by The Royal Veterinary College Ethics and Welfare Committee.

Isolation of platelets—Approximately 100 mL of venous blood was collected into 0.4M EDTA, and erythrocytes were allowed to settle for 30 minutes. The leukocyte-containing, platelet-rich plasma was removed and centrifuged at 350 × *g* for 10 minutes at 4°C to obtain platelet-rich plasma, which was subsequently centrifuged at 1,200 × *g* for 15 minutes at 4°C. The platelet pellet was washed twice in calcium-magnesium–free Hank's balanced salt solution containing 10 mmol of HEPES/L and 0.1% BSA. Platelets were counted in a hemacytometer and resuspended at a concentration of 5×10^7/mL in calcium-magnesium Hank's balanced salt solution containing 10 mmol of HEPES/L, 4 mmol $NaHCO_3$/L, and 0.1% BSA for immediate use in functional assays or at a concentration of 2.5×10^8/mL in modified homogenization buffer (10 µg of leupeptin/mL, 20mM Tris-HCl, 2mM $MgCl_2$, 1mM dithiothreitol, 5mM EDTA, 1.3mM benzamidine, 0.25mM sucrose, 20µM Nα-p-tosyl-L-lysine chloromethyl ketone, and 1% triton) prior to lysis. Platelet lysates were stored at –80°C and thawed immediately before use in PDE assays.

Measurement of platelet PDE activity—Total cAMP- and cGMP-hydrolyzing PDE activity and the influence of selective PDE inhibitors on enzyme activity were determined by use of a 2-step radioactive assay as described elsewhere for equine leukocytes,[24,25] except that the number of platelets required to yield sufficient protein was approximately 5- to 8-fold higher. In brief, [^{3}H]-cAMP[a] or [^{3}H]-cGMP[a] (final concentration, 1µM) was added to 50 µL of platelet lysate or lysis buffer in assay buffer (pH, 8) that contained a PDE inhibitor, a PDE activator, or DMSO. After incubation for 25 minutes at 30°C, the reaction was terminated by boiling for 1 minute and samples were then cooled on ice. Snake venom (0.1 mg/mL) was then added, and samples were incubated for another 10 minutes at 30°C. Samples were then placed on ice, 1 mL of methanol was added, and samples were eluted through an ion-exchange column into scintillation fluid. After flushing the columns with methanol, radioactivity in the combined elute was counted. For each assay, tubes containing tritiated substrate alone were included to measure the total radioactivity eluting from the column. Each sample was analyzed in duplicate, and each assay was performed on platelets from 5 horses. The protein content of each sample was determined by use of a colorimetric method.[28]

The isoenzyme profile was determined by use of a single high concentration (100× the reported IC_{50} value) of selective inhibitors for PDE1 (vinpocetine[b]), PDE3 (trequinsin[b]), PDE4 (rolipram[c]), and PDE5 (zaprinast[b]). Because vinpocetine is a weak PDE1 inhibitor, a PDE1-activating compound (calcium-calmodulin[c]) was additionally used, and cGMP, a dual PDE3 inhibitor and PDE2 activator, was used to evaluate the quantitative relationship between these 2 isoenzymes. The CRC for relevant agents as well as for 2 nonselective PDE inhibitors was then established. Theophylline[c] was chosen because it has been used therapeutically in equine medicine, and IBMX[c] was used for comparison because it is a more potent nonselective inhibitor. In consideration of the results obtained, CRCs were also established for 1 additional PDE3 inhibitor (milrinone[c]) and 2 additional PDE4 inhibitors (RO20-1724[c] and roflumilast[d]). All PDE inhibitors were made as stock solutions in DMSO and stored at –20°C. Serial dilutions were freshly prepared each day in DMSO.

Preparation of microtiter plates for platelet adhesion assay—Microtiter plates (96 wells) were coated (100 µL/well) with autologous serum (1:9 dilution in PBS solution), as described elsewhere.[29] Plates were stored for 48 to 72 hours at 4°C and removed from the refrigerator 1 hour before usage.

Inhibition of platelet adhesion—The influence of nonselective and selective PDE inhibitors on PAF- and thrombin-stimulated platelet adhesion was evaluated by establishing CRCs for relevant inhibitors used in the PDE assay. A direct adenylyl cyclase activator (forskolin[b]) was also evaluated as another means of increasing intracellular cAMP content. Inhibitors or DMSO (total DMSO in cell suspension, ≤ 0.1%) were added to the platelet suspension and incubated for 15 minutes at 37°C. Platelets (100 µL) were then added (triplicate or quadruplicate) for wells that contained 10 µL of medium or agonist (final concentration, 10^{-8}M PAF or 0.1 U of thrombin/mL), and adhesion was determined as described elsewhere.[e] Concentrations of PAF and thrombin were chosen because they cause maximal adhesion. The PAF and thrombin were made as stock solutions in ethanol and distilled water, respectively, and stored at –20°C. Serial dilutions were freshly prepared each day in PBS solution containing 0.25% or 0.1% BSA for PAF and thrombin, respectively.

Briefly, after incubation for 60 minutes at 37°C in a humidified atmosphere, nonadherent platelets were removed by washing 3 times (250 µL of PBS solution/wash) and then 50 µL of lysis buffer (0.2% CTAB in distilled water) and 100 µL of substrate (1 mg of the disodium salt of *p*-nitrophenyl phosphate/mL) in 0.1M

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citrate buffer (pH, 5.4) were added to each well. The enzymatic reaction was stopped after 45 minutes by the addition of 100 µL of 2M NaOH, and absorption was measured at 405 nm by use of a plate reader.[f] Platelet adherence was calculated as a percentage of the acid phosphatase activity in the lysed equivalent of the total number of platelets added to each well. Effects of the inhibitors were expressed as the percentage inhibition achieved after incubation with the inhibitor, compared with the response obtained for incubation with thrombin or PAF alone (which were assigned a value of 100%). Assays were performed by use of platelets from 3 or 6 ponies.

Determination of intracellular cAMP content— Platelets were prepared as described for the adhesion assay. The platelet suspension (5×10^7/mL) was incubated with trequinsin (10^{-5}M), forskolin (10^{-4}M), or medium alone for 15 minutes at 37°C; thrombin (0.1 U/mL) or medium was added; and suspensions were incubated for another 60 minutes. Platelets were then washed 3 times in PBS solution, and cAMP content was determined in duplicate by use of an ELISA,[g] as indicated in the manufacturer's instructions, except that the platelet concentration was increased 5-fold to enable detection of cAMP. In brief, cAMP in the platelet lysates competed with horseradish peroxidase–labelled cAMP for binding to an anti-cAMP antibody that bound to a polyclonal goat anti-mouse antibody coated onto a microplate. After removal of excess conjugate and unbound sample, substrate (hydrogen peroxide and tetramethylbenzidine) was added and intensity of color development was then determined in a plate reader at 450 nm. Detection limit of the assay specified by the manufacturer was 1.43 pmol/mL (range, 0.58 to 3.0 pmol/mL).

Data analysis—Data were evaluated to ensure that they were appropriate for analysis by use of a parametric test. Data were then analyzed by use of a repeated-measures ANOVA, followed by post hoc comparison by use of the Bonferroni test. Statistical analysis software[h] was used for all analyses. Values of $P < 0.05$ were considered significant. Data were expressed as mean ± SEM.

Results

PDE isoenzyme profile and CRCs for selected inhibitors—Use of single high concentrations revealed significant ($P < 0.001$) activation or inhibition of PDE activity with calcium-calmodulin and zaprinast, which indicated the presence of PDE1 and PDE5 (**Figure 1**). Because a more potent PDE1 inhibitor is not readily available, and in light of the high vinpocetine concentration used without significant effect, this inhibitor was not evaluated further. Because other studies have suggested that equine platelets contain PDE3 and have revealed a functional role of this isoenzyme, a higher concentration of trequinsin (10^{-6}M) was evaluated in platelets obtained from a single horse, and this caused > 50% inhibition of PDE activity. Rolipram (10^{-5}M) caused a small but insignificant inhibition of PDE activity. However, in consideration of the importance of PDE4 in regulating the function of inflammatory cells, evidence for this isoenzyme was explored further.

Concentration-response curves were next established for theophylline, IBMX, trequinsin, rolipram, and zaprinast. Concentration-dependent inhibition of cAMP- and cGMP-hydrolyzing activity was evident with trequinsin and zaprinast, and inhibition was almost complete at concentrations of 5×10^{-5}M and 10^{-4}M, respectively (**Figure 2**). The reported[30] IC_{50} for trequinsin is 3×10^{-10}M, a concentration approximately 100-fold less than that at which inhibitory effects were first detected in the assay reported here. Therefore, another PDE3 inhibitor, milrinone (IC_{50}, 3×10^{-7}M), was evaluated, and a similar decrease in sensitivity to inhibition of equine PDE3 was detected.[30] In another experiment, comparison of equine platelets obtained from 3 horses and human platelets obtained from 2 healthy volunteers revealed almost identical CRCs for trequinsin and milrinone in both species (data not shown).

Rolipram also caused concentration-dependent (but incomplete) inhibition, reaching a mean ± SEM maximum inhibition of 45.5 ± 3.5% at a concentration of 10^{-5}M. Unexpectedly, a concentration of 10^{-4}M rolipram caused significant ($P < 0.001$) PDE stimulation; this effect was evident in platelets for all 6 ponies tested and was repeatable among multiple experiments. To investigate this unusual finding, CRCs for 2 additional PDE4 inhibitors, roflumilast and RO20-1724, were established. Both compounds caused a similar

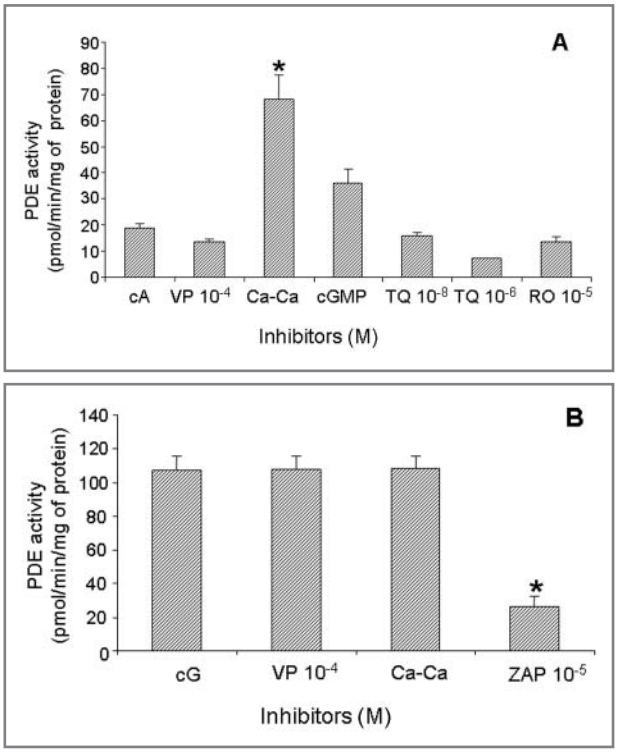

Figure 1—Mean ± SEM inhibition or stimulation of cAMP-hydrolzying PDE activity (cA; A) and cGMP-hydrolyzing activity (cG; B) in equine platelets by single high concentrations of PDE-selective agonists. Results represent duplicate assays for equine platelets obtained from 5 ponies for vinpocetine (VP), calcium-calmodulin (Ca-Ca), trequinsin (TQ) at 10^{-8}M, rolipram (RO), and zaprinast (ZAP) and from 1 pony for TQ at 10^{-6}M. *In panels A and B, value differs significantly ($P < 0.05$), compared with total cA or total cG, respectively.

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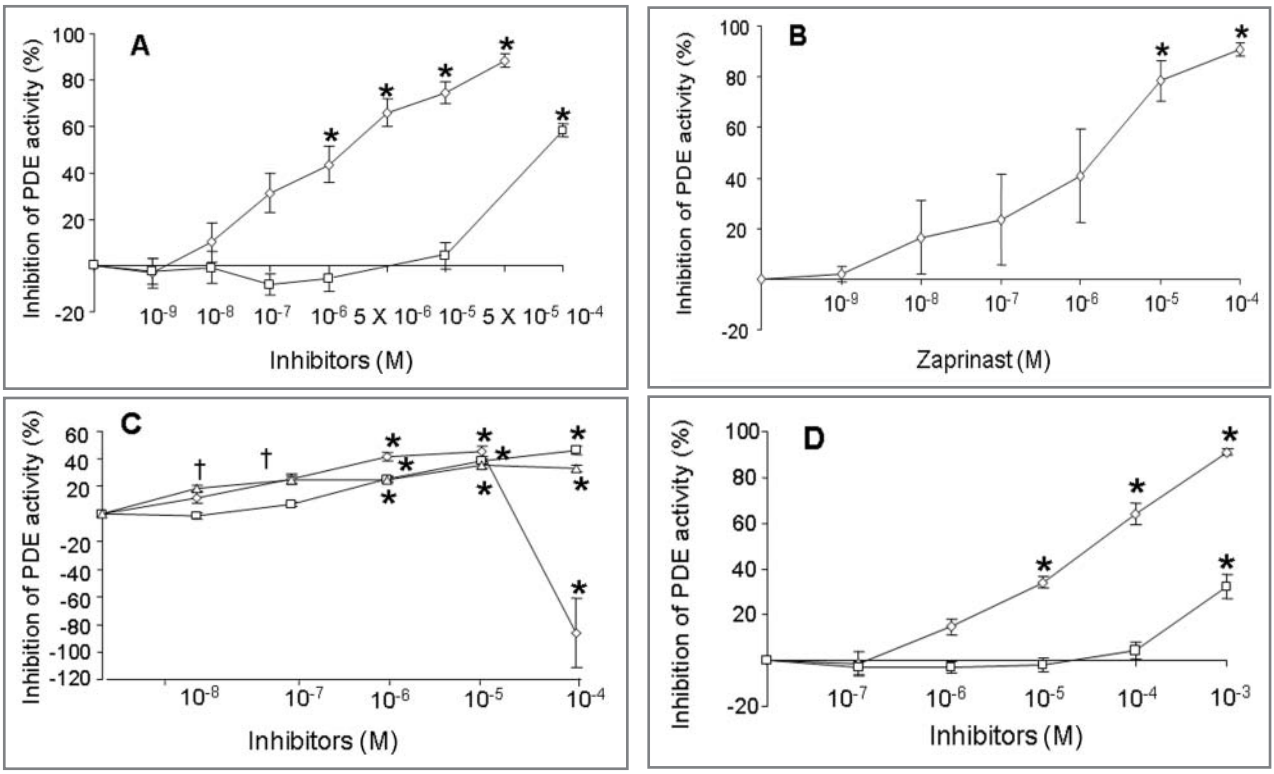

Figure 2—Mean ± SEM inhibition of PDE activity in equine platelets by the PDE3 inhibitors trequinsin (diamonds) and milrinone (squares; A); the PDE4 inhibitor zaprinast (B); the PDE5 inhibitors rolipram (diamonds), Ro 20-1724 (squares), and roflumilast (triangles; C); and the nonselective PDE inhibitors IBMX (diamonds) and theophylline (squares; D). Results represent duplicate assays for equine platelets obtained from 5 ponies for all inhibitors. *Within a concentration, value differs significantly ($P < 0.05$), compared with total cAMP-hydrolyzing PDE activity. †Within a concentration, value for roflumilast differs significantly ($P < 0.05$), compared with total cAMP-hydrolyzing PDE activity.

amount of concentration-dependent inhibition, but no stimulation at 10^{-4}M (Figure 2). The PDE activity in platelets from 2 healthy human volunteers also had no stimulation with the addition of 10^{-4}M rolipram (data not shown). Theophylline caused weak PDE inhibition, which reached approximately 30% at the highest concentration used (10^{-3}M), whereas 10^{-3}M IBMX caused almost complete inhibition of PDE activity.

Inhibition of platelet adhesion—Platelet adhesion was almost completely inhibited in a concentration-dependent manner by trequinsin, IBMX, and forskolin, whereas milrinone caused approximately 70% inhibition at the highest concentration used (10^{-5}M; **Figure 3**). In contrast, 10^{-3}M theophylline caused no inhibition (data not shown). Rolipram at 10^{-6}M and 10^{-5}M induced a mean ± SEM maximum inhibition of 32.9 ± 7.6% and 36.5 ± 3.7% for thrombin- and PAF-induced adhesion, respectively, whereas 10^{-6}M zaprinast caused a maximum inhibition of 28.0 ± 6.5% and 17.2 ± 12.5% for thrombin- and PAF-induced adhesion, respectively.

Determination of intracellular cAMP content—Forskolin and trequinsin with or without the addition of thrombin caused an increase in intracellular cAMP content (**Table 1**). Incubation with forskolin caused significant ($P < 0.001$) differences. Incubation with trequinsin and the addition of thrombin did not have a significant ($P = 0.07$) effect.

Discussion

Analysis of results obtained in the study reported here suggested that equine platelets contained PDE1, PDE2, PDE3, and PDE5 and smaller quantities of PDE4, as indicated by the effects of vinpocetine and calcium-calmodulin (PDE1), cGMP (PDE2), trequinsin and milrinone (PDE3), zaprinast (PDE5), and rolipram (PDE4) on cAMP- or cGMP-hydrolyzing PDE activity. These isoenzymes and also PDE6 have been identified[31,32] in human (PDE2, PDE3, and PDE5) and bovine (PDE1, PDE2, PDE3, PDE4, and PDE6) platelets. The cGMP-hydrolytic activity in equine platelets is approximately 6 times as high as the cAMP-hydrolytic activity, which agrees with the ratio reported for human platelets[31] (Figure 1).

Incubation with cGMP, a stimulator of PDE2 and inhibitor of PDE3, caused overall stimulation of PDE activity, which indicated more PDE2 than PDE3 in equine platelets or a greater sensitivity of PDE2 to stimulation than PDE3 to inhibition. Although PDE3 appears to be an important regulator of cAMP PDE activity in equine platelets, it was interesting that there was an apparent decrease in sensitivity of equine PDE3 to inhibition. To rule out a species difference, human and equine platelets were compared in identical assay conditions, which revealed similar sensitivity to PDE3 inhibition in response to trequinsin and milrinone. Thus, although species-specific variability to PDE inhibitors has been reported,[33] these findings suggested that dif-

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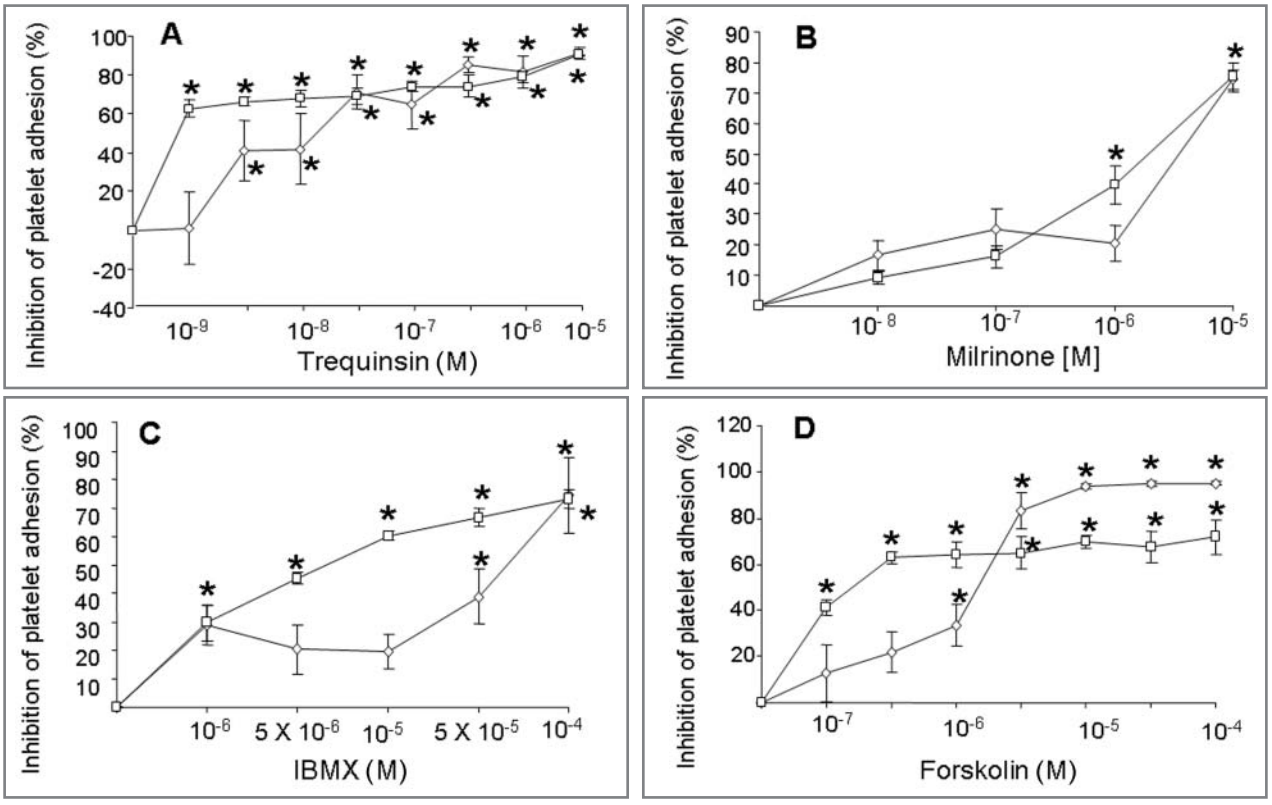

Figure 3—Mean ± SEM inhibition of thrombin- and PAF-induced adhesion of equine platelets to serum-coated plastic by the selective PDE3 inhibitors trequinsin (A) and milrinone (B), a nonselective PDE inhibitor (ie, IBMX; C), and an adenylyl cyclase–stimulating agent (ie, forskolin; D). Thrombin-induced (diamonds) and PAF-induced (squares) adhesion was 33.7 ± 6.2% and 14.8 ± 1.5%, respectively, and background adhesion was 4.1 ± 1.2%. Results represent quadruplicate assays for PAF-induced adhesion in equine platelets obtained from 3 ponies and for thrombin-induced adhesion in equine platelets obtained from 6 ponies (panels A, C, and D) and 3 ponies (panel B). *Within a concentration, value differs significantly ($P < 0.05$), compared with the value for PAF or thrombin alone.

ferences in assay conditions was the more likely explanation for the discrepancy between the reported IC_{50} and the results reported here. It should be mentioned that trequinsin at high concentrations (IC_{50}, 0.64 to 2 × 10^{-6}M and 1.6 × 10^{-6}M, respectively) reportedly inhibits PDE2 and PDE4, respectively,[34,35] and that milrinone (IC_{50}, 1.9 × 10^{-5}M) also inhibits PDE4.[36] Thus, it cannot be excluded that the almost complete PDE inhibition achieved at high concentrations was a result of a combined effect on PDE2, PDE3, and PDE4.

Surprisingly, platelet adhesion was inhibited by much lower concentrations of trequinsin and milrinone, similar to those reported to decrease aggregation of equine platelets.[26] A potential explanation is that incomplete inhibition of the total PDE3 activity was sufficient to cause a substantial functional change. Alternatively, trequinsin and milrinone could have acted through an effect unrelated to PDE3 inhibition. An increase in intracellular cAMP content after equine platelets were treated with trequinsin and forskolin was evident, although, as mentioned previously, trequinsin alone did not result in a significant increase. This supports the assumption that inhibition of platelet adhesion is attributable to an increase in intracellular cAMP content and not to an alternative mechanism unrelated to inhibition of PDE activity, particularly because forskolin (which activates adenylyl cylase) also inhibited platelet adhesion. The molecular mechanisms involved in adhesion of equine platelets to serum-coated plastic have yet to be investigated. In humans, multiple surface receptors have been implicated in adherence, with adhesion to plasma-coated plastic being dependent primarily on glycoprotein IIbIIIa and also on glycoprotein IcIIa.[37] Because glycoprotein IIbIIIa surface expression is increased in human platelets after stimulation by epinephrine, which will decrease intracellular cAMP concentrations, it is tempting to speculate that the inhibition of adhesion of equine platelets in response to PDE3 inhibition or adenylate cyclase activation involves effects on this receptor.[38]

Maximal inhibition (approx 40%) of cAMP PDE activity achieved with all of the PDE4 inhibitors tested and the limited effect of rolipram in the functional assay suggested that this isoenzyme is contained in small quantities in equine platelets and does not play a major

Table 1—Mean ± SEM intracellular cAMP content of 5 × 10^7 platelets/mL after incubation with medium alone or medium containing forskolin or trequinsin and with or without thrombin.

	cAMP (pmol/mL)	
Agonsist	**No thrombin**	**0.1 U of thrombin/mL**
Medium	3.1 ± 1.3	11.9 ± 7.3
Forskolin (10^{-4}M)	176.9 ± 26.9*	223.8 ± 29.7*
Trequinsin (10^{-3}M)	39.2 ± 8.55	81.6 ± 31.1*

*Within a column, value differs significantly ($P < 0.05$) from the value for medium alone.

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role in the regulation of platelet adhesion. In another study,[26] aggregation of equine platelets was not inhibited by 10^{-5}M rolipram. The PDE stimulation detected with 10^{-4}M rolipram was unexpected and was not evident with 2 other PDE4 inhibitors or in human platelets. In addition, this phenomenon has not been reported for equine lymphocytes in identical assay conditions,[39] which suggested that it is a specific interaction between rolipram and equine platelet PDE.

Zaprinast caused almost complete inhibition of cGMP-hydrolyzing PDE activity, which suggested that PDE5 is an important regulator of cGMP in equine platelets. This is similar to human platelets, which reportedly are among the richest sources of PDE5.[31] Zaprinast (IC_{50}, 7.6×10^{-7}M), although widely used as a PDE5 inhibitor, is not entirely selective and also inhibits PDE6 (IC_{50}, 1.5×10^{-7}M), PDE9 (IC_{50}, 2.9×10^{-5}M), and PDE11 (IC_{50}, 5 to 28×10^{-6}M).[i] Although more selective PDE5 inhibitors exist, they were not available for use in the assay reported here. The sensitivity of platelet PDE to inhibition by zaprinast was extremely variable among ponies, with 3 ponies being fairly insensitive and 2 extremely sensitive. For these 2 ponies, there was almost complete inhibition at 10^{-6}M, in contrast to 10^{-4}M for the other 3 ponies. Altered sensitivity to inhibition by PDE isoenzyme–selective inhibitors has been reported in mononuclear cells from humans with asthma and atopic dermatitis[40,41] and in lymphocytes from ponies with recurrent airway obstruction 24 hours after an antigen challenge.[39] Ponies used in the study reported here had no history of any disease and were judged to be healthy on the basis of results of clinical examination. However, an underlying or developing endocrine or metabolic disease cannot be ruled out, and this finding requires further investigation.

Lack of an effect of zaprinast on adhesion of equine platelets may be explained by its poor penetration of cells, although significant inhibition of proliferation of peripheral blood mononuclear cells has been achieved at 10^{-5}M.[25] However, in human platelets, coincubation with a guanylyl cyclase activator is required to increase basal cGMP concentrations before the effects of zaprinast on platelet function become evident.[42] It is also possible that cGMP is not an important regulator of equine platelet function because zaprinast did not inhibit aggregation of equine platelets at a concentration of 10^{-5}M.[26] An alternative PDE5-selective, cell-permeable inhibitor is necessary for further investigations.

Theophylline is a weak PDE inhibitor, as indicated by results of the PDE activity assay, and had no effect on platelet adhesion at the concentration tested. It is also a weak inhibitor of aggregation of equine platelets,[26] and some of the effects seen clinically may not be related to its ability to inhibit PDE. The second nonselective PDE inhibitor used (IBMX) inhibited PDE activity and platelet adhesion but was less effective, compared with the inhibitory effects of a PDE3-selective inhibitor (trequinsin) and an adenylyl cyclase activator (forskolin).

In the study reported here, we found that equine platelets contain PDE1, PDE2, PDE3, and PDE5 and limited amounts of PDE4. Adhesion of equine platelets can be inhibited by increasing intracellular cAMP concentrations. Functionally, PDE3 appears to be the most important isoenzyme. Inhibition of PDE3 may be beneficial for use in clinical diseases in which platelet activation is evident.

a. GE Healthcare Ltd, Buckinghamshire, England.
b. Merck Chemicals UK Ltd, Nottingham, England.
c. Sigma-Aldrich Co, Gillingham, Dorset, England.
d. Provided by Altana Pharma Ltd, Marlow, Buckhinghamshire, England.
e. Dunkel B, Rickards KJ, Page CP, et al. Regulation of equine platelet adherence by cAMP (abstr), in *Proceedings*. 75th Anniv Winter Meet Br Pharmacol Soc 2006;C013.
f. SpectraMax 250, GMI Inc, Ramsey, Mich.
g. Parameter, R&D Systems Europe Ltd, Abington, England.
h. SPSS, version 14.0, SPSS Inc, Chicago, Ill.
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